CHAPTER 1

introduction & review of literature
1.1 Introduction

Nanotechnology [1a] refers to the interactions of cellular and molecular components and engineered materials—typically clusters of atoms, molecules, and molecular fragments—at the most elemental level of biology. Such nanoscale objects—typically, though not exclusively, with dimensions smaller than 100 nanometers—can be useful by themselves or as part of larger devices containing multiple nanoscale objects.

From an evolutionary perspective, nature performed its task by creating simpler objects first and then complexity increased step by step from viruses and bacteria onwards to higher plants and mammals. In general, nature followed the rule the simpler the smaller. In contrast, man followed the concept of miniaturization i.e. the tendency to reduce size of product. Despite downsizing, the properties of products have been retained and even greatly improved in most cases. The fundamental contrast to Nanotechnology is that in this relatively new discipline the downsizing process has broken through certain barriers; beyond it, the old laws no longer necessarily apply. Any material reduced to size of nanoparticles can suddenly behave much differently than it did before. Electrically insulating materials, for example, become conductive and insoluble substances soluble. At the nanoscale, the physical, chemical, and biological properties of materials differ fundamentally and often unexpectedly from those of the corresponding bulk material because the quantum mechanical properties of atomic interactions are influenced by material variations on the nanometer scale. In fact, by creating nanometer scale structures, it is possible to control fundamental characteristics of a material, including its melting point, magnetic properties and even color, without changing the material’s chemical composition.

The nanoimpact was first foreseen by great visionary and Nobel laureate Richard Feynman as is evident by his perceptive address to the American Physical Society in 1959, “at the atomic level, there exist new kinds of forces and new kinds of possibilities, new kinds of effects. The problems of manufacture and reproduction of materials will be quite different [1b]” Since then, several significant achievements have been made towards the process of miniaturization, even though control at complexity levels manifested by biological systems is still a dream. Figure 1.1 shows
the examples of man made and natural fabrication of materials at small scale routinely. Two different approaches have been undertaken to achieve this goal, which are “top-down” [2] and “bottom up” [3].

Figure 1.1 Picture showing relative sizes of naturally occurring bioassemblies and man made materials [Courtesy: Josh Wolfe’s report on Nanotechnology: www.forbeswolfe.com]

The top-down approach thrives on the principle that large sized objects can be chiseled to obtain smaller objects. Humans have been following this approach since the beginning of civilization and with time, this art has been mastered to achieve size limits of submicron levels. However, physical constraints limit the application of this approach to achieve nano-domain precision. Thus, the bottom-up approach has taken over where small scale objects can be assembled to build up larger sized materials for various applications. This includes the synthesis of nanostructures of desired characteristics, their self-assembly and eventual formation of larger sized particles.

In nature, the bottom-up approach of creation is prevalent. The whole individual develops from single zygotic cell. The zygote multiplies and turns into multicellular embryo wherein the different organ systems develop and the single zygotic cell ultimately turns into fully developed individual.
1.2 Historical Perspective

Although the birth of Nanotechnology as a discipline is new the exposure of nanoparticles to mankind dates back to ancient times. According to Mahdihassan (1985) [4] the Chinese were the first to prepare and use red colloidal gold as the alchemic drug of longevity. According to Weigleb’s History of Alchemy (1777), they were using colloidal gold since 2500 BC. The world alchemy derives from the two Chinese words: Kim (gold) and Yeh (juice). Kimyeh (gold juice) entered the Arabic language as kimiya, and with the definite article, al, the Arabic word for red, colloidal gold was called alkimiya, which in western world gave the word alchemy. The medieval alchemist, like their Eastern counter parts, probably drew on the traditions of eastern alchemy and sought a form of metals that could be internally consumed: aurum potable (drinking gold) and luna potable (drinking silver) which they used as elixirs of life [5–7].

The ingestion of gold is mentioned in the bible (Exodus 32:20). According to verse 20, “And he took the (golden) calf which they had made, and burnt it in the fire, and ground it to powder, and strewed it upon the water, and made the children of Israel drink of it.” The mention above indicates that the potential of noble metal colloidal suspension in therapeutics. This mentioned procedure is very much similar to the traditional Indian Ayurvedic preparation which uses the serial processes of ‘Shodhan’ (extraction), ‘Jaran’ (heating) and ‘Maran’ (grinding) to produce red colloidal gold under the name of Swarna Bhasma (red gold). The Swarna Bhasma is prescribed by Ayurvedic physicians for rejuvenation and revitalization in old age.

History claims that Moses, the great physician, gave the first recipe for colloidal gold [8]. In 1818, Jeremias Benjamin Richters gave an explanation for the different colors seen in drinkable gold indicating that the pink or purple color was due to finest degree of subdivision while yellow color arises due to the aggregation of very fine particles [9]. Since earlier days, colloidal gold and silver has been used as a colorant [10]. The colorant in glasses, “Purple of Cassius”, is a colloid with eterocoagulation of gold nanoparticles and tin oxide [8]. Similarly, the Lycurgus cup of fourth century AD, which looks green in reflected and red in transmitted light, has been reported to contain colloidal gold and silver [10]. In 1857, Faraday reported the preparation of deep red colored solution of gold nanoparticles by the reduction of
aqueous chloroaurate ions using phosphorus in CS$_2$ [11]. This probably was the first rationalized report on the purposeful synthesis of colloidal gold nanoparticles. Soon thereafter, the term colloid was coined by Thomas Graham (1861) for suspended particles in liquid medium [12] and was categorized to be in the size range 1 nm to few micrometers. However, Norio Taniguchi gave the term ‘nanotechnology’ [13] for the colloidal particles, which have at least one dimension of the length scale of 1-100 nm. Ever since the work of Faraday, several different approaches have been developed towards the synthesis of colloidal noble metal nanoparticles by physical, chemical and biological routes.

1.3 Synthesis of Nanoparticles

The synthesis of inorganic nanomaterials has been demonstrated by several methods including physical, chemical and biological [Figure 1.2]. Some of the physical routes leading to successful synthesis of nanophase materials, especially the noble metal nanoparticles include vapor deposition [14], thermal decomposition [15], spray pyrolysis [16], photoirradiation [17], laser ablation [18], ultrasonication [19], radiolysis [20] and solvated metal atom dispersion [21]. However, chemical methods for synthesis of metal nanoparticles have been more popular and have gained wide acceptance. Some of the common chemical routes include sol-gel method [22], solvothermal synthesis [23], micelles based synthesis [24] and galvanic replacement reaction [25, 70]. Chemical reduction has been the most popular route towards synthesis of metal nanostructures due to easy protocols and the fine shape and size control provided by this method.

The control over size, shape, stability and the assembly of nanoparticles is achieved by incorporating different capping agents, solvents and templates. Capping agents that have been used, range from simple ions to polymeric molecules and even biomolecules [26–31]. Ever since the first report by Brust et al. [32] for the synthesis of monolayer-protected clusters (MPCs), several advances have been made in the field and a variety of capping agents have been used to prepare MPCs [33, 35] soluble in non-polar organic as well as polar solvents. As a solvent, though water is largely used, use of organic solvents [34-35], ionic liquids [36] and supercritical fluids [37-38] has also been demonstrated. Similarly, many soft and rigid templates such as micelles [39-41], polymeric molecules [42-43], DNA [44-45], Tobacco Mosaic
Virus [46-49], mesoporous materials and many more including preformed nanoparticles [50] have been employed in order to gain control over the formation and assembly of nanoparticles.

Figure 1.2 Figure summarizing different synthesis routes for nanoparticles

Although, over the past several decades, physical and chemical methods have dominated the synthesis of nanostructures, recently considerable attention has been paid towards the use of biological systems. Biological systems have been known to fabricate intricate structures at the micro and nano scales with precise control in normal environmental conditions. The exquisite siliceous exoskeletons of the diatoms and radialarians [51] and calcareous structures synthesized by the coccoliths [52] are micro scale materials, which have attracted tremendous interest. Besides, magnetite particles found in the magnetosomes [53] of the magnetic bacteria [54] are a wonderful example of functional nanomaterials that helps the microorganism to navigate in the earth’s geomagnetic field. This fact has lured scientists to understand the underlying mechanisms used by the biological systems and thus, explore the biomimetic approach towards synthesis of nanomaterials. One of the reports that opened up the gates for the use of biological systems for the synthesis of nanomaterials was a report by Klaus et al. [55] on the synthesis of silver nanoparticles in the periplasmic space of the bacteria Pseudomonas stutzeri AG259. Till date, several bacteria [56], S-layer bacteria [57], fungi [58], algae [59] and plant
systems [60] have been used for the synthesis of nanomaterials of different shapes, sizes and compositions.

1.4 Properties of Metal Nanoparticles

As the size of the metal approaches nanometer scale, the fundamental properties of matter change. The properties of atoms and molecules are not governed by the same physical laws as larger objects or even larger particles. The physical and chemical properties of nanoparticles can therefore be quite different from those of larger particles of the same substance. The following section briefly discusses the changes in the properties of the metals of nanometer size.

1.4.1 Physical Properties

It is well known that with decrease in the size of particles for a given volume of material, the number of atoms at the surface (surface area) increases tremendously, for example a 3 nm particle would have 45% of its atoms on the surface and a 1 nm particle would have 76% of the atoms on its surface. So, the surface area is greatly increased as the result of reduction in the size of material in nanometer range due to which nanoparticles become greatly advantageous for promoting the rates of chemical reactions. Thus, the reduction in the size of particles renders them excellent catalysts [61]. For example, gold is considered to be a noble metal in bulk state, but the nanoparticles of gold dispersed in alumina or iron oxide was found to be excellent catalysts for carbon monoxide oxidation [62].

Mechanical properties of a material depend strongly on the density of dislocations, grain size and the surface/interface-to-volume ratio. The strength and hardness of the material could be severely affected by any decrease in grain size. As compared to the bulk, a nanoparticle has more defects due to the high surface to volume ratio hence altered mechanical properties. Sometimes the alteration in these properties leads to generation of superplastic nanomaterials [63].

The magnetic properties of nanoparticles differ from those of bulk in two ways. The large surface to volume ratio results in a different local environment for the surface atoms in their magnetic coupling/interaction with neighboring atoms, leading to the mixed volume and surface magnetic characteristics. Unlike bulk ferromagnetic materials, which usually form multiple magnetic domains, several small ferromagnetic particles could consist of only a single magnetic domain.
In the case of a single particle being a single domain, superparamagnetism occurs, in which the magnetizations of the particles are randomly distributed, aligning only under an applied magnetic field. The alignment disappears once the external field is withdrawn. These could have important implications, for example, in ultra-compact information storage where the size of the domain determines the limit of storage density [64].

Metal nanoparticles when embedded between metal – insulator – metal junctions, or between the tip of STM and an electrode, show a differential capacitance or charging at low temperatures even at zero bias [65]. It was realized that this behavior is caused due to extremely small capacitance of the metal nanoparticles. These particles can store charge by addition or removal of electrons. Due to its low capacitance, nanometer sized metallic particles are extremely sensitive to neighboring charges [66] and therefore, could be useful as sensor materials including vapor sensors [67].

The optical properties of these nanoparticles are spectacular and, therefore, have stimulated a great deal of excitement during the last few decades. The color variations arising from changes in the composition, size, and shape of nanoparticles, surrounding medium and very high absorption cross-section promoted these materials as inorganic chromophores from visible to near infrared region [68]. Due to this reason they find applications as optical sensors and imaging agents [69-71].

These optical effects exhibited by metal nanoparticles are due to the phenomena called surface plasmon resonance, the frequency at which conduction electrons oscillate in response to the alternating electric field of incident electromagnetic radiation. This phenomenon was explained by Mie [72] and is based on the Maxwell equations on scattering. However, only gold, silver and copper nanoparticles possess plasmon resonances in the visible spectrum, which give rise to such intense colors. Nanoparticle is a complicated multi electron system, where the confinement of electronic motion due to the reduction in size leads to fascinating new effects, potentially tunable with particle size and shape.

1.4.2 Surface Plasmon Resonance

Free electrons and the cationic cores in a bulk metal constitute a plasma state. These free electrons can set into oscillations relative to the cationic lattice
when it interacts with electromagnetic radiation. Since the order of penetration depth of electromagnetic waves in metals falls in the nanometer range, it polarizes or displaces the surface electrons from its equilibrium position. Then the coulombic attraction between the cationic lattice and electrons act as restoring force to bring back the electron cloud to the equilibrium position. In this manner a dipolar oscillation of electrons is created (called plasma oscillation) with a certain frequency called \textbf{plasmon frequency}.

In a bulk metal, the electrons are free and unbound and therefore can absorb any amount of energy. When the size of the particle is decreased below the mean free path of the electron, it generates surface plasmon resonance. The surface plasmon resonance can be thought of as coherent motion of the conduction-band electrons caused by the interaction with an electromagnetic field [73-76]. In a classical description, the electric field of an incoming light wave induces polarization of the electrons with respect to the much heavier ionic core of a spherical nanoparticle. A net charge difference is only felt at the nanoparticle surface, which in turn acts as a restoring force. This creates a dipolar oscillation of all the electrons with the same phase. When the frequency of the electromagnetic field becomes resonant with the coherent electron motion, a strong absorption in the spectrum is seen, which is the origin of the observed color. The frequency and width of the surface plasmon absorption depend on the size and shape of the metal nanoparticle as well as on the dielectric constant of the metal itself and of the medium surrounding it [73-76]. For noble metals (gold, silver and copper) the plasmon resonance is strongest and shifts into the visible of the electromagnetic spectrum [20, 77].

Mie’s theory was developed for particles of spherical shape only. For cylindrical or oblate nanoparticles, Gans [78] extended Mie’s theory within the dipole approximation. The particles are usually characterized by their aspect ratio, which is defined by the ratio between the length and the width of the particle. The plasmon resonance for nanorods splits into two bands. As the aspect ratio increases, the energy separation between the resonance frequencies of the two plasmon bands increases [79-81]. The high-energy band corresponds to the oscillation of the electrons perpendicular to the major rod axis and is referred to as the transverse plasmon absorption. The other absorption band, which is red shifted to lower
energies, is caused by the oscillation of the electrons along the major rod axis and is known as the longitudinal surface plasmon absorption. Similarly, triangular nanoparticles also show two absorption bands corresponding to the transverse and longitudinal plasmon resonance. In certain cases, another peak in between the two plasmon peaks has also been reported which has been attributed to the in-plane quadrupole mode of plasmon resonance [82]. Several attempts have been made towards successful synthesis of anisotropic metal nanostructures such as rods [83], disks [84], triangular prisms [17f, 85, 188], multipods [86], cubes [87] and nanoshells [25d, 88] and their optical properties have been studied.

1.4.3 Biocompatibility

A primary interest in the idea of Nanoscience comes from its associations with biology. An ideal nanomaterial for biological purposes should be biocompatible. Nanoparticles have been used for various biological applications and otherwise. Thus, it becomes an important issue to study the short and long term effect of size, shape, and surface functional groups on the bioavailability, uptake, subcellular distribution, metabolism, and degradation of these different nanostructures. Reports on the surface properties of nanoparticles, both physical and chemical, stress that nanoparticles differ from bulk materials. Their properties depend heavily on the particle size. Therefore, nanoparticles are not merely small crystals but an intermediate state of matter placed between bulk and molecular material. Hence, the biocompatibility of nanomaterials can not be compared with the same material in bulk. Since there are numerous recipes for synthesis of nanoparticles and above that the synthesized nanoparticles are surface functionalized by various different ways each type of nanoparticle must be considered unique and tested for its biocompatibility. Attempts have been made towards this aim and studies have been undertaken to address these issues with carbon nanostructures [89], CdSe quantum dots [90] and metal nanoparticles [91, 98] in vivo and in vitro. The toxicity of gold and silver nanoparticles inside the biological system has always been an issue of concern. Despite the scientific literature available on cytotoxicity and immunotoxicology of gold (I) [92-94] and gold (III) complexes [93-97] and recent reports on cytotoxicity of various gold nanoparticles [98], little attention has been focused on the immunological response of cells to gold nanoparticles.
Size and surface area of nanoparticles

Particle size and surface area are important material characteristics from a toxicological perspective. As the size of a particle decreases, its surface area increases and allows a greater proportion of its atoms or molecules to be displayed on the surface rather than the interior of the material. This can be explained by the example given in Table 1.1 where it is shown that as particle size for a group of airborne particles with fixed mass (10 μg/m³) and unit density (1 g/cm³) decreases, their number increases exponentially along with the surface area the increase in the surface area in turn determines the potential number of reactive groups on the particles surface.

<table>
<thead>
<tr>
<th>Particle diameter (μm)</th>
<th>Particles /ml of air</th>
<th>Particle surface area (μm²/ml of air)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>0.5</td>
<td>153</td>
<td>120</td>
</tr>
<tr>
<td>0.02</td>
<td>2,390,000</td>
<td>3000</td>
</tr>
</tbody>
</table>

Much of the current knowledge of size dependent toxicity of nanoparticles comes from the studies on effect of air pollutant ultrafine particles (UFPs) on pulmonary toxicity [100]. The body displays a type of defense reaction in response to ultrafine nanoparticles that is called inflammation and is characterized by tissue injury, infection or irritation which in turn leads to swelling, redness, heat and pain at the affected area. An immune response is then normally stimulated where usually a healing process is gradually followed. However, a persistent or high inflammatory response may damage the cell layer at the surface of the tissue and other cells (such as macrophages used for particle clearance of the lungs), which can result in tissue damage and loss of function [101]. There is a correlation between a decrease in particle size and an increase in toxicity. The studies on rodents demonstrate that ultrafine particles administered to the lung cause a greater inflammatory response than do larger particles, per given mass i.e. the ultrafine particles made of low-solubility, low-toxicity materials are more inflammatory in the rat lung than fine respirable particles made from the same material [100]. For example in case of nickel, it has been shown that extent of lung injury were greater with ultrafine
nickel (20 nm) than standard nickel (5 µm) [100c] suggesting that ultrafine particles have a much more toxic effect than fine particles of the same material. Although, this is a well established correlation, the mechanisms behind are poorly understood. The theories that try to explain the mechanisms behind the increased toxicity of ultra fine particles are many. The most well established theory is that it has to do with the increased surface area and/or combination with the increasing number of particles [89a]. The increase in particle surface area is believed to be linked to lung cancer, lung fibrosis and inflammation in the lung. There is a considerable body of epidemiological studies suggesting that an increase in ambient particle concentration is related to increase in mortality and diseases in the exposed population. The strongest associations are seen for respiratory and cardiac deaths, particularly among the elderly and particulate air pollution is also associated with asthma exacerbations, increased respiratory symptoms, decreased lung function and increased medication use [102]. Table 1.2 summarizes the possible adverse effects of nanomaterials on human health at cellular level and their possible pathophysiological outcomes. It is not established what causes this yet, but a theory is that the increase in particle concentration in the air may overload the lungs and the phagocytes that are responsible for eliminating those particles. However, so far no direct relationship between manufactured nanomaterials and disease has been established due to scarcity of data. Tissue and cell culture analysis point out the role of oxidative stress in the production of inflammatory cytokines and cytotoxicity in response to nanoparticle exposure [99].

Recently, Nel and co-workers [103] compared the effects of ambient ultrafine particles with manufactured titanium dioxide (TiO₂), carbon black, fullerol, and polystyrene (PS) nanoparticles (NPs) in a cell culture system. The study was conducted in a phagocytic cell line (RAW 264.7) that represents a lung target for NPs. Physicochemical characterization of the NPs showed a dramatic change in their state of aggregation, dispersibility, and charge during transfer from a buffered aqueous solution to cell culture medium. Particles differed with respect to cellular uptake, subcellular localization, and ability to catalyze the production of reactive oxygen species (ROS) under cellular and cell free conditions. Spontaneous ROS production was compared by using an ROS quencher (furfuryl alcohol) as well as an NADPH peroxidase bio-electrode platform. Among the particles tested, ambient
ultrafine particles (UFPs) and cationic PS nanospheres were capable of inducing cellular ROS production, glutathione (GSH) depletion, and toxic oxidative stress.

**Table 1.2.** NM effects as the basis for pathophysiology and toxicity. Effects supported by limited experimental evidence are marked with asterisks; effects supported by limited clinical evidence are marked with daggers [99].

<table>
<thead>
<tr>
<th>Experimental NM effects</th>
<th>Possible pathophysiological outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROS generation*</td>
<td>Protein, DNA and membrane injury,* oxidative stress, mitochondrial perturbation*</td>
</tr>
<tr>
<td>Oxidative stress*</td>
<td>Phase II enzyme induction, inflammation, mitochondrial perturbation*</td>
</tr>
<tr>
<td>Mitochondrial perturbation*</td>
<td>Inner membrane damage,* permeability transition (PT) pore opening,* energy failure,* apoptosis,* apoptosis, cytotoxicity</td>
</tr>
<tr>
<td>Inflammation*</td>
<td>Tissue infiltration with inflammatory cells, fibrosis, granulomas, atherogenesis, acute phase protein expression (e.g., C-reactive protein)</td>
</tr>
<tr>
<td>Uptake by reticulo-endothelial system*</td>
<td>Asymptomatic sequestration and storage in liver,* spleen, lymph nodes, possible organ enlargement and dysfunction</td>
</tr>
<tr>
<td>Protein denaturation, degradation*</td>
<td>Loss of enzyme activity,* auto-antigenicity</td>
</tr>
<tr>
<td>Nuclear uptake*</td>
<td>DNA damage, nucleoprotein clumping,* autoantigens</td>
</tr>
<tr>
<td>Uptake in neuronal tissue*</td>
<td>Brain and peripheral nervous system injury</td>
</tr>
<tr>
<td>Perturbation of phagocytic function,* &quot;particle overload,&quot; mediator release*</td>
<td>Chronic inflammation, fibrosis, granulomas, interference in clearance of infectious agents</td>
</tr>
<tr>
<td>Endothelial dysfunction, effects on blood clotting*</td>
<td>Endothelial dysfunction, effects on blood clotting*</td>
</tr>
<tr>
<td>Generation of neoantigens, breakdown in immune tolerance</td>
<td>Autoimmunity, adjuvant effects</td>
</tr>
<tr>
<td>DNA damage</td>
<td>Mutagenesis, metaplasia, carcinogenesis</td>
</tr>
</tbody>
</table>

This toxicity involved mitochondrial injury through increased calcium uptake and structural organellar damage. Although active under abiotic conditions, TiO2 and fullerol did not induce toxic oxidative stress. While increased TNF-alpha production could be seen to accompany UFP-induced oxidant injury, cationic PS nanospheres induced mitochondrial damage and cell death without inflammation. They concluded that ROS generation and oxidative stress are a valid test paradigm to compare NP toxicity. Although not all materials have electronic configurations or surface properties to allow spontaneous ROS generation, particle interactions with cellular components are capable of generating oxidative stress.
Chapter 1

**Composition**

Nanoparticles may overcome solubility and stability issues for the drug and minimize drug induced side effects. But there could be significant toxicity issues associated with the composition of nano-carrier themselves. Although, there are evidences that small size and high surface area of nanoparticles are the major determinants of toxicity, there are very few reports on toxicity of nanomaterials due to their composition. Recently Warheit’s group [104] has shown toxicity of different types of titania particles was not dependent on particle size and surface area. Smaller nanoparticulate materials had effects comparable to larger nanoparticle materials. What did correlate strongly to cytotoxicity, was the phase composition of the nanoscale titania. Anatase TiO$_2$ was 100 times more toxic than an equivalent sample of rutile TiO$_2$.

**Surface chemistry**

Polycationic macromolecules show a strong interaction with cell membranes *in vitro*. Biocompatibility studies [105] revealed that the cytotoxicity of polycationic materials such as DEAE-dextran and poly-L-lysine (PLL) [106-107], dendrimers [108] and polyethylenimine (PEI) [109] increases with the increase in their molecular weight. Rotello and co-workers have also demonstrated that the cationic gold nanoparticles are cytotoxic to the mammalian and prokaryotic cells while anionic nanoparticles are inert [110]. Furthermore, the arrangement of cationic charges over nanoparticles depends on the three-dimensional structure and flexibility of the surface macromolecules and determines the accessibility of their charges to the cell surface. For example, branched molecules were found to be more efficient in neutralizing the cell surface charge than polymers with linear or globular structure, as rigid molecules have more difficulties in attaching to the membranes than flexible molecules [111]. Therefore, high cationic charge densities and highly flexible polymers should cause higher cytotoxic effects than those with low cationic charge densities. Globular polycationic macromolecules (cationized Human Serum Albumin (cHSA), ethylenediamine-core poly (amidoamine) dendrimers (PAMAM) were found to be polymers with a good biocompatibility (low cytotoxicity), whereas polymers with a more linear or branched and flexible structure (e.g. poly-diallyl-
dimethyl-ammonium chloride (DADMAC), PLL, PEI) showed higher cell damaging effects.

Geiser et al. [112] studied the influence of the particle surface chemistry on its interaction with the lung surface lining layer. They found that, regardless of the nature of their surfaces, particles submersed into the lining layer after their deposition in small airways and alveoli. This displacement was promoted by the surfactant film itself, whose surface tension falls temporarily to relatively low values [112-113]. On the other hand, reactive groups on a particle surface certainly modify the biological effects. For silica, it has been shown that surface modification of quartz affects its cytotoxicity, inflammogenicity and fibrogenicity. These differences are mainly due to particle surface characteristics [114]. Specific cytotoxicity of silica is strongly correlated to the appearance of surface radicals and reactive oxygen species (ROS), which is considered to be the key event in the development of fibrosis and lung cancer by this compound [115]. In case of silica [115] it is demonstrated that the reactive groups on nanoparticles influence their interaction with the cells.

1.5 Nanoparticles in Biology and Medicine

Nanotechnology offers unique approaches to probe and control a variety of biological and medical processes that occur at nanometer length scales, and is expected to have a revolutionary impact on biology [116] and medicine [117]. Among the approaches for exploiting nanotechnology in medicine, nanoparticles offer some unique advantages as sensing, image enhancement, and delivery agents [118-119]. In the past few years nanoparticles have attracted significant research and practical attention. They are versatile agents with a variety of biomedical applications including contrast agents for highly sensitive disease diagnostics & therapeutics [120-124], thermal ablation & radiotherapy enhancement [125, 132], as well as cancer diagnostics [69, 126-131] & therapeutics [132-134], and drug & gene delivery [135-138]. To further the application of nanoparticles in disease diagnosis and therapy, it is important that the systems be biocompatible and capable of being functionalized for recognition of specific target sites in the body after systemic administration. For biomedical applications, surface functionalization of metal nanoparticles is essential in order to target them to specific disease areas and allow them to selectively interact with cells or biomolecules. Surface conjugation of
antibodies, peptides and other targeting moieties is usually achieved by adsorption of the ligand to the gold/polymer surface. Additionally, for systemic applications, long-circulating nanoparticles are desired for passive targeting to tumors and inflammatory sites. Figure 1.3 represents a simple schematic design of multifunctional nanoparticle for drug delivery and imaging applications.

![Figure 1.3. Schematic diagram of a multifunctional nano-assembly for drug delivery, disease diagnosis and therapy.](image)

With the advancement of nanotechnology, a variety of nanostructures with different shapes and structures of different composites [139-142] have been developed including polymeric nanoparticles, metal nanoparticles, liposomes, micelles, quantum dots, dendrimers, and nano-assemblies. Presented below are brief descriptions of the nanoparticles used for biological and medical applications:

### 1.5.1 Quantum Dots

Nanoparticles of semiconductors (quantum dots) were theorized in the 1970s and were initially created in the early 1980s. If semiconductor particles are made small enough, quantum effects come into play, which limit the energies at which electrons and holes (the absence of an electron) can exist in the particles [143]. QDs typically contain a CdSe or CdTe core and ZnS shell. Quantum dots absorb white light and then re-emit it a couple of nanosecond later at a specific wavelength. By varying the size and composition of quantum dots, the emission wavelength can be tuned from blue to near infrared. For example, 2 nm quantum dots illuminate bright green, while 5 nm quantum dots luminesce red. Quantum dots have greater flexibility; when compared to other organic fluorophores quantum dots have bright...
fluorescence, narrow emission range, broad UV excitation, large molar extinction coefficients and high photo stability [122-123, 128]. Recent advances in chemistry have resulted in the preparation of monolayer-protected, high-quality, monodispersed, crystalline quantum dots as small as 2 nm in diameter, which can be conveniently treated and processed as a typical chemical reagent. The above mentioned qualities make them ideal for use in building nanoscale computing applications where light is used to process information. These structures offer new capabilities for multicolor optical coding in gene expression studies and high throughput screening. Quantum dots have been covalently linked to various biomolecules such as antibodies, peptides, nucleic acids and other ligands for fluorescence probing applications [145]. Some of the applications of QDs in biology along with their tremendous potential for imaging have already been explored in vitro [122-123, 128, 144] and in vivo [146]. However, the biocompatibility of quantum dots for in vivo applications is still a subject of debate [147].

1.5.2 Superparamagnetic Iron oxide Nanoparticles

These entities are usually prepared by the alkaline co-precipitation of appropriate ratios of $\text{Fe}^{2+}$ and $\text{Fe}^{3+}$ salts in water in the presence of suitable hydrophilic polymer such as dextran [148], poly (ethylene glycol)[149], Poly (vinyl alcohol)[148c, 150] etc. This yields an iron core which is hexagonally shaped and surrounded by polymeric molecules. These particles are called "superparamagnetic", indicating that they are attracted to a magnetic field but retain no residual magnetism after the field is removed. Therefore, suspended superparamagnetic particles tagged to the biomaterial of interest can be removed from a matrix using a magnetic field, but they do not agglomerate (i.e., they stay suspended) after removal of the field. Iron oxide nanoparticles are also amenable to surface functionalization with small surface functional groups or multivalent small molecules [148a, 149b, 151] as well as by conjugating proteins [149c-d, 152], antibodies [153] and oligonucleotides [154] for active targeting in vivo or for in vitro diagnostic procedures. A common use of superparamagnetic nanoparticles is for immuno-specific cell separations. The process is known as magnetic activated cell sorting (MACS). Typically, the nanoparticles are dispersed within the pores of larger microparticles. In the simplest (direct) method, the microparticles are coated with a
monoclonal antibody for a cell-surface antigen. The antibody-tagged, superparamagnetic microparticles are then incubated with a solution containing the cells of interest. The microparticles bind to the surfaces of the desired cells, and these cells can then be collected in a magnetic field. Methods of this type have been used to isolate or remove numerous cell types, including lymphocytes [155], stem cells [156] and tumor cells [157]. In addition to loading microparticles with superparamagnetic nanoparticles other examples of tagging the desired biomaterial with individual nanoparticles have been reported. This approach has numerous advantages. Whereas bound microparticles can affect the viability of the selected cells, nanoparticles supposedly do not affect cell viability. In addition, nanoparticles do not affect the extent of light scattering by the cell solution. The disadvantage of this approach is small magnetic moments of individual nanoparticles; however, this problem can be overcome by using a high-gradient magnetic field.

Recently, a number of small libraries of surface functionalized iron oxide nanoparticles were synthesized from parent aminated dextran caged iron oxide nanoparticles. These parent particles were first labeled with fluoresceins, thus generating particles fluorescent magnetic nanoparticles, then activated with N-succinimidyl3-(2-pyridyldithio)propionate, and re-acylated with thiol containing surface modifiers [158]. Fluorochrome attachment [148a, 154e, 159] allows the screening by wide range of high throughput fluorescence based screening methods as well as FACS.

Superparamagnetic Fe$_3$O$_4$ nanoparticles are also useful as magnetic resonance imaging (MRI) contrast agents [148a, 149b, 153f, 160]. MRI is essentially proton NMR done on tissues. Protons are excited with short pulses of radio frequency radiation; the free induction decay as they relax is measured and deconvoluted by means of a Fourier transform, which provides an image of the tissue that corresponds to proton density. Areas of high proton density, usually in the form of water or lipid molecules, have a strong signal and appear bright. Areas of bone or tendon, which have a low proton density because of the lack of water and lipids, have a weak signal and appear dark. Traditionally, a major limitation of MRI has been its inability to distinguish anomalies in soft tissue types (e.g., healthy parts of the liver from diseased lesions), as the relative proton densities can be very similar. Other regions, such as the bowel, are hard to image because air pockets and fecal
matter make the proton density inconsistent. Various contrast agents have been developed to circumvent these imaging problems. Contrast agents work by changing the strength of the MRI signal at a desired location. For example, superparamagnetic contrast agents change the rate at which protons decay from their excited state to the ground state, allowing more effective decay through energy transfer to a neighboring nucleus. As a result, regions containing the superparamagnetic contrast agent appear darker in an MRI than regions without the agent. For instance, when superparamagnetic nanoparticles are delivered to the liver, healthy liver cells can uptake the particles; diseased cells cannot. Consequently, the healthy regions are darkened, although the diseased regions remain bright. Superparamagnetic particles have many advantages over other contrast agents. Unlike agents such as perfluorochemicals, oils, and fats, superparamagnetic particles are miscible with aqueous systems, which means they can mix with material in the bowel and be used in small volumes. Immiscible agents must be used in sufficient quantity to displace intestinal matter. This miscibility also allows them to be used intravenously. Compared with other magnetic contrast agents (e.g., gadolinium chelates), they are much more potent (as much as 50 times more effective per mole) [161]. Another advantage is that the particles do not pass the blood-brain barrier; thus, they are well suited for tracking blood flow in the brain. A novel use of these nanoparticles is tracking cells in vivo. Moreover, in a recent clinical trial, Harisinghani et al. [162] successfully used ultra small superparamagnetic iron oxide (USPIO) nanoparticles to image lymph node containing micro-metastases in 80 patients with prostate cancer.

In terms of drug delivery, magnetic nanoparticles offer the possibility of use of external magnetic fields to obtain better localization than could be achieved with non-magnetic particles. The drug can be loaded on the nanoparticles [163] with or without suitable targeting molecule and can be targeted to the diseased area under the influence of external magnetic filed. So, in this way superparamagnetic nanoparticles have emerged as multifunctional nanodevices in disease diagnosis, drug targeting and delivery and tumor hyperthermia therapy [164]. Since magnetic nanoparticles are less easily destroyed or inactivated by cells than many non-magnetic ones there is the disadvantage that persistent particles may cause later cell damage and death. The same considerations apply to situations where magnetic
nanoparticles are being used for generating hyperthermia by the application of external fields [153d, 165].

1.5.3 Noble Metal Nanoparticles

"Zum Golde drängt, am Golde hängt doch alles" — “Towards gold throng all, to gold cling all”. This sentiment, from Goethe’s masterpiece *Faust*, reflects mankind’s fascination with this metal. But the lustre that makes gold so attractive in its bulk state changes entirely at the nanoscale. Gold particles of 10–100 nanometers possess optical properties that change according to their configuration: separated particles appear red in colour, and aggregated particles appear blue.

Noble metal nanoparticles devoid of any coating have received widespread interest in their use in biotechnological systems for diagnostic application and biological imaging due to its easy preparation, ready bioconjugation and highly controlled optical properties [8b, 121, 166-170]. Despite the universal view that Ag nanoparticles are better absorbers and scatterers of light than Au nanoparticles [167-169] and have the unique ability to amplify certain behaviors, e.g., Raman scattering [117, 171-173] and fluorescence [174-175] through surface enhancement effects, Au nanoparticles are used in almost every biological application of metallic nanoparticles because of their resistance to oxidation, ease of synthesis, and optical properties, the most familiar one being the red to purple color change upon aggregation. Ag remains on the sidelines because of its propensity to oxidatively corrode [176-178] and aggregate [179-181] in electrolytic solutions. The oxidative corrosion and aggregation of Ag nanoparticles can be effectively eliminated, however, with the proper protective layer, allowing stable nanoparticle solutions to exist at high NaCl concentrations and over a wide range of pH [182]. Au nanoparticles have already been used *in vivo* since the 1950s as a radiotracer [117]. Modern use of colloidal gold in biomedical applications started in 1971 when Faulk and Taylor invented the immunogold staining procedure [183]. The labeling of targeting molecules such as antibodies with gold nanoparticles truly revolutionized the visualization of subcellular components through electron microscopy, exemplifying the utility of this particular noble metal element. During the last 10 years, several groups have prepared gold nanoparticles linked with sugars [184-185], proteins, and DNA [10]. These nanoparticles are being used for assembling new
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materials, developing bioassays and as multivalent systems for interaction studies [121, 186]. Using the light scattering properties of gold nanoparticles, preliminary studies have reported their use as contrast agents for biomedical imaging using multiphoton plasmon resonance microscopy [187], third-harmonic microscopy [188], simple dark-field microscopy [131] optical coherence microscopy [189] and confocal microscopy [190-191]. Gold nanoparticles have several advantages for imaging application compared to other agents. The scattered light is very strong and they are much brighter than chemical fluorophores. They are photo bleach resistant and can be easily seen in as low as 10^{-16} M concentration. Sokolov and co-workers [191] have used confocal microscope to detect the scattering of anti-EGFR/Au nanoparticles for cervical cancer. Dickson and co-workers have fabricated highly fluorescent, water-soluble Au quantum dots (QDs) [192]. The particles are smaller in size, have narrower excitation spectra, but have comparable fluorescence to semiconductor QDs. These properties could make the Au QDs useful as biological labels, energy-transfer pairs, and as light-emitting sources for nanoscale optoelectronics. However, their derivatization and application in cell biology is eagerly awaited from a biological perspective.

Photothermal therapy using the absorption properties of antibody-conjugated gold nanoshells [69] and solid gold nanospheres [133] has been demonstrated to selectively kill cancer cells, leaving the healthy cells unaffected. To use long-wavelength laser irradiation that penetrates tissue for in vivo photothermal treatment (650-900 nm) [193], the absorption band of the nanoparticles has to be in the near infrared (NIR) region. The absorption band of core-shell particles has been tuned by adjusting the ratio of the thickness of the gold shell to the diameter of the silica core (about 120 nm in diameter) and thus enables photothermal therapy in this region. Sastry and co-workers have reported biogenic synthesis of gold nanotriangles [188] which show great promise as hyperthermic treatment for tumors.

It is has been reported that surface plasmon field enhancement of the absorption of nanoparticles changes with different shapes of gold and silver nanoparticles and is predicted to be higher for anisotropic gold triangles [188] and nanorods [194-195]. By changing the shape of gold nanoparticles one can not only change the absorption and scattering wavelength from visible to the NIR region but
also increase their absorption and scattering cross sections. The extremely flat surface and large NIR absorption make them suitable for hyperthermia treatment of tumors.

1.5.4 Dendrimers

Poor solubility and hydrophobicity of drugs/bioactive agents limit their possible applications in drug delivery and formulation development. Apart from conventional methods of solubility enhancement, there are some novel methods which can be used in solubilization. Recently, dendritic polymers [196-202] have been explored for the encapsulation of hydrophobic compounds and for the delivery of anticancer drugs. Dendrimers are globular, highly branched macromolecules possessing a well-defined core, an interior region, and a large number of end groups (Fig.1.4). These are highly branched macromolecules with controlled near monodispersed three-dimensional architecture emanating from a central core. Polymer growth stats from centre core molecule and proceeds in outward direction by a series of polymerization reactions. Hence, precise control over size can be achieved by the regulation of the extent of polymerization. Dendrimers represent a novel type of polymeric material that has generated much interest in many diverse areas due to their unique structure and properties. The physical characteristics of dendrimers, including their monodispersity, water solubility, encapsulation ability, and large number of functionalizable peripheral groups, make these macromolecules ideal candidates for evaluation as drug delivery vehicles. Dendrimer-mediated solubility enhancement mainly depends on factors such as generation size, dendrimer concentration, pH, core, temperature, and terminal functionality. Added advantage in solubilization can be achieved considering these factors. Available literature suggests that ionic interaction, hydrogen bonding, and hydrophobic interactions are the possible mechanisms by which a dendrimer exerts its solubilizing property. Different metal dendrimer nanocomposites [203-204] have been synthesized and used for biological applications. Lesniak and co-workers [203]
have synthesized fluorescent silver/dendrimer nanocomposites. The silver/dendrimer nanocomposites have potential applications as a cell labeling nanodevices.

1.5.5 Polymeric Micelles

Polymeric micelles are formed when amphiphilic polymers are placed in water [205]. These consist of an inner core of assembled hydrophobic polymeric segments capable of solubilizing lipophilic substances and an outer hydrophilic corona with hydrophilic polymeric chains exposed to the aqueous environment serving as a stabilizing interface between the hydrophobic core and the external aqueous environment [206]. Polymeric micelles can be used as efficient carriers for compounds, which alone exhibit poor solubility, undesired pharmacokinetics, and low stability in a physiological environment. The hydrophilic shell contributes greatly to the pharmaceutical behavior of polymeric formulations by maintaining the micelles in a dispersed state, as well as by decreasing undesirable drug interactions with cells and proteins through steric-stabilization effects. The size of polymeric micelles ranges from ~10 to ~100 nm, and usually the size distribution is narrow [206]. They can increase drug bioavailability and retention, since the drug is well protected from possible inactivation under the effect of their biological surroundings [206b]. Polymeric micelles have been studied extensively as delivery medium for injectable drug formulations of poorly water-soluble drugs such as paclitaxel, indomethacin, amphotericin B, adriamycin, and dihydrotosterone. Overall, they have proved to be highly effective drug delivery vehicles [207]. Kabanov’s group [209] has developed micelles formed from commercially available Pluronic® triblock co-polymers [also termed Poloxamer; poly (ethylene oxide)x-b-poly(propylene

**Figure 1.5** Polymeric micelles A) drug solubilized in hydrophobic micelle core; B) drug covalently linked to hydrophobic portion of polymer chain; C) polymeric micelle carrying antibodies attached to hydrophilic portion of polymer molecule [208]
oxides), PEO-b-PPO-b-PEO, and on block ionomer complexes as carriers for DNA.

1.6 Nanotechnology in Tissue Engineering

Tissue engineering combines biology, medicine, engineering and materials science to develop tissues that restore, maintain or enhance tissue function [210]. To recapitulate proper function and organization of native tissues in tissue engineering approaches, it is important to mimic tissue properties at the nanoscale. Nanofabricated and micro fabricated tissue engineering scaffolds have the potential to direct cell fate as well as regulate processes such as angiogenesis and cell migration. The surface topographies of tissue engineering scaffolds have been shown to induce changes in cell adhesion, morphology, motility and gene expressions [211-214]. Both, the top-down and bottom-up technologies have been used to incorporate nanoscale control for tissue engineering scaffolds.

Top-down approaches, such as soft lithography, have greatly enhanced our ability to generate microscale and nanoscale features. Approaches, such as the layer-by-layer deposition of cells and proteins using microfluidic channels [215], microsyringe deposition of PLGA polymer [216], and photo-polymerization within microfluidic channels [217] have been used to generate 3D structures with controlled geometries and properties.

Bottom-up approaches based on molecular self-assembly of small building blocks have also been used to generate tissue engineering scaffolds. Research into self-assembly of amphiphilic peptides has shown that they can self-assemble to form hydrogels for tissue engineering [218]. Self-assembled scaffolds can be easily functionalized by incorporating peptide sequences that direct cell behavior directly into the build up molecule. For example, self-assembled gels were fabricated that directed neural stem cell differentiation to neurons and repressed astrocyte differentiation without exogenous growth factors [219]. These gels were made from peptides that expressed isoleucine-lysine-valine-alanine-valine (IKVAV, an amino acid sequence found in laminin) and self-assembled to form nanofibers. Similar approaches have been used for other tissues such as cartilage, bone and cardiac applications, and show great promise in tissue engineering.
1.7 Rationale behind the Thesis

The importance of metal nanostructures is evident from the various aspects that are being pursued towards their potential applications. They have been exploited for applications such as catalysis [8b, 61-62, 220], fuel cells [221], heavy metal detection [222], photonic band-gap materials [223], single electron transistors [224], non-linear optical devices [225] and surface-enhanced Raman spectroscopy [147]; besides the biological applications enlisted above [69, 118, 133, 138, 148-149, 153, 160, 162, 164, 183, 186-191]. It is clear that there have been numerous attempts world wide to synthesize and characterize nanomaterials for use in biology and medicine. Currently, metal nanoparticles particularly gold, silver and iron oxide nanoparticles are emerging as valuable tools for early detection and therapy of various diseases including cancer. Some of the unanswered questions concerning nanoscale devices relate to their potential toxicity or their fate in the environment, neither of which has yet been studied in any concerted manner. As the field continues to advance, studies on the cellular uptake of nanoparticles, with respect to their size and shape, are required in order to facilitate nanotechnology for biomedical applications. This is important for assessing nanoparticle toxicity for their use in tissue imaging, drug delivery, and therapeutic applications, as well as for designing multifunctional nanoparticles. Detailed studies of uptake kinetics of nanoparticles by cells have not been well characterized and quantified as a function of their size and shape.

The work presented in this thesis emphasizes on assessment of biocompatibility of borohydride reduced gold nanoparticles and newly synthesized curcumin reduced silver nanoparticles in cell culture systems. This thesis focuses mainly on the following aspects:

1. Cytotoxicity studies and assessment of biocompatibility of gold nanoparticles in cell cultures.
2. Synthesis and characterization of fluorescence conjugated gold nanoparticles.
3. Study of internalization of gold nanoparticles in RAW264.7 macrophage cells.
1.8 Outline of the Thesis

The main emphasis of the thesis is to study the interaction and subsequent internalization of metal nanoparticles within the animal cells in culture with reference to borohydride reduced gold nanoparticles. The thesis also includes the development of a new method for synthesizing metal nanoparticles of different sizes and structures employing green chemistry approach and their further physico-chemical and biological characterization. The thesis consists of six chapters.

Chapter one provides a brief and general introduction to nanotechnology, with a detailed description of biocompatibility issues of metal nanoparticles, various methods of nanoparticle synthesis mentioned in the literature, their properties, surface functionalization, nanomaterials toxicity, and their applications in biology, medicine and tissue engineering.

Chapter two describes detailed materials and methods used in the following chapters of thesis in the context of characterizing nanoparticles synthesized by the curcumin and sodium borohydride methods and their interaction with cell system in vitro. The chapter also describes the detailed protocols for primary culture and cell line maintenance.

Chapter three describes the modified method of sodium borohydride reduced gold nanoparticles synthesis for cell culture applications. Nanoparticles synthesis in a perfectly sterile environment avoids the modification of nanoparticles by biological contaminants. The presence of serum in culture medium helps in avoiding the aggregation of nanoparticles against high salt concentrations. The nanoparticles synthesized this way do not induce secretion of stress induced inflammatory cytokines and release of nitric oxide in a dose and time dependent manner. The nanoparticles also do not stimulate intracellular reactive oxygen species level when treated in a dose dependent manner. Detailed studies on dose and time dependent cytotoxicity of gold nanoparticles on different types of cells including primary cultures of macrophage and islets reveal that gold nanoparticles are biocompatible in nature. Gold nanoparticles can be immobilized on to the surface of poly-L-lysine coated alginate hydrogel and support the growth of cells. These gold coated alginate hydrogel beads represent a model system for tissue engineering and fermentor applications.
Chapter four deals with the study of time dependent kinetics of gold nanoparticle internalization inside the macrophage cells using different sophisticated microscopic tools viz. AFM, CFLSM, TEM and FACS. The early events of particle internalization, tracked by AFM, suggests pinocytotic mode of internalization. The cell membrane invaginates in the form of small cup like nanopits followed by particle internalization. The pits are transient and the cell membrane attains its original topography after particle internalization. Synthesis and characterization of different types of fluorescent gold nanoparticles for CFLSM suggests that lysine capped gold nanoparticles are suitable for FITC conjugation. Confocal microscopic analysis suggests that gold-lysine-FITC nanoparticles are taken up by the macrophages and start accumulating in lysosomes in a time dependent manner. Lysosomes are arranged in a perinuclear fashion. The inability of gold nanoparticles endocytosis at reduced temperature indicates that it is an active process. At later time points, TEM studies confirm gold nanoparticle accumulation in the perinuclearly arranged lysosome. The mechanism of internalization of gold nanoparticles has been discussed using phagocytosis specific inhibitor cytochalasin B.

Chapter five deals with the simple one step room temperature synthesis of silver and gold nanoparticles using curcumin as a reducing as well as surface capping agent. Silver nanoparticles synthesized this way are aqueous, spherical in shape, nearly monodispersed and stable at variable pH and salt concentrations. Curcumin is a common Indian spice and is has used as a curry pigment through the ages. Consumption of curcumin has been associated with various beneficial effects on human health. The idea behind using curcumin as reducing agent for synthesis of nanoparticles is to increase the biocompatibility of the latter and if possible, to use the synthesized nanoparticles for therapeutic purposes. UV-Vis-NIR spectra of silver nanoparticles shows a characteristic peak at ~416 nm while in case of gold nanoparticles along with the peak at ~530 nm for spherical nanoparticles, an additional broad peak in the NIR region corresponds to nanoparticles of different geometries and shapes. Curcumin is a diferuloyl methane having two o-methoxy phenolic -OH groups attached to an α, β-unsaturated β-diketone (heptadiene-dione) moiety. Detailed studies on curcumin reduced nanoparticles synthesis reveal that
both the phenolic and biketone groups are involved. The toxicity of curcumin reduced silver nanoparticles assessed on different cell lines in a dose and time dependent manner indicate biocompatibility of silver nanoparticles. Studies on further biological characterizations to check anti-microbial and antiangiogenic activities reveal that the nanoparticles are non-antimicrobial but have therapeutic potential.

Chapter six summarizes the method used for the synthesis of nanoparticles, their interaction with cell systems and the process of endocytosis with reference to gold nanoparticles. The methods of assessment of biocompatibility and synthesis of fluorescent gold nanoparticles as lysosomal markers are described as future prospects in this area.
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Chapter 1


Chapter 1


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