CHAPTER 2

REVIEW OF LITERATURE
2. Review of Literature

- A simple biological method for the synthesis of gold nanoparticles (GNPs) using *Cassia auriculata* aqueous leaf extract was reported. Reduction of Aauric chloride led to the formation of GNPs within 10 min at room temperature. The size, shape and elemental analysis were carried out using X-ray diffraction, TEM, FE-SEM-EDAX, FT-IR and Visible absorption spectroscopy. Stable triangular and spherical crystalline GNPS with well-defined dimensions of average size of 15–25 nm were synthesized. Effect of pH was also studied to check the stability of GNPs (Kumar et al., 2011)

- Green synthesis of pure metallic silver and AuNPs and bimetallic Au/Ag nanoparticles by aqueous solutions of *Azadirachta indica* shows rapid formation of stable silver and AuNPs with flat, plate like morphology in polydisperse form (Shankar et al., 2004).

- *Bankar et al. (2010)* synthesized GNPs by using banana peel extract (BPE) as a simple, non-toxic, eco-friendly ‘green material’. They have reported the formation of variety of GNPs when the reaction conditions were altered with respect to pH, BPE content, chloroauric acid concentration and temperature of incubation. Dynamic light scattering (DLS) studies revealed that the average size of the nanoparticles under standard synthetic conditions was around 300 nm. X-ray diffraction studies of the samples revealed spectra that were characteristic for gold. Fourier transform infra red (FTIR) spectroscopy indicated the involvement of carboxyl, amine and hydroxyl groups in the synthetic process.

- Anisotropic nanoparticles were synthesized by using apiin as the reducing and stabilizing agent. It has been reported that the size and shape of the nanoparticles can be controlled by varying the ratio of metal salts to apiin compound in the reaction medium. GNPS were
characterized by UV–Vis–NIR spectroscopy, transmission electron microscopy (TEM), FT-IR spectroscopy, X-ray diffraction (XRD) and thermo gravimetric analysis (TGA). By TEM analysis the average size of the gold and silver nanoparticles was found to be at 21 and 39 nm. Moreover it has discussed that NIR absorption property of the synthesized gold Nano triangles can be exploited in the hyperthermia treatment for cancer cells and also in IR-absorbing optical coatings (Kasthuri et al., 2009)

➢ Biogenic gold nanotriangles and spherical silver nanoparticles were synthesized by a simple procedure using Aloevera leaf extract as the reducing agent. It has been found that multiply twinned particles (MTPs) play an important role in the formation of gold nanotriangles. It has been confirmed that the slow rate of the reaction along with the shape directing effect of the constituents of the extract was responsible for the formation of single crystalline gold nanotriangles (Chandran et al., 2006).

➢ An eco-friendly simple approach for the synthesis of GNPs, amenable for large scale commercial production and technical applications using coriander extract as the reducing agent for biosynthesis of GNPs (6.75–57.91 nm) has been reported by Narayanan et al. (2008)

➢ Gold nanoparticles were synthesized using a simple, efficient, economic and nontoxic method by employing aqueous extract of fenugreek (Trigonellafoenum-graecum) as reducing and protecting agent. It was found that different sizes of gold nanoparticles could be obtained by controlling the synthesis parameters. The presence of different functional groups present in the biomolecules as the capping agents in nanoparticles is confirmed by FTIR analysis. The synthesized gold nanoparticles showed good catalytic activity for the reduction of 4-nitrophenol to 4-aminophenol by excess NaBH4. The catalytic activity is found to be size-dependent, the smaller nanoparticles showing faster activity (Aromal & Philip 2012).
As Biologically inspired experimental process in synthesizing nanoparticles is of great interest in present scenario, an in situ green biogenic synthesis of gold nanoparticles using aqueous extracts of *Terminalia chebula* as reducing and stabilizing agent is reported. Gold nanoparticles were confirmed by surface plasmon resonance in the range of 535 nm using UV–visible spectrometry. TEM analysis revealed that the morphology of the particles thus formed contains anisotropic gold nanoparticles with size ranging from 6 to 60 nm. Hydrolysable tannins present in the extract of *T. chebula* were found to be responsible for reductions and stabilization of gold nanoparticles. Antimicrobial activity of gold nanoparticles showed better activity towards gram positive *S. aureus* compared to gram negative *E. coli* using standard well diffusion method. (Kumar, et al., 2012)

*Kumar et al., 2011* synthesized gold nanoparticles, with a particle size ranging from 5 to 15 nm, using *Zingiber officinale* extract which acts both as reducing and stabilizing agent. Gold nanoparticles synthesized using citrate and *Z. officinale* extract demonstrated very low protein adsorption. Both nanoparticles were non platelet activating and non complement activating on contact with whole human blood. They also did not aggregate other blood cells, however, they found that nanoparticles synthesized with *Z. officinale* extract was highly stable at physiological condition compared to citrate capped nanoparticles, which aggregated.

Green synthesis of GNPs using honey as reducing and capping agents was reported. The concentration of HAuCl4 and honey in aqueous solutions was varied and colloids having a larger propensity of either anisotropic or spherical nanocrystals were obtained. The nanoparticles obtained were characterized by UV–visible spectra, high-resolution TEM and XRD. The spherical particles (15nm) showed high crystallinity with fcc phase is evidenced by bright
circular spots in SAED pattern and clear lattice fringes in the high-resolution TEM image. FTIR measurements were identified the possible biomolecules responsible for capping and efficient stabilization of the GNPs synthesized using honey. The carboxylic acid group vibrations and amide I and II bands indicate the binding of protein with Au surface through the amine group rather than the carboxyl group. (Philip 2009)

- Reduction capability of phytochemicals occluded in tea was exploited to reduce gold salts to the corresponding gold nanoparticles was reported. It has been found that the phytochemicals present in tea serve a dual role as effective reducing agents to reduce gold and also as stabilizers to provide a robust coating on the gold nanoparticles in a single step. The tea-generated gold nanoparticles (T-AuNPs), have demonstrated remarkable in vitro stability in various buffers. By cellular internalization studies the authors showed that synthesized AuNPs enters in to cells through endocytosis process. As the generation of T-AuNPs follows all principles of green chemistry it was found that T-AuNPs was non toxic and it has been concluded by the authors that T-AuNPs have potential to be used in anticancer drug delivery (Nune, et al., 2009)

- Shukla et al. (2005) employed theRAW264.7 macrophage cells and evaluated the cytotoxicity and immunogenic effects of gold nanoparticles. The cytotoxicity of gold nanoparticles has been correlated with a detailed study of their endocytotic uptake using various microscopy tools such as atomic force microscopy (AFM), confocal-laser-scanning microscopy (CFLSM), and transmission electron microscopy (TEM). They found that Au (0) nanoparticles reduce the production of reactive oxygen and nitrite species, and do not elicit secretion of proinflammatory cytokines. Through AFM measurements they confirmed that gold nanoparticles are internalized inside the cell via a mechanism involving pinocytosis. They concluded gold
nanoparticles were noncytotoxic, nonimmunogenic, and biocompatible in nature and thus having potentials for application in nanoimmunology, nanomedicine, and nanobiotechnology.

- **Voliani, et al. (2013)** covalently conjugated the Doxorubicin to peptide encapsulated GNPs by click chemistry in order to localize its therapeutic action and minimize side effects. The loaded Doxorubicin was photo-released in a controlled fashion by a multiphoton process. Their results suggest that Gold nanospheres act both as a cage and a carrier for the drug and provide a nontoxic conjugate that effectively releases the payload upon irradiation with a 561 nm CW laser at mW power and showed that this approach yields excellent spatial and temporal control on the release process.

- **Housni, et al. (2008)** synthesized Bovine Serum Albumin (BSA) stabilized, water soluble gold nanoparticles that will be ideal for the application in field of biomedicines and biotechnology. BSA stabilized gold nanoparticles are synthesized in one step, using Irgacure (I-2959) as photoinitiator. They reported that polydispersity of mixed monolayer stabilized gold nanoparticles is largely dependent on the concentration of BSA in mixed monolayer. UV visible spectrum obtained for mixed monolayer stabilized gold nanoparticles indicates aggregation with the increase in BSA content. Finally, PAGE and fluorescence spectrum have confirmed that BSA is not denatured during the photochemical process and it still possess its native conformation.

- **Kattumuriet al. (2007)** synthesized and stabilized Gold nanoparticles (AuNPs) within the nontoxic phytochemical gum-arabic matrix (GA–AuNPs) and they performed detailed *in vitro* analysis and *in vivo* pharmacokinetics studies of GA–AuNPs in pigs to gain insight into the organ-specific localization of this new generation of AuNP vector. Their results demonstrate that naturally occurring GA can be used as a nontoxic phytochemical construct in the production of
readily administrable biocompatible AuNPs for diagnostic and therapeutic applications in nanomedicine.

Bar Ilan, et al. (2009) addressed the issue of potential toxicity of nanoparticles by utilizing a putative attractive model in developmental biology and genetics: the zebra fish (*Danio rerio*). Using They synthesized colloidal silver (cAg) and gold nanoparticles (cAu) in a panoply of sizes (3, 10, 50, and 100 nm) and by employing semiquantitative scoring system, it is found that cAg produces almost 100% mortality at 120 h postfertilization, while cAu produces less than 3% mortality at the same time point. They found that parallel sizes of cAg and cAu induce significantly different toxic profiles, with the cAg being toxic and the cAu being inert in all exposed sizes. Therefore, they proposed that nanoparticle chemistry is as, if not more, important than specific nanosizes at inducing toxicity *in vivo*.

Patra et al. (2007) investigated whether GNPs, even in the absence of any specific functionalization, induce any cell specific response. They found out that GNP-induced death response in human carcinoma lung cell line A549 but not affected the BHK21 (baby hamster kidney) and HepG2 (human hepatocellular liver carcinoma). They concluded that GNPs do not universally target all cell types.

Goodman, et al. (2004) employed MTT, hemolysis, and bacterial viability assays to explore differential toxicity among the cell types used, using 2 nm core particles. These studies show that cationic particles are moderately toxic, whereas anionic particles are quite nontoxic.

Khlebtsov & Dykman (2011) reported that all organs of the reticuloendothelial system are the basic primary target for accumulation of GNPs within the size range of 10 to 100 nm, and the uniformity of distribution increases with a decrease in particle size. Similarly it has been reported that in recent years, Danio rerio embryos have become a popular model for toxicity
experiments. Because during in-vivo experiments GNPs can be passively transferred by diffusion into the chorionic space of the embryos and can retain their random-walk motion through chorionic space and into the inner mass of the embryos.

- **Iram et al. (2014)** utilised glucoxylans isolated from seeds of *Mimosa pudica* for the green synthesis of gold and silver nanoparticles. They reported that average particle size was about 40 and 6 nm for gold and silver, respectively. The size of gold particles obtained in this work is suitable for drug delivery as they are non-cytotoxic.

- **Hien et al. (2012)** synthesised gold nanoparticles (AuNPs) with diameter from 4 to 10 nm, capping by hyaluronan (HA) using a γ irradiation method. The maximum absorption wavelengths at 517–525 nm of colloidal AuNPs/HA solutions were measured by UV–Vis spectroscopy. The size and size distribution of AuNPs were determined from TEM images. Their results indicated that higher dose rate and HA concentration favor smaller sizes of AuNPs whereas the size increases with Au³⁺ concentration. The colloidal AuNPs/HA solution was fairly stable more than 6 months and can be applied in biomedicines and cosmetics.

- **Sen et al. (2013)** synthesized Gold nanoparticles with a glucan, isolated from an edible mushroom *Pleurotus florida*. Glucan was reported to be acted as reducing as well as stabilizing agent. The synthesized gold nanoparticles were characterized by UV–visible spectroscopy, HR-TEM, XRD, SEM, and FT-IR analysis. The results indicated that the size distribution of gold nanoparticles (AuNPs) changed with the change in concentration of chloroauric acid (H AuCl₄). The resulting Au NPs-glucanbioconjugates function as an efficient heterogeneous catalyst in the reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP), in the presence of sodium borohydride.
Chairam et al. (2009) reported the Size- and shape-controlled synthesis of silver and gold nanoparticles using partially hydrolyzed starch vermicelli templates as green nanoreactors for the growth of nanoparticles. Mung bean vermicelli is of interest due to the higher amylose content and its transparency, allowing the formation of coloured particles on the vermicelli to be observed. The as-prepared silver and gold nanoparticles were characterized by UV–Visible spectroscopy, transmission electron microscopy (TEM), and X-ray diffraction (XRD). They reported that their approach had a great potential to design new fine structures of vermicelli and utilize its structure as a template for the large-scale synthesis of size- and shape-controlled silver and gold nanoparticles for chemical and biological applications.

Zheng Het al. (2011) prepared a series of 6-mercaptopurine-carboxymethyl chitosans (6-MP-CMC) characterized. In vitro drug release behaviors in the buffer solutions containing glutathione (GSH) were investigated. They reported that 6-MP-CMC did not release any 6-MP in the media without GSH and containing 2µM GSH. By comparison, the obvious 6-MP release could be observed within an hour in the media containing 2mM and 10mM GSH. Moreover it was stated that buffer pH and the 6-MP content in 6-MP-CMC had obvious influences on the 6-MP release. They observed maximum release rate at pH 5 than at pH 7.4.

Cuinet al. (2011) synthesized silver and gold complexes with 6-mercaptopurine (H2MP). The Ag (I) and Au(I) complexes with HMP-, AgHMP and AuHMP, were obtained by mixing an acidified H2MP aqueous solution with an equimolar aqueous solution of AgNO3 or Au(CN)2. They found that the AuHMP and KAu(MP)2 complexes decreased cell viability of HeLa cancer cells in vitro. The IC50 values for AuHMP and KAu(MP)2 are 3.0 and 30.0 mM, respectively. Anti-M.tuberculosis assays showed a MIC value of 2.24 mM for AuHMP and 5.12 mM for free MP while AgHMP is active at the concentration 93.2 mM.
Ghoshal et al. (2014) developed 6MP (6-Mercaptopurine) loaded CNTs solvent method; as a carrier for drug targeting to cancer tissues for exhibiting antineoplastic activity. Non-covalent functionalization of Multiwalled Carbon Nanotubes (MWCNTs) was achieved using basic treatment followed by treatment with HCL. The loaded nanotubes were shown to release the drug for more than 10h, thus controlling the release and the amount of drug released from the best performing formulation was 59.2%. The release was found to follow the zero-order release pattern.

Dorniani et al. (2013) synthesized 6-mercaptopurine (FCMP) loaded superparamagnetic nanoparticles. They reported that the synthesized Fe$_3$O$_4$ nanoparticles and the FCMP nanocomposite were generally spherical, with an average diameter of 9 and 19 nm, respectively. They reported that the release of 6-mercaptopurine from the FCMP nanocomposite was found to be sustained and governed by pseudo-second order kinetics. Moreover to improve drug loading and release behavior, they prepared a novel nanocomposite (FCMP-D), ie, Fe$_3$O$_4$ nanoparticles containing the same amounts of chitosan and 6-mercaptopurine but using a different solvent for the drug. The resultant FCMP-D did not demonstrate “burst release” and the maximum percentage release of 6-mercaptopurine from the FCMP-D nanocomposite reached about 97.7% and 55.4% within approximately 2,500 and 6,300 minutes when exposed to pH 4.8 and pH 7.4 solutions, respectively. By MTT assay, they shown that the FCMP nanocomposite was non-toxic to a normal mouse fibroblast cell line.

Kevadiya et al. (2013) intercalated 6-mercaptopurine (6-MP), an antineoplastic drug in interlayer gallery of Na+-clay (MMT) and further entrapped in poly (l-lactide) matrix to form microcomposite spheres (MPs) in order to reduce the cell toxicity and enhance in vitro release and pharmacokinetic proficiency. In vitro drug releases showed controlled pattern, fitted to
kinetic models suggested controlled exchange and partial diffusion through swollen matrix of clay inter layered gallery. The *in vitro* efficacy of formulated composites drug was tested in Human neuroblastoma cell line (IMR32) by various cell cytotoxic and oxidative stress marker indices. *In vivo* pharmacokinetics suggested that the intensity of formulated drug level in plasma was within remedial borders as compared to free drug.

- **Serpe et al. (2004)** synthesized solid lipid nanoparticles (SLN) carrying cholesteryl butyrate (chol-but), doxorubicin and paclitaxel and the antiproliferative effect of SLN formulations versus conventional drug formulations was evaluated on HT-29 cells. They reported that *In vitro* cytotoxicity of SLN carrying chol-but and doxorubicin was higher than that of conventional drug formulations. Intracellular doxorubicin was double after 24 h exposure to loaded SLN versus the conventional drug formulation, at the highest concentration evaluated by flow cytometry. Moreover, they reported that the combination of low concentrations of chol-but SLN (0.1–0.2 mM) and doxorubicin (1.72 nM) or paclitaxel (1.17 nM) exerted a greater-than-additive antiproliferative effect at 24 h exposure, while the combination of Na-but and doxorubicin or paclitaxel did not.

- **Wanget al. (2013)** prepared mesoporous silica nanoparticles with amino and thiol groups (MSNSN) and covalently modified with methotrexate and 6-mercaptopurine to form 6-MP–MSNSN–MTX. In the presence of DTT, 6-MP–MSNSN–MTX gradually releases 6-MP. It has been shown that in rat plasma, 6-MP–MSNSN–MTX effectively inhibits the metabolic deactivation of 6-MP and MTX.

- **Senthil et al. (2010)** used chitosan and carrageenan like polymers to prepare hydrogel nanoparticles and encapsulated the mercaptopurine by the counter polymer gelation method. The diameter of hydrogel nanoparticles was reported as 370 – 800 nm with a positive zeta potential
of 26 – 30 mV. The hydrogel nanoparticles were almost spherical in shape, as revealed by scanning electron microscopy (SEM). Drug loading varied from 9 to 17%. Mercaptopurine released from the nanoparticles at the end of the twenty-fourth hour was about 69.48 – 76.52% at pH 7.4. The drug release from the formulation was following zero order kinetics, which was evident from the release kinetic studies and the mechanism of drug release was anomalous diffusion, which indicated that the drug release was controlled by more than one process.

- Zhang et al. (2013) synthesized magnetic Fe₃O₄ nanoparticles by co-precipitation method and the mercaptopurine (MER) drug-loaded magnetic microspheres were obtained through emulsion cross-linking methods. The microspheres showed good loading capacity values of 11.8%, as well as good Encapsulation efficiency values of 79.4%. The release profiles showed an initial fast release rate, which decreased as time progressed and about 84 % had been released after 48 h. The experimental results indicated that the prepared magnetic microspheres may be useful for potential applications of MER for magnetically targeted chemotherapy.

- Podsiadlo et al. (2008) reported that anticancer drugs have short biological half-life and severe side effects can be loaded on gold nanoparticles to overcome their problems. They prepared gold nanoparticles conjugated with 6-Mercaptopurine and its riboside derivatives to treat leukemic cancer. Synthesised mercaptopurine loaded GNPs showed substantial enhancement of the antiproliferative effect against K-562 leukemia cells than the free drug.

- Jang et al. (2013) developed a strategy to prepare dextran-coated AuNPs (Gold Nanoparticle) with control over its size by simply boiling an aqueous solution of Au salt and dextran, in which dextran serves as both reducing agent for AuNP (Au(0)) formation from Au(III) and AuNP surface coating material. The prepared dextran-coated AuNPs (dAuNPs) maintained its colloidal stability under high temperature, high salt concentration, and extreme
pH. Importantly, the dAuNP remarkably improved efficacy of an anti-cancer agent, doxorubicin (Dox), when harnessed as a Dox delivery carrier. The half-maximal inhibitory concentration (EC50) of Dox-conjugated dAuNP with diameter of 170 nm was 9pM in HeLa cells, which was $1.1 \times 10^5$ times lower than that of free Doxorubicin and lower than any previously reported values of Dox-nanoparticle complex. Interestingly, smaller AuNPs with 30 and 70 nm showed about 10 times higher EC50 than 170 nm AuNPs when treated to HeLa cells after conjugation with Doxorubicin.

She et al. (2013) developed Heparin drug conjugates loaded with Doxorubicin. Dynamic light scattering (DLS) and transmission electron microscope (TEM) studies demonstrated the dendronized heparine DOX conjugate self assembled into compact nanoparticles with negatively charged surface. The nanoparticles with 9.0 wt% (weight percent) of doxorubicin (DOX) showed pH-sensitive property due to the faster drug release rate at pH 5.0 and slow release rate at pH 7.4 aqueous. The nanoparticles were shown to effectively kill cancer cells in vitro. Notably, the nanoparticles resulted in strong antitumor activity, high ant angiogenesis effects and induced apoptosis on the 4T1 breast tumor model due to the evidences from mice weight shifts, tumor weights, tumor growth curves, immunohistochemical assessment and histological analysis. It’s also noteworthy that dendronized heparin and its nanoparticle with drug demonstrated no significant toxicity to healthy organs of both tumour bearing and healthy mice, which was confirmed by histological analysis compared with free drug DOX. The dendronized heparine DOX conjugate based nanoparticle with high antitumor activity and low side effects may be therefore a potential nanoscale drug delivery vehicle for breast cancer therapy.

Deshpande et al. (2013) has carried out preliminary phytochemical screening of ethanol and petroleum ether extract of stem bark of Acacia nilotica. It revealed the presence of alkaloids,
carbohydrates, saponins, Tannins, Flavonoids, cardiacglycosides and anthraquinone in both ethanol and ether extracts while fixed oils and fats, proteins and amino acids were absent. The antimicrobial activity of the extracts was determined by agar diffusion method. The ethanol extract showed more significant activity against Streptococcus mutans as compared to petroleum ether extract. The minimum inhibitory concentration of ethanol extract was 5 mg/ml while 10 mg/ml for ether extract.

Shalviri et al. (2012) investigated the capability of a new nanoparticulate system, based on terpolymer of starch, polymethacrylic acid and polysorbate 80, to load and release doxorubicin (Dox) as a function of pH and to evaluate the anticancer activity of Dox-loaded nanoparticles (Dox-NPs) to overcome multidrug resistance (MDR) in human breast cancer cells in vitro. The Dox-NPs were characterized by Fourier transform infrared spectroscopy (FTIR), isothermal titration calorimetry (ITC), transmission electron microscopy (TEM), and dynamic light scattering (DLS). The cellular uptake and cytotoxicity of the Dox-loaded nanoparticles were investigated using fluorescence microscopy, flow cytometry, and a 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay. The Dox-NPs were taken up by the cancer cells in vitro and significantly enhanced the cytotoxicity of Dox against human MDR1 cells with up to a 20-fold decrease in the IC50 values.

Kratzet al., (2012) have discussed about the barriers that hinder nanoparticles penetration deeply and evenly into solid tumors: a chaotic, tortuous vascular compartment resulting in tumor tissue distant from microvessels, a heterogeneous blood flow distribution with a concomitant defective microcirculatory exchange process, and a high interstitial fluid pressure. Furthermore, a resulting hostile tumor microenvironment characterized by hypoxia and/or extracellular acidosis can reduce the efficacy of anticancer drugs and confer drug resistance.
Conversely, the enhanced permeation and retention effect has become the gold standard for developing macromolecular prodrugs and nano-sized drug delivery systems. In this respect, combining low-molecular weight cytostatic drugs with nano-sized drug delivery systems appears to be a natural choice for combination therapy that aims at distributing anticancer drugs at higher concentrations in the tumor in a more even manner.

- Vigderman et al. (2012) reviewed the various covalent strategies that have been developed to attach drugs to gold nanoparticles as well as the strengths and limitations of such strategies. After examining general strategies for the synthesis of gold nanoparticles and their subsequent covalent functionalization, they discussed about nanoparticle conjugates for gene therapy, antibacterial, and anticancer applications.

- Joshi et al. (2012) have conjugated 11-mercaptoundecanoic acid-modified gold nanoparticles (~7 nm) with chloroquine to explore their potential application in cancer therapeutics. The anticancer activity of chloroquine-gold nanoparticle conjugates (GNP-Chl) was demonstrated in MCF-7 breast cancer cells. The MCF-7 cells were treated with different concentrations of GNP-Chl conjugates, and the cell viability was assayed using trypan blue, resulting in an IC50 value of 30 ± 5g/mL. Flow cytometry analysis revealed that the major pathway of cell death was necrosis, which was mediated by autophagy. The drug release kinetics of GNP-Chl conjugates revealed the release of chloroquine at an acidic pH, which was quantitatively estimated using optical absorbance spectroscopy.

- Lal et al. (2012) carried out the green synthesis of gold nano particle using various plant extracts and spices extracts was done in which extracts reduces aqueous HAuCL4.3H2O to Au and stabilized by itself at certa in crystalline phase,synthesized nano particle was confirmed by the change of colour of auric chloride which is yellow in colour,and growth of nano particle was
monitored by surface plasmon behaviour using UV-Visible spectroscopy. Furthermore they have reported that green synthesis approach was rapid and better alternative to chemical synthesis and also effective for the large scale synthesis.

Verma et al. (2012) have reported that Acacia nilotica (linn.) wild. Ex Del., is a medicinal tree belonging to leguminoseae family and sub family mimosaceae, a moderate sized, spiny evergreen tree found throughout India, known to be rich in phenolics consisting of condensed tannins and phlobatannin, gallic acid, (+) catechin, (-) epigallocatechin-7-gallate, and has been used for the treatment of viral (cold, bronchitis), bacterial (diarrohea), amoeboid (dysentery) fungal, bleeding files and leucoderma diseases. They summarized the information concerning the botany, ethnopharmacology, phytochemistry, biological activity, and toxicity of *Acacia nilotica* linn.

Gautier et al. (2012) have developed polyethylene glycol-coated superparamagnetic iron oxide nanoparticles for improved cancer chemotherapy. Doxorubicin (DOX) anticancer drug, was loaded in prepared nanoparticles. The DOX loaded particles present a hydrodynamic size around 60 nm and a zeta potential near zero at physiological pH, both parameters being favourable for increased colloidal stability in biological media and decreased elimination by the immune system. At physiological pH of 7.4, 60% of the loaded drug is gradually released in ~2 h. The intracellular release and distribution of DOX is followed by means of confocal spectral imaging (CSI) of the drug fluorescence. The in vitro cytotoxicity of the DLPS on MCF-7 breast cancer cells is equivalent to that of a DOX solution. The reversible association of DOX to the SPION surface and the role of polymer coating on the drug loading/release were discussed, as both being critical for the design of novel stealth magnetic nanovectors for chemotherapy.
➢ **Rana et al. (2012)** studied the Gold nanoparticles (AuNPs) provide attractive vehicles for delivery of drugs, genetic materials, proteins, and small molecules. AuNPs feature low core toxicity coupled with the ability to parametrically control particle size and surface properties. In their review, they discussed on engineering of the AuNP surface monolayer, highlighting recent advances in tuning monolayer structures for efficient delivery of drugs and biomolecules. They discussed about particle functionalization such as organic monolayers and biomolecule coatings, with their applications in drug, DNA/RNA, protein and small molecule delivery.

➢ **Manju et al. (2012)** devised a simple method for the fabrication of water soluble Curcumin(Cur) conjugated gold nanoparticles to target various cancer cell lines. Cur was conjugated to hyaluronic acid (HA) to get a water soluble conjugate (HA–Cur). They generated gold nanoparticles (AuNPs) by reducing chloroauric acid using HA–Cur, which played the dual role of a reducing and stabilizing agent and subsequently anchored folate conjugated PEG. These entities were probed using different analytical techniques, assayed the blood compatibility and cytotoxicity. Their interaction with cancer cell lines (HeLa cells, glyoma cells and Caco 2 cells) was followed by flow cytometry and confocal microscopy. Blood–materials interactions studies showed that the nanoparticles are highly hemocompatible. Flow cytometry and confocal microscopy results showed significant cellular uptake and internalization of the particles by cells. HA–CurAuNPs exhibited more cytotoxicity comparing to free Curcumin.

➢ **Gu et al. (2012)** developed a gold-doxorubicin (DOX) nanoconjugates system to overcome MDR. Gold nanoparticles (AuNPs) were first PEGylated as Au-PEG-NH2, and DOX was then grafted onto AuNPs via a cleavable disulfide linkage (Au-PEG-SS-DOX). Confocal images revealed that the extent of intracellular uptake of Au-PEG-SS-DOX was greater than that of free DOX in the MDR cells, and inductively coupled plasma mass spectroscopy analysis
further confirmed that AuNPs significantly increased the level of drug accumulation in MDR cells at a nanoparticles dose greater than 15 μM. The cytotoxicity study demonstrated that the Au-PEG-SS-DOX nanoconjugates system efficiently released the anticancer drug DOX and enhanced its cytotoxicity against MDR cancer cells.

MubarakAli et al. (2011) has done bioreduction of silver nitrate (AgNO₃) and chloroauric acid (HAuCl₄) using plant extract, Mentha piperita (Lamiaceae). The nanoparticles were characterized using UV–Vis spectroscopy, FTIR, SEM equipped with EDS. They found that silver nanoparticles synthesized were generally found to be spherical in shape with 90 nm, whereas the synthesized gold nanoparticles were found to be 150 nm. Their results showed that the leaf extract of menthol is very good bioreductant for the synthesis of silver and gold nanoparticles.

Kievit et al. (2011) described the fabrication and characterization of a drug-loaded iron oxide nanoparticle designed to circumvent Multi Drug Resistance. Doxorubicin (DOX), an anthracycline antibiotic commonly used in cancer chemotherapy and substrate for ABC-mediated drug efflux, was covalently bound to polyethylenimine via a pH sensitive hydrazone linkage and conjugated to, an iron oxide nanoparticle coated with amine terminated polyethylene glycol. Drug loading, physiochemical properties and pH lability of the DOX-hydrazone linkage were evaluated in vitro. Nanoparticle uptake, retention, and dose-dependent effects on viability were compared in wild-type and DOX-resistant ABC transporter over-expressing rat glioma C6 cells. They found that DOX release from nanoparticles was greatest at acidic pH, indicative of cleavage of the hydrazone linkage. DOX-conjugated nanoparticles were readily taken up by wild-type and drug-resistant cells. In contrast to free drug, DOX-conjugated nanoparticles persisted in drug-resistant cells, indicating that they were not subject to drug efflux. Greater
retention of DOX-conjugated nanoparticles was accompanied by reduction of viability relative to cells treated with free drug. Our results suggest that DOX-conjugated nanoparticles could improve the efficacy of chemotherapy by circumventing MDR.

- **Mirza et al. (2011)** have presented the demonstration of gold nanoparticles (Au NPs) functionalized with an anticancer drug, doxorubicin. Doxorubicin was assembled on gold via amino group. The reaction proceeded under mild acidic conditions. Au NPs could not be adsorbed on doxorubicin in alkaline solution because amino group was not protonated. However, under acidic conditions, protonation created a positively charged amino group thus adsorption was easier. The interaction between Au colloids and doxorubicin is believed to be electrostatic. High-resolution TEM was used for visualization of nanoparticles, which were found to retain their average size and shape. The method, demonstrated that doxorubicin could be attached to Au NPs in a controlled manner.

- **Inbakandan et al. (2010)** in their article investigated the growing trend of exploring bacteria, fungi, actinomycetyes and plant materials for the synthesis of nanoparticles They synthesised gold nanoparticle (Au) from gold precursor using the extract derived from the marine sponge. water soluble organics present in the marine sponge extract mainly responsible for reduction of gold ions to nano sized Au particles. High resolution transmission electron micrograph (HR-TEM) confirmed the monodispersed and spherical shaped with the size range from 7 to 20nm, Through FTIR analysis, the reducing agent in marine sponge extract was indentified which is attributed for the biosynthesis of gold colloids. They confirmed the crystalline nature of the synthesized gold nanoparticles by XRD analysis.

- **Qi et al. (2010)** have developed biocompatible bovine serum albumin (BSA)-dextran–chitosan nanoparticles by Maillard reaction. The nanoparticles were characterized by light
scattering, potential, atomic force microscopy and pyrene fluorescence. The nanoparticles having a spherical shape and hydrodynamic diameters of 130–230 nm are stable in physiological condition. Doxorubicin was effectively loaded into the nanoparticles after changing the pH of the mixture to 7.4. The antitumor effects of doxorubicin loaded nanoparticles were investigated by the tumor inhibition and survivability of murine ascites hepatoma H22 tumor-bearing mice. The loaded nanoparticles largely decreased the toxicity of doxorubicin and significantly increased the survivability of the tumor-bearing mice.

第三个 bullet point: Cai et al. (2010) have studied about metastatic breast cancer. They mentioned that patients with metastatic breast cancer have a five-year survival rate of 27% compared to 98% for localized cancer, and the presence of even a few cancer cells in lymph nodes, known as isolated tumor cells or nanometastases, significantly increases the risk of relapse in the absence of aggressive treatment. They demonstrated a unique delivery system for localized doxorubicin chemotherapy to the breast tissue. The hyaluronan–doxorubicin nanoconjugate exhibits a sustained release characteristic in vitro and in vivo in the breast tissues of rodents bearing human breast cancer xenografts. In addition, the conjugate reduces dose-limiting cardiac toxicity with minimal toxicity observed in normal tissues. Finally, the conjugate dramatically inhibits breast cancer progression in vivo, leading to an increased survival rate. Thus, localized chemotherapy to the breast lymphatics with a nanocarrier may represent an improved strategy for treatment of early stage breast cancers.

第四个 bullet point: Zhou et al., (2010) have reported that the biosynthesis of nanoparticles has arisen as a promising alternative to conventional synthetic methodologies owing to its eco-friendly advantages. They confirmed that an electrostatic force or ionic bond-based interaction between
the chloroauric ions and bioconstituents (reducing sugars and flavonoids) was the reason behind the formation of gold nanoparticles.

- **Kalaivaniet al. (2010)** compared the two extraction methods and evaluates the free radical scavenging activity of Acacia nilotica. Their results indicated that the sequential extraction method was effective in concentrating the active principles in the ethanol extract as compared to the maceration method in DPPH assay. Based on the results, free radical scavenging property of the extracts obtained from sequential extraction method was analyzed in different assays to find out the possible antioxidant mechanism. They indicated that ethanol extract rich in phenolic and flavonoid contents had potent antioxidant activity. The possible antioxidant mechanism of the ethanol extract can be due to its hydrogen or electron donating and direct free radical scavenging properties. Hence, the ethanol extract represents a source of potential antioxidants that could be used in pharmaceutical and food preparations.

- **Brahma N. Singh et al., (2000)** have evaluated the antioxidant and anti-quorum sensing activities of Acacia nilotica. The specific phenolic compositions and their quantifications were performed by HPLC and MS/MS, which showed that the HEF (pH 4) was higher in gallic acid, ellagic acid, epicatechin, rutin, and GTs. In order to find antioxidant potential of various extracts, their activities were studied for TPC, AOA, FRSA, RP, inhibition of LPO, FIC activity. The results obtained strongly indicate that green pod of A. nilotica are important source of natural antioxidants.

- **Prabaharan et al. (2009)** developed Gold nanoparticles stabilized with a monolayer of folate-conjugated poly(L-aspartate-doxorubicin)-b-poly(ethylene glycol) copolymer (Au-P(LA-DOX)-b-PEG-OH/FA) as a tumor targeted drug delivery carrier. The anticancer drug, doxorubicin, was covalently conjugated onto the hydrophobic inner shell by acid-cleavable
hydrazone linkage. The DOX loading level was determined to be 17 wt%. The NPs formed stable unimolecular micelles in aqueous solution. The conjugated DOX was released from the micelles much more rapidly at pH 5.3 and 6.6 than at pH 7.4, which is a desirable characteristic for tumor-targeted drug delivery. Cellular uptake of the micelles facilitated by the folate-receptor-mediated endocytosis process was higher than that of the micelles without folate. This was consistent with the higher cytotoxicity observed with the developed micelles against the 4T1 mouse mammary carcinoma cell line.

References


