CHAPTER 1: PART A

INTRODUCTION TO CARBOHYDRATES AND BIOACTIVE POLYSACCHARIDES

1A.1. Carbohydrates

Carbohydrates, the most naturally occurring organic biomolecules, are essential constituents of all living organisms. These have general molecular formula $C_m(H_2O)_n$, but any intact water molecules was not detected. Robyt, 1998 defined "Carbohydrates are polyhydroxy carbonyl compounds or compounds that can be produced from them through oxidation, reduction or replacement of different functional groups" [1]. Depending on the number of sugar residues present in entire chain, they are divided into three categories: monosaccharides, oligosaccharides and polysaccharides.

Monosaccharides are chiral polyhydroxy carbonyl compounds which cannot be hydrolyzed into smaller units. Generally, they are white crystalline solids and sweet, containing single carbonyl functional groups. e.g.; glucose, mannose, galactose, fructose, ribose, arabinose, etc.

Oligosaccharides are composed of two to ten monosaccharide linked together glycosidically. On acid hydrolysis, they cleaved into constituent monosaccharide units. According to the number of units, they are called disaccharides (e.g.; sucrose, maltose, lactose, etc.), trisaccharides (e.g.; raffinose, maltotriose, etc.), tetrasaccharides (e.g.; stachyose, maltotetraose, nystose, etc.), and so on.

Polysaccharides are made up of more than ten monosaccharide linked together glycosidically. Living systems often converts monosaccharides into various kinds of polysaccharides through biological synthesis when they are not instantly used.

1A.2. Polysaccharides

The great quantity of the carbohydrates in nature is present mainly as polysaccharides. Polysaccharide was first reported biopolymer [2]. They are often branched with high molecular weight and large numbers of monosaccharides units impose complexity in structure, leading to different characteristics from their constituents; like amorphous nature, insoluble in water, having no sweet taste, etc. [3]. The physicochemical natures of polysaccharides depend on chain conformation, intermolecular hydrogen bonding, etc. For example, the chains in amylopectin tend to adopt helical conformations while some (1→3), (1→6)-β-D-glucan chains adopt triple helical conformation, due to extensive internal hydrogen bonds among hydroxyl groups.

❖ Classification of polysaccharides:

- Depending on the type monomeric unit present in their structure, polysaccharides are divided into two categories:
  - **Homopolysaccharides**, which are constituted of only one type of monosaccharide, e.g. starch, cellulose, dextran, glycogen, etc.
  - **Heteropolysaccharides**, which are composed of different monosaccharide units, e.g. galactomannan, xyloglucan, glucomannan etc.
- Depending on the structure polysaccharides are divided into two categories:
  - **Linear -** alginates, amylose, cellulose etc.
  - **Branched** – amylopectin, arabinoxylan, gum arabic etc.
- Depending on their biological function, polysaccharides are divided into two categories:
  - **Storage polysaccharides** which store energy in animals and plants, e.g.; glycogen, starch, and inulin, etc.
  - **Structural polysaccharides** which play roles in structure construction in plant cell wall or exoskeleton of insects. e.g.; cellulose, chitin, etc.

1A.3. Structure and function of some common Polysaccharides

Starch

It contains two components, amylose and amylopectin, both are homopolysaccharide of D-glucose [4]. Amylose is unbranched, helically coiled polysaccharide, composed of (1→4)-α-D-glucopyranoside residue (**Figure 1A.1**). Amylopectins are also possessed same main chain of (1→4)-α-D-glucopyranoside residue, but branched with (1→6)-α-D-glucopyranoside units at about every 25-30 D-glucopyranoside resiues. All plant cells is rich resource of it. Human and other animals excrete amylase enzyme which helps to digest starch, consumed from different food sources.

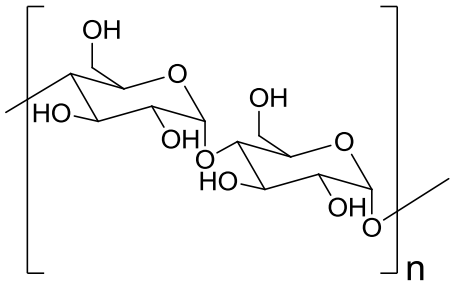

Figure 1A.1. α-amylose, a polymer of α-(1→4) linked D-glucose.

Cellulose

Cellulose is the most abundant compound in the plant territory, forming the basic component of the plant cell walls and provides the structural strength [5-6]. It is a highly insoluble, rigid and fibrous homo polysaccharide of D-glucose units linked through (1→4)-β-glycosidic linkages (**Figure 1A.2**). It is interesting that due to the inactivity of the digestive enzyme α-amylase to (1→4)-β-Glc*p* linkages, humans cannot digest cellulose, but, these are used as dietary fibres.

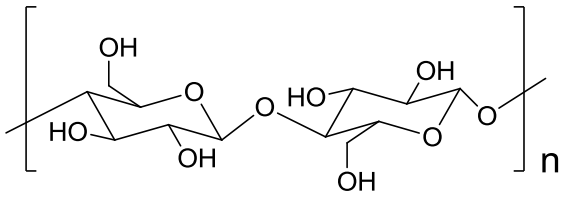

Figure 1A.2. Cellulose, a polymer of β-(1→4) linked glucose.

Glycogen

It is an extensively branched homopolysaccharide made up of D-glucose residues linked together by α-(1→4) glycosidic linkages with α-(1→6)-linked branches at few points. It is found in nearly all animal cells and in few protozoa and algae. It is mainly used for short-term storage energy in muscles and liver of humans and other vertebrates [7-8].

Pectin

Pectins are a complex set of heterogeneous water soluble polysaccharides, consist of α-(1→4)-linked galacturonic acid or its ester (**Figure 1A.3**). They are the cementing

materials of cell walls of terrestrial plants. Apple, guava, quince, plum, gooseberries, and citrus fruits are rich source of pectins. Pectins are widely used in medicines, sweets and as thickening agent of jams and jellies, stabilizer in fruit juices, gelling agent. They serve as source of dietary fiber [9]. It can exhibit anti-inflammatory activities by reducing blood cholesterol [10].

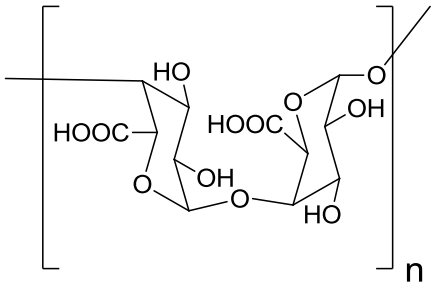

Figure 1A.3. Pectin, a polymer of α-(1→4) galacturonic acid

Chitosan

Chitin is a linear water-insoluble homopolysaccharide of β-(1→4)-linked N-acetyl-D-glucosamine residues [11]. Hydrolysis with NaOH solution of chitin yields water-soluble deacetylated homopolysaccharide, chitosan (**Figure 1A.4**). The outer skeletons of shellfish, including crab, lobster, etc. are the rich sources of chitin. Chitosan is used to control cholesterol and obesity and in the treatment of patients of kidney failure, appetite, trouble sleeping (insomnia), Crohn's disease, etc. It is high biodegradability, high biocompatibility and non-toxic nature, for these properties the pharmaceutical industries used it as filler in tablets as carrier in controlled drug delivery [12].

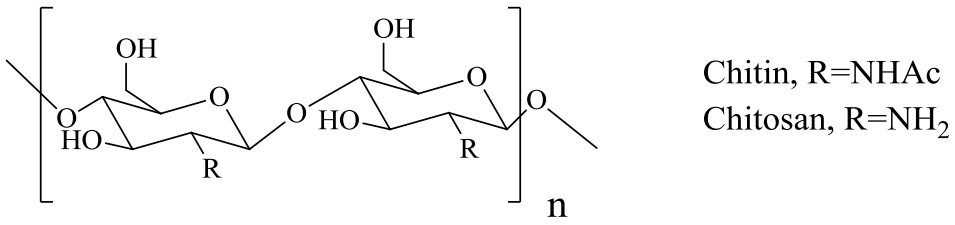

Figure 1A.4. Structure of Chitin, β-(1→4)-linked N-acetyl-D-glucosamine and Chitosan, β-(1→4)-linked D-glucosamine.

Heparin

It is an acidic mammalian polysaccharide consisting of highly sulfated (1→4)-linked hexosamine and uronic acid residues (**Figure 1A.5**). It is commonly extracted from animal tissues like, bovine lung and porcine intestine. It has a variety of biological functions like mitogenesis, blood anticoagulation, anti-inflammation and cell migration [13].

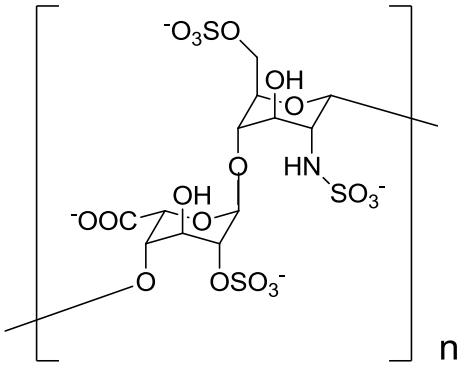

Figure 1A.5. Heparin, a polymer of (1→4)-linked sulfated hexosamine and uronic acid.

Guar gum

It is water soluble, yellowish white, nearly odourless, free-flowing powdered polysaccharide. It is obtained from the ground endosperm of the seed of the guar plant. It is mainly galactomannans, comprises of a linear bacbone of (1→4)-linked β-D-man*p* units with a side chains of (1→6)-linked α-D-gal*p*, where the ratio of mannose and galactose is nearly 2:1(**Figure 1A.6**) [14]. It is employed as emulsifier, stabilizer, formulation aid and thickener in different foods like, processed vegetable, vegetable juices, breakfast cereals, sweet sauces, syrups, cheeses, Jams and jellies, etc.

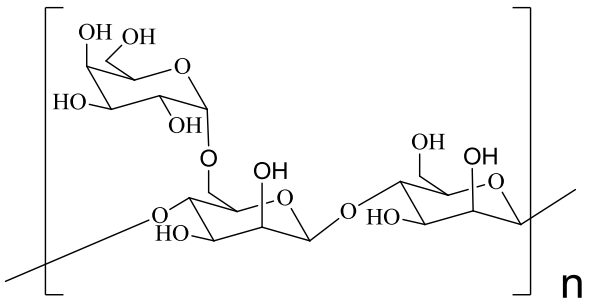

Figure 1A.6. Guar gum, a polysaccharide of (1→4)-linked β-D-man*p* units with side chains of (1→6)-linked α-D-gal*p*

1A.4. Mushrooms

1A.4.1. Definition and description

Mushroom is "a macro fungus with a distinguishing fruiting body that can be hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand" [15]. Mushrooms are the higher fungi with fruiting bodies, which are commonly umbrella like, but others may have various sizes, shapes and colour. Normally, it consists of a stalk or stipe and a cap or pileus. Mushrooms can be roughly divided as edible mushrooms, medicinal mushrooms and poisonous mushrooms.

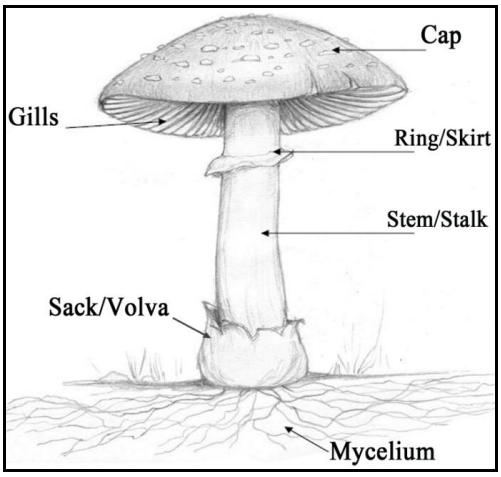

Figure 1A.7. Different parts of Mushroom [Mushroom structure: Teacher's notes, www.fungi4school.org]

1A.4.2. Edible mushroom *Termitomyces heimii*

It is a non-poisonous wild mushroom, grows with termite's guts through symbiotic relationship [16]. It is mainly found at forests of laterite soil of india and different part of the world [17-19]. *Termitomyces heimii* is a wealthy source of ergosterol and linoleic acid. So, it can be employed for control blood pressure, immune response, inflammation, and apoptosis through the linoleic acid assisted prostaglandins production and ergosterol is the precursor which made it as food supplement for hypocalcinaemia and osteoporosis suffering people [18]. For our studies the fruiting bodies of mushroom *T. heimii*, was collected from the laterite forest of Moyurbhonj area of Odisha in the month of August [19].

1A.4.3. Edible mushroom *Lentinus fusipes*

The non-poisonous wild mushroom, *Lentinus fusipes* (genus 'Lentinus', family 'Polyporaceae'), grows beneath bamboo clumps in rainy season at the forest of Karlapat sanctuary, Banei, Belghar and Kotagarh in Odisha, India india and different part of the world. Thus, it is locally called baunsa chattu. For our studies the fruiting bodies of mushroom *Lentinus fusipes* was collected from the Banei forest of Odisha, India [19].

1A.4.4. Chemical Composition and nutritional value

Mushrooms have been consumed as flavoured tasty food by human for their chemical and nutritional characteristic since times immemorial [20-21]. These are rich source of protein, glycoproteins, vitamins, minerals, fibers and trace elements but lack of cholesterol [22-24]. Mushrooms are the rich source of various of bioactive compounds e.g., polysaccharides, ceramides, phenolic compounds, sterols, etc.[25]. Mushrooms possess ~90% water by weight. The remaining ~10% consists of carbohydrate, protein, fat with essential minerals like potassium, calcium, iron, zinc, magnesium, copper, phosphorous, etc. [13]. The amino and fatty acids, vitamins, minerals, etc. contain in the commercial mushrooms is comparable to meats and higher than fruits and vegetables [26-27]. Research has shown that nutrient contents can significantly differ in mushrooms even within the same genus [28].

1A.5. Mushroom polysaccharides

Mushrooms are an essential resource of secondary metabolites [29-33]. Among different bioactive functional compounds occurring in mushrooms, polysaccharides plays a great function for health benefits [25]. Bioactive polysaccharides are present in different mushrooms and most have unique chemical structures with variation of genus. A wide range of polysaccharides were extracted from different mushroom, but, majority of them are glucans with variation of linkages, such as (1→6)-β-D-glucan, (1→3)-, (1→6)-β-D-glucans, (1→3)-α-D-glucans, and some are true heteroglycans. In some mushrooms, proteins or peptides bounds with active polysaccharides, known as

polysaccharide-protein or polysaccharide-peptide complex shows enhanced bio-eficiency [34].

1A.5.1. Structures and bioactivities

The most unique features of polysaccharides derived from mushroom are antibacterial, antifungal, antioxidant, antiinflammatory, antiviral, anticancer, antitumour, antidiabetic, antitumour, anti-HIV and immunostimulatory activities [24, 35-39].

β-D-glucans are the most significant mushrooms isolated polysaccharide. There are reports of several linear and branched β-D-glucans possessing beneficial biological activities [40-42]. A series of investigation revealed that the most active forms of β-D-glucans contained (1→3), (1→6) linkages and have the ability of immunoenhancing and immunostimulating properties; thus, they are recognised biological response modifiers (BRM) and used for the treatment of various infectious diseases including cancer [43-47]. Glucans having α- or both α- and β- linkages are also isolated from different mushrooms [48-51]. There have been also reports on antitumor activities of β-D-glucan-protein complexes [52], α-manno-β-glucan [53], α-glucan-protein complexes and heteroglycan-protein complexes [54], heteroglycans [55-56]. There are some reports of immunostimulating and antioxidant property of water soluble β-D-glucans, which can terminate the chain reaction of ROS by the donation of hydrogen to the free radicals and diminish the adverse effect of ROS [57-61]. The bioactivities of polysaccharides derived from mushroom are greatly influenced by the structure, size, molecular weight, conformation, branching pattern, structural modification, and solubility [62-63]. The special structural features of glucans, like high molecular weight, β-(1→3) linkages in the main chain and additional β-(1→6) branching made them potent antitumor agent [64-65]. Solubility in water is also an important aspect of mushroom polysaccharides. It has been observed that water soluble β-glucans has more prominent effect on the immune systems of humans and animals than water insoluble form [66]. Polysaccharides that form triple-helical conformation have clinical used for the treatment of cancers like human breast cancer, liver cancer and promyeloctytic leukemia [39].

The structural modifications of mushroom polysaccharides sometime improve the medicinal properties, water solubility of mushroom polysaccharides. A water insoluble but alkali soluble linear (1→3)-α-D-glucan obtained from *Amanita muscaria* and *Agrocybe aegerita* exhibits minor antitumor effect, while carboxymethylated products showed powerful antitumor activity [67-68].

1A.5.2. Some commercialized mushroom derived polysaccharides

Mushroom derived polysaccharides have been drawn the research interest of chemist and immunobiologists because of their immunomodulating and antitumor properties [36, 66]. Polysaccharides of mushrooms are able to increase the life time of cancer patients and used in cancer therapeutic, since, unlike other available anticancer chemical medicines these do not have toxic side effects [69]. Several mushroom polysaccharides are commercialized worldwide as anti-tumor agents, like **Agarican** (from *Agaricus blazei***, USA**), **Grifron-D** (from *Grifola frondosa***, Japan**), **Krestin** (from turkey tail mushroom *Trametes versicolor*), **Lentinan** (from *Lentinus edodes***, Japan**) and **Schizophyllan** (from *Schizophyllum commune)* and have been used clinically.

Agarican

This polysaccharide was isolated from mushroom *Agaricus blazei***.** The antitumor and immunostimulating activity of *A. blazei* extracts were investigated on Sarcoma 180 and fibrosarcoma tumor bearing mice [70-71]. Then, it has been commercialized and used clinically as antitumor agents [52, 72-73]. It also competent to prevent of broad spectrum of diseases like allergies, asthma and chronic hepatitis, etc.[74-76]. Total, seven polysaccharides were isolated from fruiting bodies of *A. blazei*, all of them have established antitumor action [77]. The most recognized fraction from *A. blazei* was (1→2),(1→3)-β-D-glucomannan, has antitumor action against Sarcoma 180 [48].

Grifron-D or GD

This polysaccharide was isolated from mushroom *Grifola frondosa* (also known as Maitake). **GD** is actually a (1→3),(1→6)-β-D-glucan. GD has *in vitro* cytotoxic affect on prostate cancer cells of human (PC9) and causes 95% cell death by an apoptosis [78] and strong antitumor activity [79].

Krestin

This was developed from turkey tail mushroom *Trametes versicolor*. It is a protein bound polysaccharide (PSK), contains 75% β-glucan and 25% protein [34, 66]. PSK has amazing immunostimulating activity and exhibits direct effect against various types of tumors in animals such as, adenosarcoma, sarcoma, melanoma, carcinoma, plasmacytoma, fibrosarcoma, mastocytoma [80] and indirect effect to improve cellular immunity in host [37,81].

Lentinan

This polysaccharide was first isolated from Shiitake mushroom, *Lentinus edodes*. It is actually a β-(1→3), β-(1→6)-glucan. Lentinan exhibited significant antitumor activity against allogenic tumors, a variety of synergic and autochtonous tumors [82]. It has also been used for prolong life span of colorectal cancer patients [83-84].

Schizophyllan

This polysaccharide was derived from the mushroom *Schizophyllum commune* [85]. It shows antitumor activity against Sarcoma 180, Sarcoma 37, Yoshida Sarcoma and Erlich Sarcoma [37]. It is effective in the uterine cervix cancer therapy; also considerably efficient in median survival of gastric cancer patients in combination with conventional chemotherapy (tegafur or mitomycin C and 5-fluorouracil) and overall cure of stage II cervical cancer patients in combination with prolonged radiotherapy [86-88].

1.A.5.3. Mechanism of bioactivity of mushroom polysaccharides

Several β-D-Glucans [89] and α-D-Glucans [90] are world widely used as antitumor and immunomodulating agents. Polysaccharides do not directly attack cancer cell, but, by immunostimulation it can activate natural Killer cells (NK-cell), T-cell, B-cell in the host and exhibits carcinostatic activities. Therefore, these are regarded as biological response modifiers (BRM) [89]. β-Glucans can enhance both, innate and adaptive immune response [40]. The mechanisms of bioactivity of polysaccharides are

still not clear. A possible pathway of the biological action of β-D-Glucan has been represented by T. Mizuno, 2002 [91] (shown in Figure 1A.7).

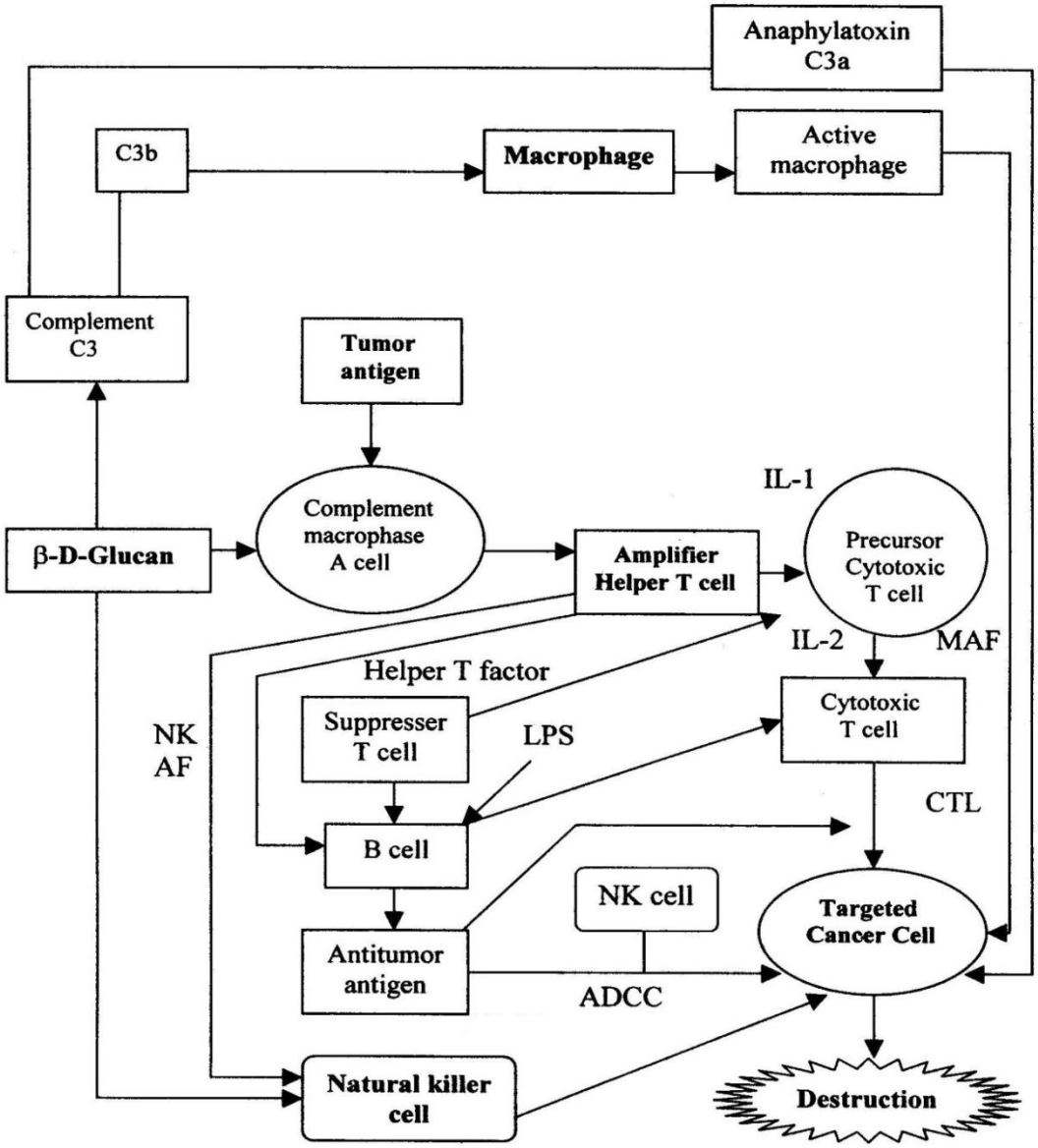

Figure 1A.8. Possible immune mechanism: β-D-glucan as BRM to target cancer cells [91].

CHAPTER 1: PART B

INTRODUCTION TO NANOPARTICLES AND POLYSACCHARIDE AS CAPPING AGENT

1B.1. Introduction to Nanoscience and nanotechnology

Nanotechnology is a multidisciplinary field of physics, chemistry, biology, materials science, health sciences, electronics and engineering. It involves the manufacture, processing and application of materials that are in size ranging from 0 to 100 nm in at least one dimension. At present it is the most rapid growing area of research throughout the entire world for versatile applications of nanoparticles (NPs) in numerous fields of science. Practically, all the revolution of nanoscience and technology was triggered after the Nobel lecture by Richard Feynman (Nobel Laureate in Physics, 1965) during a conference of American Physical Society,1959 entitled ***"There is a plenty of room at the bottom"***, in which he described the possibility of storing bits of information in atomic dimensions [92]. Nanomaterials often show abruptly different optical, mechanical, magnetic, electronic, electrical and chemical properties from their conventional bulk counterparts of identical substance due to their very smaller size and larger surface to volume ratio [93]. These materials show superior properties, e.g., enhanced catalytic, adsorption, enhanced optical properties, increased hardness, compressive strength, etc. [94].

Surface Plasmon Resonance

According to solid state physics, the plasmon is the collective oscillation of a free charge in a metal. In an applied external electric field, produced by light source influence the free electrons present at the surface to vibrate collectively and also create an electric field. When the generated electric field and the applied external electric field resonate the consequential phenomenon is called surface plasmon resonance (SPR) at the metal surface (**Figure 1B.1**). Metal nanoparticles shows surface plasmon resonance absorption in the UV–Vis. region.

The particle size, adsorbed species, chemical environment and dielectric constant have controlled over SPR band shift of nanoparticles [95-96]. Thus, design and synthesis of metal nanoparticles with potential applications can be achieved by controlling their size and shape.

1B.2. Synthesis of Nanoparticles

Several chemical and physical methods have been developed for the synthesis of nanoparticles using liquid, solid, or gas phase precursors [97-98]. The physical methods which are also known as "top down'' approaches (**Figure 1B.2**), involves broking down of bulk material into nano-sized particles. These approaches include vapour deposition, etching, grinding, ball milling, laser ablation, photo-lithography, and electron beam lithography etc. The "top down'' approach is not appropriate for nanoparticle synthesis, since, this method causes defected or damaged surface structure and contaminations, in addition internal stress to the nanomaterials which can affect the surface dependent properties. The chemical methods which are also known as "bottom up" approach involves organised association of atoms or small molecules (**Figure 1B.2**). The "bottom up" syntheses mostly include chemical reduction, photochemical reduction, co-precipitation, thermal decomposition, hydrolysis, *etc.* Studies have shown that the experimental conditions, nature of reducing agents and adsorption processes of stabilizing agent have direct influence on the size, morphology, stability and properties (chemical and physical) of the nanoparticles [99-100].

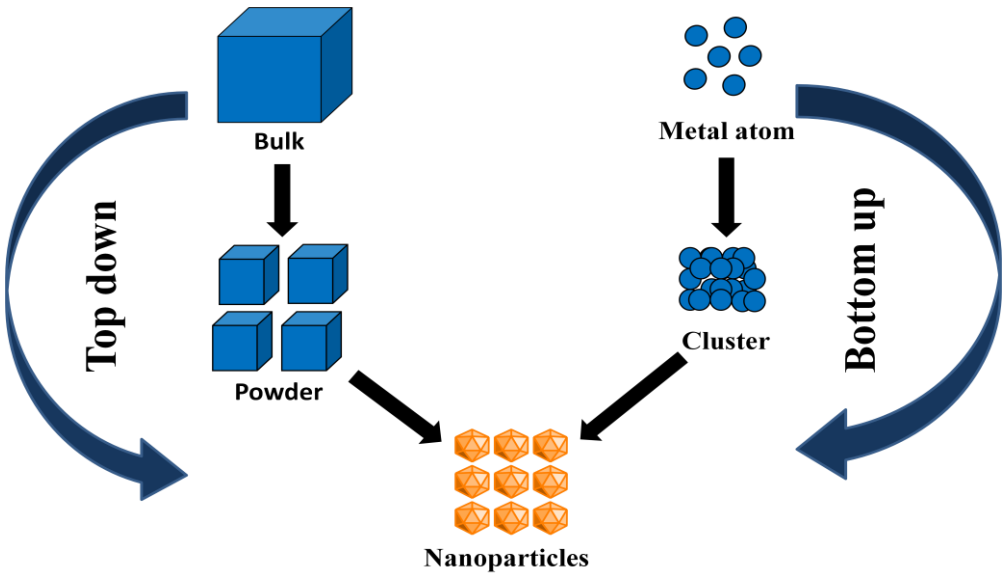

Figure 1B.2: Top down and bottom up approach for the synthesis of metal nanoparticles

1B.3. Green Synthesis of Nanoparticles

One of the simple approaches for bottom-up synthesis of metal nanoparticles invoves the use of chemical reducing agents like $NaBH_4$, hydrazine, N,N-dimethyl formamide, trisodium citrate, etc. [101-110]. All these reducing agents associated with potential environmental risk as they possess chemical toxicity and biological hazards. Over the past two decades, many attempts have been made to total elimination or minimization of waste and to reduce threats to health and environment through the adoption of 12 fundamental principles of green chemistry [111]. Uses of renewable materials, nontoxic chemicals, solvents which are environmentally benign, etc. are the few of the important implementation in a green synthetic approach [112]. Now a days, biomolecules and bioorganisms (**Figure 1B.3**) are the valuable alternative for large-scale green synthesis of nanoparticles because of their eco-friendliness, biocompatibility and very cost effectiveness [113-115].

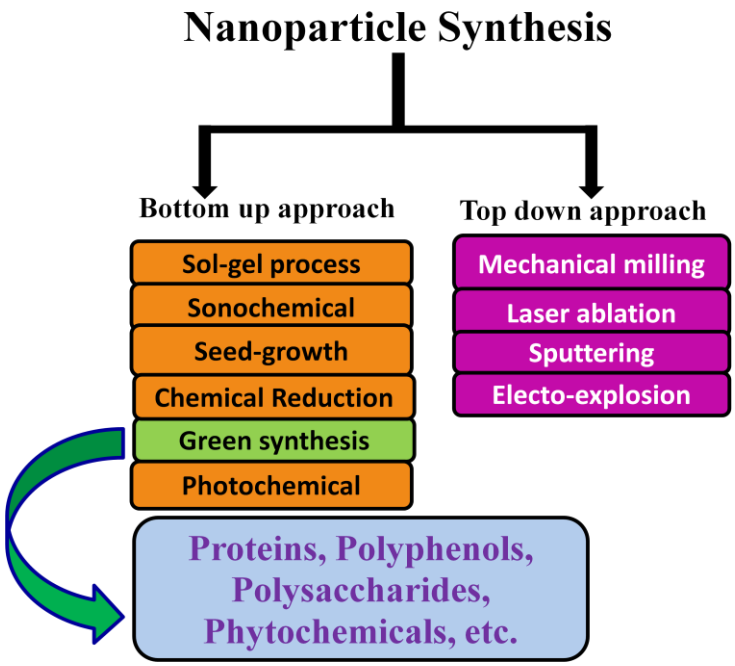

Figure 1B.3. Various approaches adopted in the synthesis of metal nanoparticles

1B.4. Polysaccharide capped nanoparticles

Plants synthesise carbohydrates by photosynthesis and major part of these carbohydrates are polysaccharides [116]. Polysaccharides represent an excellent scaffold for nanoparticles synthesis as they have hemiacetal end to reduce metal salt precursors and lot of hydroxyl group and other functionalities to stabilize the synthesized metal nanoparticles. The hydroxyl groups of polysaccharides also play an important function to reduce gold salts [117].

Polysaccharide with hydroxyl and amino groups helps to bind tightly to the metal nanoparticles surface giving a hydrophilic surface [118]. There are series of reports as shown below where heparin, hyaluronic acid (HA), alginic acid, chitosan as well as plant polysaccharides like cellulose, dextran and starch were employed for synthesis and stabilization of AgNPs.

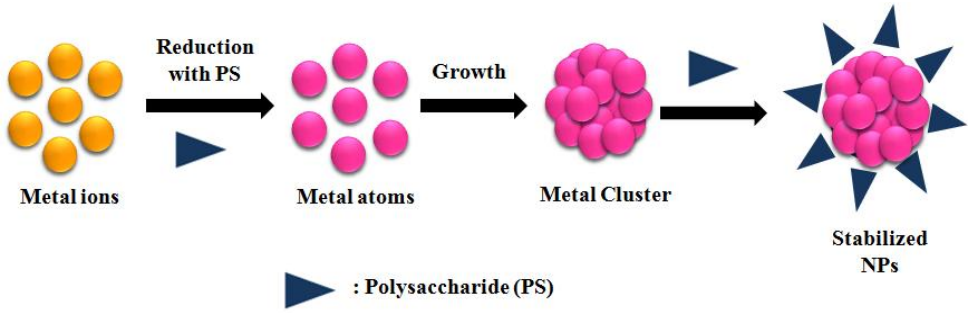

Figure 1B.4. A schematic representation of polysaccharides mediated synthesis and stabilization of metal nanoparticles.

Among several metal-NPs, AgNPs have recognized importance for their unique physical characteristics such as easy to synthesis and fabrication, biocidal activity, chemical stability, optical and electrical properties [119-121].

1B.4.1. Starch capped AgNPs

Highly stable AgNPs of an average size 5.3 nm were prepared from Ag salt by using mixture of starch and glucose where glucose reduce the Ag+ and starch serve as capping agent [122]. Another method showed that soluble starch has been employed for both purposes (reducing and stabilizing agent). Research revealed that the AgNPs entrapment inside the helical array of amylose chain attained stability [123].

1B.4.2. Cellulose capped AgNPs

Cellulose is the main component of plant cell walls consisting of a linear chain of β-(1,4)-linked D-glucose. Cai et al. [124] synthesize AgNPs from salt solutions of AgNO3 using nanoporous cellulose gels obtained from an aqueous alkali hydroxide-urea solution. These nanomaterials have effective applications in catalysis, electro-optical devices and as antibacterial agents.

1B.4.3. Dextran capped AgNPs

Aminodextran was employed both as reducing agent and surface stabilizer for the preparation of biocompatible AgNPs of controllable size to develop a biosensor to detect concanavaline-A [125]. Morrow et al. showed the morphology of AgNPs can be controlled by altering the pH of aminodextran solution [126]. In alkaline pH (pH~12) aminodextran solution yields spherical AgNPs of mean size of 20 nm while acidic pH favors the formation of large AgNPs of other shapes.

1B.4.4. Chitosan capped AgNPs

Chitosan-capped AuNPs was first reported by Huang and Yang in 2004 [127]. The primary amine group of chitosan effectively supports the immobilization of metallic nanoparticles. Sun et al. reported that during the synthesis of AuNPs, the intrinsic viscosity of chitosan decreased indicates the degradation of some chains [128]. Wei et al. reported the enhanced catalytic activity of Au and Ag NPs-chitosan conjugates for the reduction of 4-nitrophenol in combination with NaBH4 [129]. In an earlier report it was observed that the size, shape and crystalline structure of AgNPs-chitosan composites can be controlled by controlling the reaction temperature [130]. Dendritic Ag-chitosan film prepared by mixing the chitosan solution with Ag salts was also used for surface-enhanced Raman spectroscopy (SERS) [131-132]. Another report showed that Ag-chitosan film synthesized from chitosan contaiing acidic AgNO3 solution have exhibited antibacterial activity against E. coli and Bacillus [133]. Carboxymethyl chitosan (CMC) was employed as a matrix material for the preparation Ag nanoparticles [134]. These nanocomposites were successfully employed to detect trace of amino acids by SERS.

1B.4.5. Heparin capped AgNPs

Heparin capped AgNPs were prepared using heparin as reducing and surface stabilizing agent without any modification by thermal treatment of Ag-salt [127]. The morphology and size of the AgNPs was found to be dependent on concentration of heparin and the precursor salt. The red-shift of the SPR band of the AgNPs were observed with increase in heparin and the Ag+ ion concentration, indicates increased size. There is also report of preparation of AgNPs-heparin composites by the use of

2,6-diaminopyridinyl heparin (DAPHP) as both reducing and surface stabilizing agent [135]. Both the Au-DAPHP and Ag-DAPHP nanoparticles showed narrow size distribution. The smaller size distribution is due to stronger binding interaction between the diaminopyridine moieties of DAPHP to the nanoparticles. Both Au and AgNPs-DAPHP composites showed anticoagulant and anti-angiogenesis [128] activities. Local anti-inflammatory activities were also observed for these nanocomposites. Moreover, Ag-DAPHP nanoparticles showed effective antimicrobial against Staphylococcus aureus and E. coli [136].

1.B.4.6. Hyaluronic acid capped AgNPs

Hyaluronic acid (HA) is a linear, high molecular weight polysaccharide commonly found in soft tissues of animals. HA was used for the synthesis of AgNPs (size range 5 to 30 nm), where it played the role of both, the reducing and surface stabilizing agent [135]. Ag-HA nanoparticles are effective antimicrobial against S. aureus and moderate against E. coli [136].

1B.4.7. Alginic acid capped AgNPs

Alginic acid is an acidic polysaccharide often found in the cell walls of brown algae [137]. Alginic acid has many applications including in drug industry as drug delivery agent and in food industries as stabilizer and thickener [137-138]. Photochemical synthesis of AgNPs was achieved using calcium alginate both as reducing and surface stabilizing agent. The resulting AgNPs were spherical with a size of less than 10 nm. The effective catalytic action of this NPs were assessed on 4-nitrophenol to covert nitro grouo into amino group, this implies that it can be utilised as solid-phase heterogeneous catalyst for industrial applications [139].

1B.5.Dimensionality and Classification of Nanomaterials

Nanoparticles are classified on the basis of their dimensionality, morphology, composition, uniformity, and agglomeration. According to Richard W. Siegel [140], Nanostructure materials are divided mainly in three different classes with respect to dimension as: 1) Zero dimensional or 0D (quantum dots, core–shell quantum dots, hollow spheres, onions and nano-lenses spheres, cubes, and polyhedrons, etc.),

quantum dots have been widely employed in light emitting diodes (LEDs) [141], solar cells [142], single-electron transistors [143] and lasers [144].

2) One dimensional or 1D e.g. nanowires, nanorods, nanotubes, nanobelts, nanoribbons, nanostrands or fibres, etc. have profound application in nanoelectronics, nanodevices, nanocomposite materials and alternative energy resources etc. [145-152].

3) Two dimensional or 2D (eg. nanoprisms, nanoplates, nanosheets, nanowalls, thin films and nanodisks discs,etc. These have novel applications in developing sensors, photocatalysts, nanoreactors, etc. [153-158].

4) Three dimensional or 3D (eg. nanoballs, nanocoils, nanocones, and nanoflowers, etc.). They have broad spectrum of applications in the area of catalysis, sensing, drug delivery, cell imaging, and other biomedical applications [159-167].

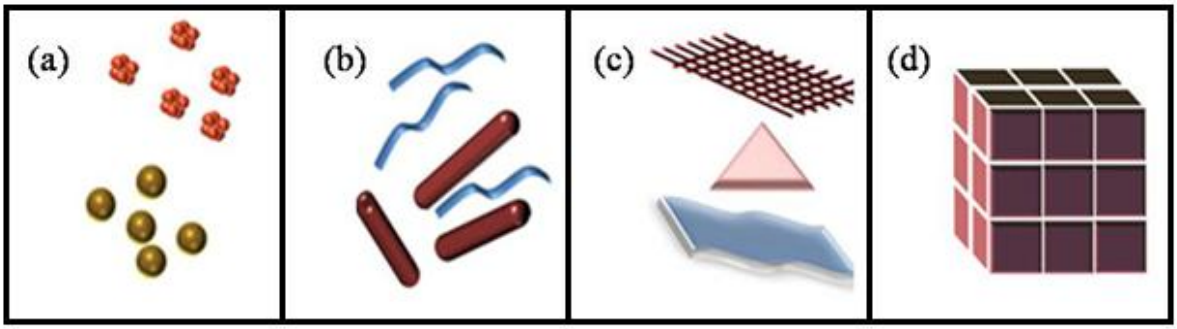

Figure 1B.5. Classification of Nanomaterials (a) 0D spheres and clusters, (b) 1D nanofibers, wires, and rods, (c) 2D films, plates, and networks, (d) 3D nanomaterials (Ref-140).

1B.6. Application of nanoparticles

The self-dependent superior characteristics are the driving force behind the explosion in research interest in nanoscale materials and endless applications. Some diverse areas are sensing, drug delivery, gene therapy, cancer cells imaging, agriculture, degradation of pesticides, antimicrobial agent, catalysis, computation, electronics, information technology, biotechnology and national security etc.

1B.6.1. In Drug delivery and Gene therapy utility

Nanotechnology in medicine is a very promising and rapidly-growing field. Novel nano-devices have enormous applications in various biological, biomedical and pharmaceutical fields [168-170]. The well defined surface structure of the nanoparticles provides an effective

drug delivery in the optimum dosage range, controlled release and specific cells targeting often resulting in increased therapeutic efficiency of the drugs, weakened side effects [171]. The nanoparticles having high magnetization value, small size (≤ 100 nm) and a narrow particle size distribution, these properties are implemented to several in vivo applications e.g., MRI contrast enhancement, cell separation, tissue repair, detoxification of bio-fluids, drugs delivery and immunoassay [172]. PEG coated Au-NPs loaded with TNF-α was reported to enhance tumor cell apoptosis rate. Au-NPs functionalized with oligonucleotides have the potential to activate immune system and unblock pathways in human blood cells [173]. Unique property AuNPs to increase the local temperature triggers the modulated release of encapsulated drug in AuNPs-thermosensitive synthetic polymer composites [174].

1B.6.2. Cell imaging

The nanoparticles based devices can overcome limits of usual organic dyes, such as poor hydrophilicity, low quantum yield and inadequate stability in biological systems [175]. Moreover, the high scattering power of NPs can improve the contrast of imaging. Taking advantage of this feature, Zhang et al. [176] detected single microRNA in lung cancer cells by using fluorescent metal nanoshells as molecular imaging system. Loo et al. established the NIR scattering of Au-nanoshells as a contrast enhancing agent in dark-field to target antihuman epidermal growth factor receptor 2 (HER2) [177]. SERS of Au or AgNPs unveiled cellular structural information in live cells [178-179]. Ray et. al. reported a and highly sensitive colorimetric analyse for selective and sensitive recognition of breast cancer (SK-BR-3 cell lines) using oval-shaped Au-NP based nanoconjugate [180].

1B.6.3. Biological sensing

Distinct properties of metal nanoparticles have made them precious scaffolds for the invention of novel sensors. In last twenty years several researches were performed utilizing metal nanoparticles, especially AgNPs and AuNPs in the idenfication of metal ions, organic molecules, nucleic acids, proteins and microorganisms. Hydrogel composites containing nanoparticles have been also reported for the manufacture of optical biosensors. For example, chitosan and alginate Au-NPs composites have shown outstanding results in glucose sensing [181-182] also in trace analysis of substrates using SERS [183-185].

1B.6.4. Antimicrobial activity

Both Au and AgNPs have been commonly found to have broad spectrum of antimicrobial activity against human and animal pathogens [186-188]. The use of metallic silver in \ antimicrobial products is well known. Studies have revealed that AgNPs are superior antibacterial than bulk silver and that even in nanomolar concentrations is more effective than micromolar concentrations of silver ions [189], which lead to an increased attention on materials comprising AgNPs. In this context, numbers of polysaccharide based AgNPs-composites have been reported [190-193] which exhibited antimicrobial activity in wound dressings and for water purification purposes. Moreover, NPs-PS composites may show a synergistic antibacterial activity and exhibit elevated antibacterial activity than their separated components, as recently observed for AgNPs composites [194-195].

1B.6.5. Catalytic activity

Metallic nanoparticles (1-100 nm) offer very high surface to volume ratio which make them efficient catalist. Recently, AuNPs are used for catalytic reduction of nitro group of 4- at room temperature instead of $NaBH_4$ [196]. Now, it is broadly used catalyst for various types of organic transformation reactions such as: a) selective hydrogenation reaction b) selective oxidation reaction of alkanes c) selective oxidation of amines d) nitro group hydrogenation e) epoxidation reaction f) oxidation of alcohols g) aromatic ring hydrogenation h) oxidation of polyols [196-202].