



## Applications of Nanotechnology in Pharmaceuticals

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### ABSTRACT

The present study focuses on an application of Nanotechnology in Pharmaceuticals. Drug particles in the Nano meter size range have unique characteristic that can lead to enhanced performance in a variety of dosage forms. Nature is the ultimate in nanotechnology producing nanostructures that offer function proteins and many others compounds. Nanotechnology is an industrial revolution based on integration of disciplines that could change every facets of human life. There are number of nanoparticles used as carrier for therapeutic and diagnostic agents namely polymeric nanoparticles, solid lipid nanoparticles, Nano suspension and Nano crystals, polymeric micelles, ceramic nanoparticles, liposomes, dendrimers, magnetic nanoparticles, Nano shells, nanowires, Nano pores, quantum dots, Ferro fluids. In this review the main scientific and technical aspects of nanotechnology are introduced and some of its applications are discussed.

**Keywords:** Nanotechnology, Nano meter, Types of nanoparticles, Applications.

### INTRODUCTION

The present study is an attempt at an analysis of an application of nanotechnology in Pharmaceuticals. Nanotechnology can be defined as the science and engineering involved in the design, synthesis, characterization, and application of materials and devices whose smallest functional organization in at least one dimension is on the Nano meter scale or one billionth of a meter. At these scales, consideration of individual molecules and interacting groups of molecules in relation to the bulk macroscopic properties of the material or device becomes important, since it is control over the fundamental molecular structure that allows control over the macroscopic chemical and physical properties. Applications to medicine and physiology imply materials and devices designed to interact with the body at subcellular (i.e., molecular) scales with a high degree of specificity. This can potentially translate into targeted cellular and tissue specific clinical applications designed to achieve maximal therapeutic affects with minimal side effects. Nanotechnology and Nano engineering stand to produce significant scientific and technological advances in diverse fields including medicine and physiology. In a broad sense, they can be defined as the science and engineering involved in the design, syntheses, characterization, and application of materials and devices whose smallest functional organization in at least one dimension is on the Nano meter scale, ranging from a few to several hundred Nano meters.

A Nano meter is one billionth of a meter or three orders of magnitude smaller then a micron, roughly the size scale of a molecule itself (e.g., a DNA molecule is about 2.5 nm long while a sodium atom is about 0.2 nm). To give an appreciation of just how significant an order of magnitude is, let alone three orders when going from

micron to Nano meter scales, consider that no one would ever walk from New York to San Diego, but with a single order of magnitude change in speed (the equivalent of changing speed from walking to driving), you would get to San Diego across the United States in about 2 days. Flying, which would be two orders of magnitude faster than walking, would get you across the United States in a few hours and in a supersonic plane (or three orders faster than walking), it would take you minutes. (Walking a straight line between the two cities would take about 42 days at an average speed of 3 miles per hour).

The potential impact of nanotechnology stems directly from the spatial and temporal scales being considered: Materials and devices engineered at the Nano meter scale imply controlled manipulation of individual constituent molecules and atoms in how they are arranged to form the bulk macroscopic substrate.

### Nanotechnology in Nature

Nature has enough evidence of nanotechnology, based on its ability to work at the atomic, molecular and supramolecular levels. The mechanisms of the biological and physical world operate mainly at the range of 1 to 100 nm. The diameter of a hydrogen atom is about 0.1 nm which is too small to be seen with human eyes. A molecule (such as water molecule) may be made up of 20 to 30 atoms and has a diameter of about 1nm. The width of a DNA molecule is about 2.5 nm, a typical protein is between 1 to 20 nm and ATP biochemical motor is 10nm in diameter. The human hair is about 10,000 nm thick while human cells range from 5,000 to 200,000 nm in size. Although this is larger than Nano scale, the viruses that attack human cells fall within 10 to 200 nm, which is within the Nano meter region. Nature is the ultimate in nanotechnology, producing nanostructures that offer

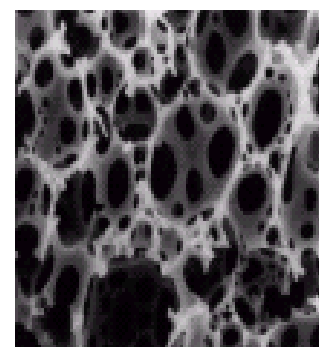
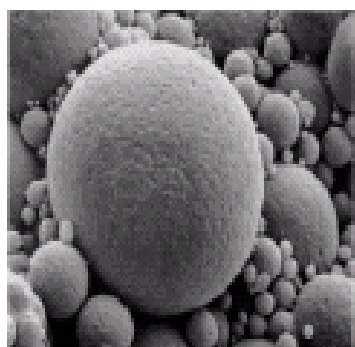


functional proteins and many other compounds at cellular level of great significance to life on earth. It is thought that one of the functions of proteins and compounds that exist at cellular level is that of Nano technological separations. Biological systems are thought by some scientists to have come about through a process of dynamic self-assembly comprising separation and compartmentalization of many substances into the desired pattern or device. Some biological systems contain Nano systems that are devoted to specific functions such as locomotion, where actin moves along myosin and kinesin moves along microtubules. Thus a DNA molecule can be seen as a self-assembly machine which replicates itself and also produces complex organisms under the right conditions. Ribosomes construct protein molecules with precision following instructions from DNA.

Molecular motors that make up the human muscles are sophisticated Nano machines that can convert chemical energy to mechanical energy with high efficiency. Ribosomes, another molecule, can construct protein molecules with precision following instructions from DNA. Photosynthesis is carried out in green plants by cells of Nano size, which employ energy to synthesize organic compounds by using cheap raw materials they pick up. Sufficient knowledge and insight into the principles of natural systems would enhance the design and fabrication of manmade nanostructures that may mimic the functions of natural systems. For instance, biomolecules such as proteins, peptides, DNA, lipids and carbohydrates can act as templates their shapes and chemical properties can be employed to arrange inorganic substances such as metals on Nano scale.

**Table 1:** Types of nanoparticles used as carrier for Therapeutic and diagnostic agents<sup>3</sup>

S. No.	Type of Nanoparticles	Material Used	Applications
A	Polymeric Nanoparticles	Biodegradable Polymers	Controlled and targeted drug Delivery
B	Solid Lipid Nanoparticles	Melted lipid dispersed in an aqueous surfactant	Least toxic and more stable colloidal carrier systems as alternative materials to polymers
C	Nano suspensions & Nano crystals	The drug powder is dispersed in a surfactant solution	Stable system for controlled delivery of poorly soluble drugs.
D	Polymeric micelles	Amphiphilic block copolymers	Systemic and controlled delivery of water-insoluble drugs
E	Liposomes	Phospholipid Vesicles	Controlled and targeted drug Delivery
F	Dendrimers		Carriers for site specific drug Delivery
G	Magnetic nanoparticles	An inorganic core of iron oxide (magnetite Fe <sub>2</sub> O <sub>3</sub> , maghemite or other insoluble ferrites) coated with polymer such as dextran.	Drug targeting, Diagnostic tool in biology and Medicine
H	Nano shells coated with Gold	Dielectric (typically gold sulfide or silica) core and a metal (gold) shell	Tumor Targeting
I	Quantum Dots	CdSe–CdS core-shell	Targeting, Imaging Agent
J	Ferro fluids	Iron oxide magnetic nanoparticles surrounded by a polymeric layer	For capturing cells and other biological targets from blood or Other fluid and tissue samples.



**Figure 1:** Polymeric Nanoparticles

## Polymeric Nanoparticles

Polymeric nanoparticles are colloidal solid particles with a size range of 10 to 1000nm and they can be spherical, branched or shell structures. The first fabrication of nanoparticles was about 35 years ago as carriers for vaccines and cancer chemotherapeutics. They are developed from non-biodegradable and biodegradable polymers. Their small sizes enable them to penetrate capillaries and to be taken up by cells, thereby increasing the accumulation of drugs at target sites. Drugs are incorporated into nanoparticles by dissolution, entrapment, adsorption, attachment or by encapsulation, and the nanoparticles provide sustained release of the drugs for longer periods, e.g., days and weeks. Nanoparticles enhance immunization by prevention of degradation of the vaccine and increased uptake by immune cells. One of the determinants of the extent of uptake by immune cells is the type of polymer employed. In a study<sup>4</sup> comparing Poly (caprolactone) (PCL), poly (lactide-coglycolide) (PLGA) and their blend, PCL nanoparticles were the most efficiently taken up by immune cells due to their hydrophobicity. However, all polymeric nanoparticles elicited vaccine (diphtheria toxoid) specific serum IgG antibody response significantly higher than free diphtheria toxoid. To target drugs to site of action, the drug can be conjugated to a tissue or cell specific ligand or coupled to macromolecules that reach the target organs. To target an anticancer agent to the liver, polymeric conjugate nanoparticles which comprised biotin and diamine terminated poly (ethylene glycol) with a galactose moiety from lactobionic acid were prepared.

Some other applications of nanoparticles include possible recognition of vascular endothelial dysfunction; oral delivery of insulin; brain drug targeting for neurodegenerative disorders such as Alzheimer's disease; topical administration to enhance penetration and distribution in and across the skin barrier; and pH-sensitive nanoparticles to improve oral bioavailability of drugs such as cyclosporine A. Some polymers used in the fabrication of nanoparticles include chitosan, alginate, albumin, gelatin, polyacrylates, polycaprolactones, poly (D, L-lactide-co-glycolide) and poly (D, L-lactide). However, there are concerns about polymeric nanoparticles including cytotoxicity of by-products (although some, such as polyanhydrides, degrade into products that are biocompatible) and scalability<sup>4</sup>.

## Applications<sup>5</sup>

### Corticoids release

Corticoids are anti-inflammatory drugs with high efficiency in the treatment of posterior segment eye diseases such as uveitis. It has also been proved that corticoids can improve the wound healing and they may be effective in the case of fibrosis (proliferative vitreoretinopathy and sub retinal neovascularization). Systemic administration of corticoids determines a series of side effects, topic administration being preferred. In

the eventuality of topic administration, only a small amount of the drug reaches the posterior segment of the eye. Direct injections in the vitreous can increase the therapeutic efficiency but usually repeated injections are required, generating a great discomfort for the patient. Some risks are also associated with this technique, such as vitreous hemorrhage or retinal detachment. Some local toxic effects have been also observed.

Another modality to insure a therapeutic concentration of the drug is to use drug releasing implants. There are also disadvantages associated with this technique: a large surgical incision is required to install the implant; the implant is very difficult to remove and it exists the possibility that the implant would migrate, endomaging the epithelium. An alternative to these rather complicated methods is to use corticoids loaded nanoparticles. Some of the most promising polymers are PLGA due to their very low toxicity. Gomez presented the synthesis of dexamethasone loaded PLGA nanoparticles. Dexamethasone is a poorly soluble crystalline corticoid generally used in the treatment of diabetic macular edema (as an implantable device).

### Anticancer therapies

In the anticancer therapy, one of the worst problems is the low tumors answer to treatment, because of the nonspecific bioavailability of the administered anticancer agent. By using nanoparticles it is possible to achieve the bioaccumulation of the drug in the target tissue. In the most cases, the EPR (enhanced permeation and retention) effect is responsible for the accumulation of drug in the tumors tissue. Hapca synthesized PLA nanoparticles on which they have grafted monoclonal antibodies with a high specificity for the treatment of ovarian cancer and lymphomas. A current application of nanoparticles carriers is the controlled release of heme oxygenase (HO- 1) inhibitors. HO-1 is an enzyme involved in the oxidation of heme, by cleavage of the porphyrin ring leading to formation of biliverdin, carbon monoxide and free iron. Biliverdin is subsequently hydrogenated by the cytosolic enzyme biliverdin reductase to form bilirubin (a potent antioxidant). Although high levels of bilirubin in the blood can cause toxic effects to central nervous system, in a normal quantity, the bilirubin protects the human cells against oxidation. Tumors cells can also use HO-1 to protect themselves from oxidative processes. Especially the renal and prostatic tumors are characterized by a high concentration of HO-1. These observations led to the hypothesis that the administration of a heme inhibitor could increase the tumor's sensibility to oxidative processes.

The oxidative processes can then be easily increased by certain usual drugs: cisplatin, anthracyclin, camptothecin etc. One of the most interesting inhibitor of HO-1 is the zinc protoporphyrin (ZnPP). Apart from inhibiting HO, ZnPP also induces cell death by a secondary mechanism. The protoporphyrin derivates are also known to be efficient photosensitizers. These derivatives absorb light



in the UV- visible region and being excited to a long-lived triplet state can interact with molecular oxygen, which on becoming singlet oxygen exerts cytotoxic effect. The use of ZnPP is limited by its poor solubility in water. Macromolecular porphyrin compounds were also synthesized but the molecular weight was not high enough to allow the tumor targeting.

Other drugs that have been successfully used in the treatment of cancer are paclitaxel and doxorubicin. In the case of paclitaxel, after the oral administration, the bioavailability is as low as 1%. Paclitaxel is eliminated from bloodstream at the first hepatic passage, by cytochrome P-450. By synthesizing small enough nanoparticles, it is possible to attach and maintain the nanoparticle paclitaxel complex to the intestinal mucosa until the complete absorption of paclitaxel to portal vein. The particles dimensions have an important role in their capacity to cross through biological membranes. Several studies confirmed that particles with a diameter of 5000 nm can reach the lymphatic circulatory system. Particles with a diameter lower than 500 nm can cross the epithelium membrane by endocytosis and nanoparticles with diameter lower than 50 nm can reach the interstitial spaces. These observations allowed the development of novel pharmaceutical formulations for oral administration of anticancer drugs.

#### **Blood-brain barrier**

It is known that between the blood streamline and the central nervous system there is a barrier known as the blood-brain barrier (BBB). BBB allows only the exchange of ions in order to maintain a constant osmotic pressure and the passage of nutrients. Its role is to protect the brain and the spinal axis from any chemical or bacteriological threats. The protection offered by the BBB comes at a certain price: it is impossible to get drugs through the barrier, so the therapy for the central nervous system is very difficult. In the brain, endothelial cells are packed more tightly together, due to the existence of tight zonulae occludentes junctions between them. The blood brain barrier recognizes therapeutic agents as foreign particles and doesn't allow their passage. Because of the blood-brain barrier, finding a way to deliver bioactive substances to brain has become a real challenge. One of the methods to achieve drug delivery to the central nervous system is to entrap the drugs into nanoparticles. Because of their reduced size, nanoparticles are able to pass through the vascular endothelium of BBB. There are several studies that showed good result in the treatment of brain tumors by drug loaded nanoparticles.

#### **Vaccines and gene therapy**

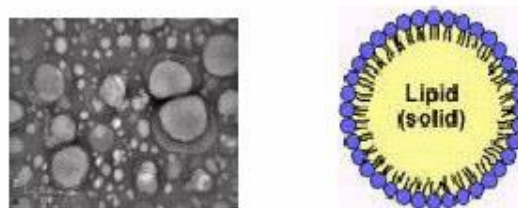
Another field where nanoparticles are very important is gene delivery. By encapsulating the genes into nanoparticles it is possible to protect them from degradation in the presence of certain factors (pH, bile, proteolytic enzymes). The entrapment of genes into

nanoparticles has encountered some problems regarding the stability of the synthesized structures during the preparation as well as after administration. One method to ensure the stability is to bind the genes to the surface of nanoparticles or Nano capsules. The binding must be reversible in order to allow the cleavage of the complex once the target has been reached. In order to improve the biocompatibility of the product it is better to avoid the use of surfactants. A method to obtain nanoparticles without using a surfactant was proposed by Castadello who obtained nanoparticles able to bind DNA without using a surfactant. The nanoparticles have a PMMA core and a shell of PEG and positively charged groups. The PEG chains are both biocompatible and biodegradable and provide steric stability while the positively charged groups bind DNA. By using these complex structures, the risk of physical desorption is greatly decreased. Such a vaccine is also stable and non-toxic while it is possible to administer it orally.

Gene therapy is a potential method for treating neurodegenerative diseases such as Parkinson. The controlled delivery of genes responsible for GDNF (Glial Cell Line-Derived Neurotrophic Factor) formation stops the disease evolution and maintains a constant level of dopamine despite cells lost because of the disease.

#### **Diagnostic**

Sun presented the modality to obtain copper chlorophyll labeled nanoparticles. These nanoparticles can be directly traced *in vivo* by analytical electron microscopy (AEM). Nanoparticles covered with Polisorbate T-80 have been detected in the brain, what proves the existence of endocytosis and/or transcytosis at some extent. Therefore this type of nanoparticles can be used in the functional exploration of the brain.



**Figure 2:** Solid Lipid Nanoparticles

#### **Solid Lipid Nanoparticle (SLN)**

Colloidal particles ranging in size between 10 and 1000 nm are known as nanoparticles. They are manufactured from synthetic/natural polymers and ideally suited to optimize drug delivery and reduce toxicity. Over the years, they have emerged as a variable substitute to liposomes as drug carriers. The successful implementation of nanoparticles for drug delivery depends on their ability to penetrate through several anatomical barriers, sustained release of their contents and their stability in the nanometer size. However, the scarcity of safe polymers with regulatory approval and



their high cost have limited the wide spread application of nanoparticles to clinical medicine.

To overcome these limitations of polymeric nanoparticles, lipids have been put forward as an alternative carrier, particularly for lipophilic pharmaceuticals. These lipid nanoparticles are known as solid lipid nanoparticles (SLNs), which are attracting wide attention of formulators world-wide. SLNs are colloidal carriers developed in the last decade as an alternative system to the existing traditional carriers (emulsions, liposomes and polymeric nanoparticles). They are a 3<sup>rd</sup> generation of submicron sized lipid emulsions where the liquid lipid (oil) has been substituted by a solid lipid. SLN offer unique properties such as small size, large surface area, high drug loading and the interaction of phases at the interfaces, and are attractive for their potential to improve performance of pharmaceuticals, nutraceuticals and other materials. SLNs are attracting major attention as novel colloidal drug carrier for intravenous applications. The SLNs are submicron colloidal carrier which is composed of physiological lipid, dispersed in water or in an aqueous surfactant solution. So if systematically investigated, SLNs may open new vista in research and therapy<sup>6</sup>.

#### **Advantages and problems of SLNS and other nanoparticles**

SLNs combine the advantages and avoid the drawbacks of several colloidal carriers of its class. Potential disadvantages such as poor drug loading capacity, drug expulsion after polymeric transition during storage and relatively high water content of the dispersions (70-99.9%) have been observed. The drug loading capacity of conventional SLN is limited by the solubility of drug in the lipid melt, the structure of the lipid matrix and the polymeric state of the lipid matrix. If the lipid matrix consists of especially similar molecules (i.e. tristearin or tripalmitin), a perfect crystal with few imperfections is formed. Since incorporated drugs are located between fatty acid chains, between the lipid layers and also in crystal imperfections, a highly ordered crystal lattice cannot accommodate large amounts of drug. Therefore the use of more complex lipids is more sensible for higher drug loading<sup>6</sup>.

#### **Applications<sup>7</sup>**

##### **SLN for Parenteral Application**

Wissing intensively reviewed parenteral use of SLN. SLN are very suitable for systemic delivery because they consist of physiologically well-tolerated ingredients and they have good storage capabilities after lyophilization and/or sterilization. When injected intravenously, SLN are sufficiently small to circulate in the micro vascular system and prevent macrophage uptake in case of hydrophilic coating. Therefore, SLN have been suggested for viral and non-viral gene delivery. Cationic SLN has been demonstrated to bind genes directly via electrostatic interactions, and have potential benefits in targeted gene

therapy in treatment of cancer. The charge of particles can also be modulated via the composition, thus allowing binding of oppositely charged molecules (Olbrich; Tabatt; Pedersen). Treatment of central nervous system diseases such as brain tumors, AIDS, neurological and psychiatric disorders is often constrained by the inability of potent drugs to pass blood brain barrier (BBB). Hydrophilic coating of colloids improves the transport of these through BBB and tissue distribution (Kreuter 2001; Wang). Fundaro prepared doxorubicin loaded stealth and non-stealth SLN and observed that the stealth nanoparticles were present in blood at higher concentrations than non-stealth SLN after 24h following intravenous administration.

##### **SLN for Nasal Application**

Nasal administration was a promising alternative noninvasive route of drug administration due to fast absorption and rapid onset of drug action, avoiding degradation of labile drugs (such as peptides and proteins) in the GI tract and insufficient transport across epithelial cell layers (Lee). In order to improve drug absorption through the nasal mucosa, approaches such as formulation development and prodrug derivatization have been employed. SLN has been proposed as alternative Tran's mucosal delivery systems of macromolecular therapeutic agents and diagnostics by various research groups (Muller and Keck; prego). In a recent report, coating polymeric nanoparticles with PEG gave promising results as vaccine carriers (Vila). The role of PEG coating of polylactic acid nanoparticles in improving the Trans mucosal transport of the encapsulated bioactive molecule reported to be successful by Tobio. This concept can be useful for solid lipid nanoparticles.

##### **SLN for Respiratory Application**

The lungs offer a high surface area for drug absorption by avoiding first-pass effects. Rapid drug absorption by aerosolization of drugs (in the 1-3  $\mu\text{m}$  size range) occurs since the walls of alveoli in the deep lung are extremely thin (Agu; Banga). Lymphatic drainage plays an important role in the uptake of particulates in the respiratory system. SLN can be proposed as carriers of anti-cancer drugs in lung cancer treatment or peptide drugs to improve their bioavailability. Assessment of inhaled radio-labeled SLN bio distribution has been described and the data showed an important and significant uptake of the radio-labeled SLN into the lymphatic after inhalation (Videira). In a recent study, ant tubercular drugs (rifampicin, isoniazid and pyrazinamide) were incorporated into various formulations of solid lipid particles ranged from 1.1 to 2.1  $\mu\text{m}$  and formulations were nebulized to guinea pigs by mouth for direct pulmonary delivery (Pandey 2005a and 2005b). Nebulization of solid lipid particles carrying ant tubercular drugs was observed to be successful in improving drug bioavailability and reducing the dosing frequency for better management of pulmonary tuberculosis.



### **SLN for Ocular Application**

Ocular drug administration via SLN has been reported several times (Friedrich). Bio-compatibility and mucoadhesive properties of SLN improve their interaction with ocular mucosa and prolong corneal residence time of the drug, with the aim of ocular drug targeting. Cavalli evaluated SLN as carriers for ocular delivery of tobramycin in rabbit eyes. As a result SLN significantly enhanced the drug bioavailability in the aqueous humor. Cavalli also studied pilocarpine delivery via SLN, which is commonly used in glaucoma treatment, earlier. They reported very similar results in order to enhance the ocular bioavailability of drug.

### **SLN for Rectal Application**

A few reports are available on the rectal drug administration via SLN in the literature (Sznitowska). Sznitowska incorporated diazepam into SLN for rectal administration in order to provide a rapid action. They applied SLN dispersions on rabbits and performed bioavailability studies. They found that lipid matrix which is solid at body temperature is not an advantageous system for diazepam rectal delivery. They decided to employ lipids which melt around body temperature in their next experiments. This area seems very open to investigation, especially when the benefits of rectal route are taken into consideration. PEG coating seems to be a promising approach on rectal delivery and consequently, enhancement of bioavailability.

### **SLN in Cancer chemotherapy**

From the last two decades several chemotherapeutic agents have been encapsulated in SLN and their *in-vitro* and *in-vivo* efficacy have been evaluated. Tamoxifen, anticancer drugs have been incorporated in SLN to prolong the release of drug following i.v. administration in breast cancer (Murthy, 2005). Tumor targeting has been achieved with SLN loaded with drugs like methotrexate and camptothecin. Metoxantrone SLN local injections were formulated to reduce the toxicity and improve the safety and bio efficacy of the drug in treating breast cancer and lymph node metastases (Wong).

### **Oral SLN in anti-tubercular chemotherapy**

Antitubercular drugs such as rifampin, isoniazid, and pyrazinamide-loaded SLN systems were able to reduce the dosing frequency and improve patient compliance. Antitubercular drugs loaded SLNs were prepared using solvent diffusion technique (Pandey).

### **Nano suspension and Nano crystals**

Poorly water soluble drugs pose a great challenge in drug formulation development. The low saturated solubility and dissolution velocity lead to poor bioavailability. With the increasing number of newly developed lipophilic drug compounds, many techniques have been proposed, such as solid dispersions, co-solvents, emulsions, liposomes and nanoparticles based on lipidic or polymer carriers.

However, the use of large amounts of excipients or organic solvents is limited in pharmaceutical formulations due to possible toxicity of the compounds. A pharmaceutical Nano suspension is defined as very finely dispersed solid drug particles in an aqueous vehicle for either oral or topical use or parenteral and pulmonary administration. The particle size distribution of the solid particles in Nano suspensions is usually less than one micron with an average particle size ranging between 200 and 600 nm Nano suspension is a sub-micron colloidal dispersion of drug particles which are stabilized by surfactants, polymers or a mixture of both.

Hydrophobic drugs such as naproxen, bupravaquone, nimesulide, amphotericin B, omeprazole, nifedipine are formulated as Nano suspension. The stability of the particles obtained in the Nano suspension is attributed to their uniform particle size which is created by various manufacturing processes. The absence of particles with large differences in their size in Nano suspensions prevents the existence of different saturation solubility's and concentration gradients, consequently preventing the Ostwald ripening effect.

### **Applications<sup>9</sup>**

#### **Oral drug delivery**

The oral route is the preferred route for drug delivery because of its numerous well-known advantages. The efficacy or performance of the orally administered drug generally depends on its solubility and absorption through the gastrointestinal tract. Hence, a drug candidate that exhibits poor aqueous solubility and/or dissolution-rate limited absorption is believed to possess low and/or highly variable oral bioavailability. Owing to low oral bioavailability, such a drug candidate would have to be administered in a larger excess than actually required if it were completely bioavailable in order to achieve a therapeutically active concentration, thus making the therapy costly. Orally administered antibiotics such as atovaquone and bupravaquone reflect this problem very well. Nano sizing of such drugs can lead to a dramatic increase in their oral absorption and subsequently bioavailability. The amelioration in oral bioavailability can be attributed to the adhesiveness of the drug Nano suspension, increased surface area (due to reduction in particle size by 10–50-fold), and increased saturation solubility, leading to an increased concentration gradient between the gastrointestinal tract lumen and blood, and increased dissolution velocity. This enhancement in bioavailability will lead to a subsequent reduction in drug dose, rendering the therapy cost-effective and obliterating any undue drug dumping in the body.

Some milestones Atovaquone, an antibiotic indicated for treating opportunistic *Pneumocystis carinii* infections in HIV patients, non-complicated *P. falciparum* malaria and leishmanial infections (Looareesuwan), shows poor bioavailability (10–15%) because of its dissolution- rate



limited absorption and has to be administered in high doses (750 mg twice daily). Administration of atovaquone as a Nano suspension resulted in a 2.5-fold increase in oral bioavailability as compared to the commercial product Wellvone, which contains the micronized drug (Schoeller). Danazol, a poorly bioavailable gonadotropin inhibitor, showed a drastic improvement in bioavailability when administered as a Nano suspension as compared to the commercial danazol macro suspension Danocrine (Liversidge & Cundy 1995). Danazol Nano suspension led to an absolute bioavailability of 82.3%, whereas the marketed danazol suspension Danocrine was 5.2% bioavailable.

In addition, danazol Nano suspension resulted in a reduction in the inter subject variability and fed/fasted ratio of danazol. Amphotericin B, a highly effective polyene antibiotic used for systemic mycoses and leishmaniasis lacks oral bioavailability. However, oral administration of amphotericin B as a Nano suspension produced a substantial improvement in its oral absorption in comparison to orally administered conventional commercial formulations such as Fungizone, AmBisome and micronized amphotericin B (Kayser). Orally administered amphotericin B Nano suspension brought about a high uptake of Nano particulate drug through the gastrointestinal tract. This is reflected by the considerable reduction it brought about in the number of *L. donovani* parasites in the liver of infected female Balb/c mice as compared to other commercial formulations.

#### **Parenteral drug delivery**

The parenteral route is an invasive route. Parenteral administration of drugs is critical and often associated with the problems such as the limited number of acceptable excipients, restrictions on the quantities of excipients approved for parenteral use, the stringent requirements of the aseptic production process, safety issues, patient noncompliance and biological problems such as allergic reactions and thrombophlebitis. Despite all these limitations, the parenteral route still retains its value because of its special advantages, such as quick onset of action in case of emergency, reduction in dose of the drug and the ability to target the drug quickly to the desired site of action, especially in the case of severe infections. The parenteral route is often employed as an alternative when the drug is either not absorbed through the gastrointestinal tract or undergoes extensive first-pass metabolism. For administration by the parenteral route, the drug either has to be solubilized or have particle/globule size below 5 $\mu$ m to avoid the capillary blockade.

The current approaches for parenteral delivery include salt formation, solubilization using co-solvents, micellar solutions (Kim), complexation with cyclodextrins and recently liposomes (Dupont 2002). However, there are limitations on the use of these approaches because of limitations on their solubilization capacity and parenteral acceptability. In this regard, liposomes are much more

tolerable and versatile in terms of parenteral delivery. However, they often suffer from problems such as physical instability, high manufacturing cost and difficulties in scale-up. Nano suspensions appear to be a unique approach to solving the problems mentioned above. From the formulation perspective, Nano suspensions meet almost all the requirements of an ideal drug delivery system for the parenteral route. Since the drug particles are directly Nano sized, it becomes easy to process almost all drugs for parenteral administration. Moreover, the absence of any harsh solvents/co-solvents and/or any potentially toxic ingredient in Nano suspensions enables them to bypass the limitations of parenteral administration attributed to conventional formulations strategies. Hence, Nano suspensions enable significant improvement in the parentally tolerable dose of the drug, leading to a reduction in the cost of the therapy and also improved therapeutic performance.

The maximum tolerable dose of paclitaxel Nano suspension was found to be three times higher than the currently marketed Taxol, which uses Cremophore EL and ethanol to solubilize the drug. In the MV-522 human lung xenograft murine tumors model, paclitaxel Nano suspensions at doses of 90 and 100mg kg<sup>-1</sup> showed no cases of death (n/9) whereas Taxol at a concentration of 30mg kg<sup>-1</sup> showed a 22% death rate (Merisko-Liversidge). Similarly, the Nano suspensions of other anticancer agents, such as etoposide and camptothecin, revealed an improvement in the tolerance level of the drug compared to the marketed preparations. In addition, Nano suspensions have been found to increase the efficacy of the parenterally administered drug (Merisko-Liversidge). A comparison of the efficacy of paclitaxel Nano suspension with Taxol using the mammary 16-C murine tumors model (n/5 for each experimental group) revealed the superiority of paclitaxel Nano suspension over Taxol in reducing the median tumors burden (Merisko-Liversidge). Similarly, aphidicolin, a poorly water-soluble new anti-parasitic lead molecule, when administered as Nano suspension revealed an improvement in EC<sub>50</sub> from 200 to 40 ngmL<sup>-1</sup> in comparison to DMSO-dissolved drug (Kayser 2000).

Recently, clofazimine, a poorly water-soluble anti leprotic drug, has been successfully formulated as a Nano suspension. Nano suspensions can be administered via different parenteral routes, ranging from intra-articular to intraperitoneal to intravenous injection. Currently, studies are in progress to identify strategies for manipulating the surface properties, size and shape of drug Nano suspensions in order to eliminate sequestration by the phagocytic cells of MPS-enriched organs whenever desired.

#### **Ocular drug delivery**

Nano suspensions can prove to be a boon for drugs that exhibit poor solubility in lachrymal fluids. For delivery of such drugs, approaches such as suspensions and ointments have been recommended. Although



suspensions offer advantages such as prolonged residence time in a cul-desac (which is desirable for most ocular diseases for effective treatment) and avoidance of the high tonicity created by water soluble drugs, their actual performance depends on the intrinsic solubility of the drug in lachrymal fluids. Thus, the intrinsic dissolution rate of the drug in lachrymal fluid governs its release and ocular bioavailability. However, the intrinsic dissolution rate of the drug will vary because of the constant inflow and outflow of lachrymal fluids. Hence, suspensions may fail to give consistent performance. However, Nano suspensions, by their inherent ability to improve the saturation solubility of the drug, represent an ideal approach for ocular delivery of hydrophobic drugs. Moreover, the Nano particulate nature of the drug allows its prolonged residence in the cul-de-sac, giving sustained release of the drug.

To achieve sustained release of the drug for a stipulated time period, Nano suspensions can be incorporated in a suitable hydrogel base or mucoadhesive base or even in ocular inserts. An approach that has recently been investigated to achieve the desired duration of action of the drug is the formulation of polymeric Nano suspensions loaded with the drug. The bio erodible as well as water-soluble/ permeable polymers possessing ocular tolerability (Pignatello) could be used to sustain the release of the medication. The Nano suspensions can be formulated using the quasi-emulsion and solvent diffusion method. The polymeric Nano suspensions of flurbiprofen and ibuprofen have been successfully formulated using acrylate polymers such as Eudragit RS 100 and Eudragit RL 100 (Bucolo; Pignatello). The polymeric Nano suspensions have been characterized for drug loading, particle size, zeta potential, *in-vitro* drug release, ocular tolerability and *in-vivo* biological performance. The designed polymeric Nano suspensions revealed superior *in-vivo* performance over the existing marketed formulations and could sustain drug release for 24 h. The scope of this strategy could be extended by using various polymers with ocular tolerability.

### Targeted drug delivery

The need to target drugs to specific sites is increasing day by day as a result of therapeutic and economic factors. Nano particulate systems have shown tremendous potential in targeted drug delivery, especially to the brain (Schroeder; Kreuter 2001). Successful targeting of the peptide dalargin to the brain by employing surface modified polyisobutyl cyanoacrylate nanoparticles has been a major achievement in targeted delivery (Kreuter). Likewise, Nano suspensions can be used for targeted delivery as their surface properties and *in-vivo* behavior can easily be altered by changing either the stabilizer or the milieu. Their versatility and ease of scale-up and commercial production enables the development of commercially viable Nano suspensions for targeted delivery. Natural targeting of MPS by Nano suspensions has already been described. However, the natural

targeting process could pose an obstacle when macrophages are not the desired targets. Hence, in order to bypass the phagocytic uptake of the drug, its surface properties need to be altered, as in the case of stealth liposomes (Gregoriades 1995; Allen 1997; Lasic; Papisov 1998; Woodle 1998; Vyas). The engineering of stealth Nano suspensions (analogous to stealth liposomes) by using various surface coatings for active or passive targeting of the desired site is the future of targeted drug delivery systems. Another good example is targeting of *Cryptosporidium parvum*, the organism responsible for cryptosporidiosis, by using surface-modified mucoadhesive Nano suspensions of bupravaquone (Kayser 2001; Müller & Jacobs 2002a).

A considerable difference has been observed in the efficacy of bupravaquone Nano suspensions when delivered with and without mucoadhesive polymers (Kayser 2001; Müller & Jacobs 2002a). Mucoadhesive bupravaquone Nano suspensions, because of their prolonged residence at the infection site, revealed a 10-fold reduction in the infectivity score of *Cryptosporidium parvum* as compared to the Bupravaquone Nano suspensions without mucoadhesive polymers. Similarly, conditions such as pulmonary aspergillosis can easily be targeted by using suitable drug candidates, such as amphotericin B, in the form of pulmonary Nano suspensions instead of using stealth liposomes (Kohno). With the advent of polymeric Nano suspensions loaded with drug (as described in the section on ocular delivery), it should be possible to target sites, such as the colon, or bacteria, such as *H. pylori*, by suitable modifications in the formulation strategy. Overall, Nano suspensions have indicated a good potential in targeted drug delivery but this has yet to be fulfilled.

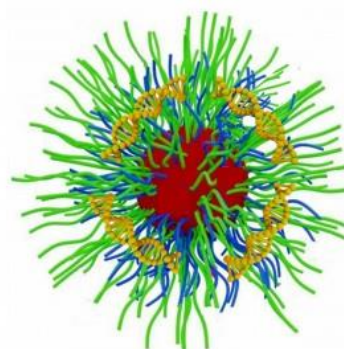


Figure 3: Polymeric Micelles

### Polymeric Micelles

Despite the great amount of scientific data amassed on tuberculosis (TB), an infection that has afflicted the human race for thousands of years, there is still no victory in sight over the disease, which today represents a severe problem for public health in many parts of the world, especially in the poorer countries. With the constantly growing number of multidrug resistant strains and the spread of AIDS, this destroys patients' immunity, leaving them much more susceptible to mycobacterial infection;



the rates of incidence and prevalence of TB have been rising and could threaten the socioeconomic development of affected countries, in view of the fact that both TB and AIDS affect, in the main, people in their most productive years, aged 15-49. While it is true that very useful tuberculostatic products exist, both for treatment and prophylaxis, the known toxic side-effects of these drugs, the infection of patients by the *M. avium* complex and their lack of adherence to the course of treatment, are all factors that make the ongoing search for improved drugs absolutely indispensable.

As a rule, new drugs are introduced by modifying molecules with known characteristics, often with the help of computer-aided design techniques. Synthetic routes that are designed to begin with a known drug and make planned improvements in some of its properties, particularly in its pharmacokinetics (as is the case in delayed action prodrugs) are often used. Prodrugs are chemicals with little or no pharmacological activity, undergoing biotransformation to a therapeutically active metabolite. In the prodrug approach, the physicochemical properties of the parent drug are transiently modified with a promoiety or carrier<sup>4</sup>. The objectives of a prodrug strategy are: to solve problems resulting from poor solubility, insufficient chemical stability or poor organoleptic properties; reduced systemic toxicity; improved oral bioavailability, by improving the oral absorption of the drug and/or by decreasing its presystemic metabolism.

Other objectives are to improve absorption, lengthen the duration of action of the drug by slow metabolic release, and finally achieve the organ/tissue-selective delivery of an active agent. Even though great advances have been made in polymeric drugs, the practice of conjugating hydrophobic drug moieties in dense clusters along the polymer chain of a carrier molecule and, not infrequently, the low water solubility of the carrier itself, can easily result in the precipitation of the polymer derivative, and often does. To overcome this problem, a promising technique is to make polymer drugs that take the form of micelles. In line with this approach, micelle-forming polymer derivatives of the initial-phase anti-tuberculous drugs pyrazinamide, isoniazid and rifampin were synthesized in this study. These derivatives were characterized by determining the critical micelle concentration (CMC) and the mean diameter of the micelles, and then tested for activity against several mycobacterial strains, such as virulent *M. tuberculosis* and *M. avium*<sup>10</sup>.

### Application<sup>11</sup>

#### Solubilization

The micellar core is a compatible micro environment and a hub for incorporating water-insoluble guest molecules. The hydrophobic molecules can be covalently coupled to the block copolymers or physically incorporated into the hydrophobic core of micelles. The solubilization process

leads to enhancement of their water solubility and there by bioavailability. It is often observed that the gastrointestinal (GI) uptake of particles is affected significantly by particle size. A 15 to 250-fold higher uptake efficiency of particles approximately 100 nm in diameter by the GI tract was noted than that of the micrometer-sized particles. Thus, polymeric micelles (Nano sized) elevate uptake and enhance bioavailability. The extent of solubilization depends upon the micellization process, the compatibility between the drug and the core forming block, chain length of the hydrophobic block, concentration of polymer, and temperature. Above CMC, there is a sharp increase in the solubility of drug as it gets more space to occupy in the aggregates of the hydrophobic part of the micelle.

The occupancy of the core region by drug leads to an increased RC of the micelle. It is worth mentioning that the core region has limited capacity for accommodation, for instance, Pluronic P85 has a core region which is 13% of the whole micelle weight. The influence on solubilization capacity of hydrophobic block length has been examined for griseofulvin in polyoxyethylene and polyoxybutylene copolymer micelles with varying number of hydrophobic block lengths and hydrophilic block lengths sufficient for formation of spherical micelles. It was found that the solubilization capacity was dependent on the hydrophobic block length up to a certain extent (15 units of hydrophobic block), after which the solubilization capacity became independent of the same. Dong and coworkers also studied the effect of hydrophobic block length on solubilization of toluene in di block and tri block polyurethane surfactants. It was concluded that solubilization capacity of polyurethane surfactants increased with an increase in the hydrophobic segment for the same block chain structure.

#### Targeting

Targeting via polymeric micelles is usually achieved by one of the following approaches; the enhanced permeability and retention effect, stimuli sensitivity, complexing specific targeting ligand molecules to the micelle surface, or by coupling monoclonal antibodies to the micelle corona, i.e. active targeting using immunomicelles.

#### Enhanced Permeability and Retention Effect (EPR Effect)

Owing to their nanoscopic size, polymeric micelles passively accumulate at the interstitial spaces of various pathological sites by extravasating leaky capillaries (especially of solid tumors). They also have been shown to distribute to some of the cytoplasmic organelles, and infarct tissues, infected areas, inflammatory sites that have compromised barrier function. As the polymeric micellar drug carriers cannot pass through walls of normal blood vessels, decreased side effects of the drug are observed. In tumor neovasculature, there is a poorly developed lymphatic drainage system that leads to enhanced retention of polymeric micelles within the solid



tumor as micelles are not efficiently cleared. This feature allows prolonged circulation of polymeric micelles in the circulatory system upon administration. Due to these characteristics, it is possible to achieve passive drug targeting using polymeric micelles. The hyper permeability of tumors associated with the EPR effect is based on excessive production and secretion of vascular permeability factors stimulating extravasation within cancerous tissue.

Commonly secreted chemicals are vascular endothelial growth factor bradykinins, nitric oxide, prostaglandins, enzyme collagenase, and peroxyxynitrite. Vetvicka and his associates formulated a micellar drug delivery system designed to prolong the blood circulation time and maximize the efficiency of the EPR effect. They prepared doxorubicin conjugated poly (ethylene oxide) block-poly (allyl glycidyl ether) micellar system that circulated for long time and released doxorubicin efficiently at the tumor site because of the acidic pH prevailing at the tumor site. This also leads to destabilization and disruption of the micellar system generating free diblock unimers that could be excreted. Maitani developed polymeric micelles composed of various poly (ethylene glycol)- poly(aspartate ester) block copolymers incorporating camptothecin, a naturally occurring cytotoxic alkaloid. The micellar system solubilized the poorly water soluble drug and a stable formulation of camptothecin-loaded micelles was obtained. The stability of the formulation was found to strongly depend on the amount of benzyl esters and length of the PEG. The drug-loaded micelles were potentially delivered to tumor sites owing to the EPR effect.

### **Stimuli-Sensitivity**

For ideal drug targeting, there should not be any drug release from the micelle during circulation. The drug should be released only after the polymeric micelles accumulate at the targeted tissue, by means of some internal trigger such as pH, particular enzyme, etc. or by an external trigger including temperature, light, ultrasound or magnetic field. Depending on the stimulus applied varied responses may be observed including disruption of the structure, changes in shape, volume, permeation rates, hydration state, swelling/collapsing, hydrophilic/hydrophobic surface, or conformational changes. Destabilization of micelles as a result of stimulation by either physiological or external trigger is termed as 'stimuli-sensitivity' or 'environmental sensitivity' of the micelles. Release of drug from the micellar system is dependent on the exploitation of differences that exist in normal tissues and pathological tissues. Such a release mechanism from polymeric micelles is also termed as 'intelligent delivery' or 'smart delivery' by other researchers.

### **Acid-Sensitive Polymeric Micelles**

There are a number of pH gradients that exist in normal and pathophysiological states inside the body. Acid-

sensitive or pH-sensitive polymeric micelles exploit these differences in pH for drug targeting. In tumors and inflammatory tissues a mildly acidic pH is encountered (pH approx.6.8). This is a slightly low value as compared with the pH of blood and normal tissues (pH approx.7.4). Micelles can also be taken up into the cell by the process of endocytosis and may as well enter cell organelles as endosomes, lysosomes, etc. The pH value inside these organelles is nearly 5.5. This has served as the basis for the development of pH-sensitive polymeric micelles. e.g., negatively charged oligo/poly (nucleic acids) can be delivered intracellularly by complexing them with cationic polymers. Once into endosomes, these are deprotonated causing disruption of endosomal membrane and releasing nucleic acids in the cytosol.

Two main approaches that have been used for developing pH sensitive systems are: involvement of a titrable group into the copolymer, and inclusion of labile linkages that are destabilized in acidic conditions. Incorporation of titrable groups such as amines, carboxylic acids into the backbone of the copolymer leads to an alteration of the solubility of the polymer upon protonation. This in effect may disrupt the micellar structure. Inclusion of acid-labile linkages, such as benzoic imine linkage, in polymeric structures has shown to cause change in micellar integrity or complete destruction of the micellar structure when these polymers encounter low-pH environment.

### **Complexing Targeting Ligand Molecules to Micelles**

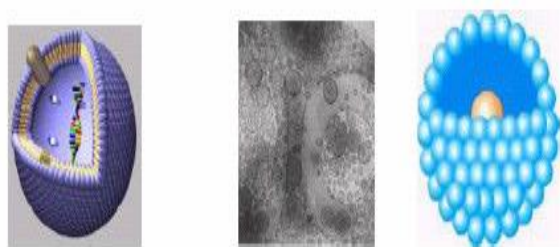
An impressive strategy to enhance cellular internalization of polymeric micelles at desired target tissue is attachment of cell-specific ligands on the surface of these Nano carriers.

Thus, covalent attachment of cell specific ligands, e.g. sugars, peptides, and monoclonal antibodies, on the surface of polymeric micelles has been pursued to enhance drug delivery to various cells. For tumor targeting, cancer-specific peptides are more appropriate as peptides can easily be derivatized and engineered to achieve better *in vivo* stability and tissue specificity. In this context, Lavasanifar conjugated an arginine-glycine-aspartic acid (RGD) containing peptide as a ligand that can recognize adhesion molecules overexpressed on the surface of metastatic cancer cells, to the surface of poly(ethylene oxide)-block- poly(caprolactone) micelles. It was found that micelles were good ligand-targeted carriers for enhanced drug delivery to metastatic tumor cells.

Torchilin used the overexpression of Peripheral Benzodiazepine Receptor (PBR) in certain cancers for 70 targeting such tissues. Selective ligands to the PBR may induce apoptosis and cell cycle arrest.

Thus, polyethylene glycol-phosphatidylethanolamine PBR-targeted micellar drug delivery system loaded with paclitaxel was prepared. They demonstrated the use of this system to reveal significantly enhanced toxicity against some cancerous cells.





**Figure 4:** Liposomes

### Liposomes

Liposomes were first produced in England in the 60's, by Bangham who was studying phospholipids and blood clotting. According to legend, he was experimenting with new laboratory equipment, and he made a noted observation about phospholipids forming closed multilamellar vesicle spontaneously in aqueous solution which took two years to be proved. The phospholipid reorganisation in aqueous solution is mainly driven by the hydrophobic effect which organizes amphiphilic molecules (phospholipids) so as to minimize entropically unfavorable interactions between hydrophobic acyl-chains and surrounding aqueous medium. This effect is further settled by various intermolecular forces such as electrostatic interactions, hydrogen bonding, as well as Vanderwaals and dispersion forces. Liposomes were defined as an artificial microscopic vesicle consisting of a central aqueous compartment surrounded by one or more concentric phospholipid layers (lamellas). Furthermore, hydrophilic (in the aqueous cavity), hydrophobic (within lipidic membrane) and amphiphilic substances are able to be incorporated within these vesicles developing large potential applications. Numerous researchers have worked with these structures since Bangham's discovery, making of liposomes the most popular Nano carrier systems. Liposomes can be classified in terms of composition and mechanism of intracellular delivery into five types

- (i) Conventional liposomes;
- (ii) pH-sensitive liposomes;
- (iii) Cationic liposomes;
- (iv) Immune liposomes and
- (v) Long-circulating liposomes<sup>12</sup>.

### Applications<sup>13</sup>

New drug delivery systems such as liposomes are developed when an existing formulation is not satisfactory and reformulation offers superior therapeutic efficacy and safety over the existing formulation. Indeed, liposome formulations of some drugs have shown a significant increase in therapeutic efficacy and/or therapeutic indices in preclinical models and in humans, compared to their non-liposomal formulations. The therapeutic applications of liposomes generally fall into several categories briefly described below.

### Formulation aid

Hydrophobic drugs such as cyclosporin and paclitaxel are usually formulated in surfactants and organic co-solvents for systemic administration in humans. These solubilizers may cause toxicity at the doses needed to deliver the drug. In contrast, liposomes are made up of lipids which are relatively non-toxic, non-immunogenic, biocompatible and biodegradable molecules, and can encapsulate a broad range of water-insoluble (lipophilic) drugs. Currently, liposomes or phospholipid mixtures are being used as excipients for preparing better-tolerated preclinical and clinical formulations of several lipophilic, poorly water-soluble drugs such as amphotericin B. In preclinical studies, liposomes have been evaluated as a vehicle for the delivery of paclitaxel and its analogs as an alternative to the cremophor/ethanol vehicle. Paclitaxel liposomes were able to deliver the drug systemically and increase the therapeutic index of paclitaxel in human ovarian tumor models.

### Intracellular drug delivery

Drugs with intracellular targets/receptors are required to cross the plasma membrane for pharmacological activity. Liposomes can be used to increase cytosolic delivery of certain drugs such as N-(phosphonacetyl)-L-aspartate (PALA) which is normally poorly taken up into cells. PALA is taken up into the tumor cells through fluid-phase endocytosis (pinocytosis) and it diffuses out into the cytoplasm as the endosome pH drops. However, pinocytosis is very limited in its efficiency. Liposomal delivery of drugs which normally enter the cells by pinocytosis can be very effective because liposomes can contain greater concentrations of drug compared to the extracellular fluid and the endocytosis process by which negatively charged liposomes are predominantly taken up by the cells, is more efficient than pinocytosis. For example, the potency of PALA encapsulated liposomes was up to 500-fold greater against human ovarian tumor cell lines than that of free PALA.

### Gene therapy

A number of systemic diseases are caused by lack of enzymes/factors which are due to missing or defective genes. In recent years, several attempts have been made to restore gene expression by delivery of the relevant exogenous DNA or genes to cells. Cationic liposomes have been considered as potential non-viral human gene delivery system. They are usually composed of a cationic lipid derivative and a neutral phospholipid (DOPE). The latter is required by certain cationic lipids to form stable liposomes. Some of the widely used cationic liposome formulations are: lipofectin (DOTMA: DOPE, 1:1); lipofectamine (DOSPA: DOPE, 3:1); transfectace (DDAB: DOPE, 1:3); cytofectin (DMRIE: DOPE); transfectam (DOGS) and DC-cholesterol. The negatively charged genetic material (e.g., plasmid) is not encapsulated in liposomes but complexed with cationic lipids by electrostatic interactions. Plasmid-liposome complexes

are thought to enter the cell by fusion with the plasma or endosome membrane. Allovectin-7, a gene transfer product is currently in clinical trials (phase I/II) as an immunotherapeutic agent for the treatment of metastatic melanoma, renal cell and colorectal carcinoma. Allovectin-7 is composed of a plasmid containing the gene for the major histocompatibility complex antigen HLA-B7 with fl-2 microglobulin formulated with the cytofectin (DMRIE: DOPE).

The on-going clinical trials have indicated that intralesional injection of Allovectin-7 can be performed safely and have demonstrated antitumor activity in some patients. Plasmid-liposome complexes have many advantages as gene transfer vehicles over viral-based vectors: (i) these complexes are relatively nonimmunogenic because they lack proteins; (ii) liposomes or lipid complexes can be used for transfection of large-sized genetic material; and (iii) viruses, unlike plasmid-liposome complexes, may replicate and cause infections. However, there are several problems limiting the application of liposomes as a gene delivery system.

#### Site-specific targeting

Site-specific delivery, the concept first proposed by Paul Ehrlich (Ehrlich, 1906) involves the delivery of a larger fraction of drug to the target site and therefore, reducing exposure to normal tissues. Liposomes have been employed for accomplishing both passive and active targeting of drugs.

#### Immunological adjuvants in vaccines

Liposomes can encapsulate antigens in their aqueous space or incorporate in the bilayer depending on the lipophilicity of the antigen. Liposomes were first used as immunological adjuvants in order to enhance the immune response to encapsulated diphtheria toxoid. Since then, liposomes have been used as nontoxic adjuvants with bacterial, viral, protozoan, tumor and other antigens. The tendency of liposomes to interact with macrophages in RES is exploited in this approach (passive targeting). The mechanism by which liposomes cause increases in antigens immune response is not fully understood. However, augmentation of liposomal adjuvant city can be achieved by co-administration of liposome encapsulated antigen with other adjuvants such as lipid A, lipopolysaccharides, muramyl dipeptide and interleukin (IL-2).

Furthermore, antibody-mediated targeting of liposomal to antigen-presenting cells may also improve immunostimulatory effects. The influence of physicochemical properties of the liposomes such as charge density, membrane fluidity and epitope density, on the immune response of the antigen has been extensively studied. For instance, liposome formulations of inactivated encephalomyocarditis and Semliki Forest viruses were significantly more immunogenic when charged phospholipids were used compared to neutral lipids. The phase transition temperature ( $T_o$ ) of the lipids

also appears to influence immunogenicity. For example, immunogenicity of haptens was higher in liposomes composed of lipids with a high  $T_c$  than in those with a low  $T_c$ . Recently, the first liposome-based vaccine (liposomes containing inactivated hepatitis A virions) was approved for human use in Switzerland and currently, several other liposome-based vaccines are in clinical trials.

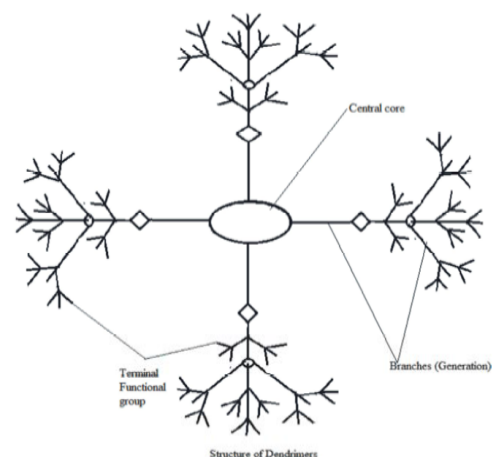


Figure 5: Dendrimers

#### Dendrimers

The discovery and creation of new drugs is timely and costly process. It is estimated that every new drug takes 12 to 15 years to develop, at a cost of over \$800 million. A more efficient approach would be the devising of effective drug delivery systems for already developed experimental drugs that failed to make it to the market. Controlled release systems can improve the effectiveness of drug delivery by sustained release of the compound over time or by release at a specific target. By controlling the time and location of delivery, side effects can be minimized and drug efficacy can be maximized, thus leading to a lower dosage for patients. Methods to achieve controlled release include chemical or enzymatic reaction, diffusion through a matrix, or solvent activation. Currently the two common drug delivery systems are liposomes and polymeric systems. These both have limited applications, as liposome-based systems have poor stability and difficulty targeting specific tissues and linear polymers are polydisperse.

Dendrimers offer advantages including a lower polydispersity index, multiple sites of attachment, and a controllable, well-defined size and structure that can be easily modified to change the chemical properties of the system. In addition, macromolecules such as dendrimers have an enhanced permeability and retention effect that allows them to target tumor cells more effectively than small molecules. Dendrimers have applications in gene and antisense therapy, magnetic resonance imaging, and in boron neutron capture therapy.

Advances in dendrimer delivery systems, biodegradable dendrimers, and release from dendrimers can be applied to drug delivery in addition to other applications<sup>14</sup>.



**Application<sup>15</sup>****Therapeutic Application**

- o Dendrimer in photodynamic therapy
- o Dendrimers for Boron Neutron capture therapy

**Diagnostic Application**

- o Dendrimers as MRI contrast agent
- o Dendrimers as X-Ray contrast agent
- o Dendrimer as molecular probe

**Pharmaceutical Application**

- o Dendrimers in pulmonary drug delivery
- o Dendrimers in Transdermal drug delivery
- o Dendrimers in ocular drug delivery
- o Dendrimers in oral drug delivery
- o Dendrimers for controlled release drug delivery
- o Dendrimers in targeted drug delivery
- o Dendrimers in gene delivery
- o Dendrimers as solubility enhancer
- o Cellular delivery using dendrimers carrier
- o Dendrimers based product in cosmetics
- o Dendrimers based commercial products

**Dendrimers in Gene Delivery**

Dendrimers can be used as a carrier in gene therapy. Example- PAMAM dendrimers have terminal amino groups which interact with phosphate group of nucleic acid. So PAMAM dendrimers have been tested as a genetic material vector. Super Fect™ is a transfection reagent, it consist of activated dendrimers. Activated dendrimers can carry a large amount of genetic material than viruses. Super Fect-DNA complex are highly stable and have high transfection efficiency. The high transfection efficiency is due to their well-defined shape and low pk of the amines.

**Dendrimers in Targeted and Controlled Release Drug Delivery**

The dendrimers facilitate the passive targeting of drug to solid tumors. This is due to their enhanced solubility and plasma circulation time. EPR (Enhanced Permeation and Retention) in tumors tissues leads to reduce cytotoxicity of anticancer drug and increased uptake by cancer cell lines. Example-Doxorubicin.

**Applications of Dendrimers in Waste Water Treatment**

Dendritic polymers are used in the purification of water contaminated by toxic metal ion, inorganic solute and organic solutes.

**Magnetic nanoparticles**

Magnetic nanoparticles offer some attractive possibilities in biomedicine. First, they have controllable sizes ranging from a few nanometers up to tens of nanometers, which places them at dimensions that are smaller than or comparable to those of a cell (10–100µm), a virus (20–450 nm), a protein (5–50 nm) or a gene (2 nm wide and 10–100 nm long). This means that they can ‘get close’ to a biological entity of interest. Indeed, they can be coated with biological molecules to make them interact with or bind to a biological entity, thereby providing a controllable means of ‘tagging’ or addressing it. Second, the nanoparticles are magnetic, which means that they obey Coulomb’s law, and can be manipulated by an external magnetic field gradient. This ‘action at a distance’, combined with the intrinsic penetrability of magnetic fields into human tissue, opens up many applications involving the transport and/or immobilization of magnetic nanoparticles, or of magnetically tagged biological entities. In this way they can be made to deliver a package, such as an anticancer drug, or a cohort of radionuclide atoms, to a targeted region of the body, such as a tumor. Third, the magnetic nanoparticles can be made to resonantly respond to a time-varying magnetic field, with advantageous results related to the transfer of energy from the exciting field to the nanoparticle.

For example, the particle can be made to heat up, which leads to their use as hyperthermia agents, delivering toxic amounts of thermal energy to targeted bodies such as tumor’s; or as chemotherapy and radiotherapy enhancement agents, where a moderate degree of tissue warming results in more effective malignant cell destruction. These, and many other potential applications, are made available in biomedicine as a result of the special physical properties of magnetic nanoparticles<sup>16</sup>.

**Nano shells coated with Gold**

Gold Nano shells are spherical particles with diameters typically ranging in size from 10 to 200nm. They are composed of a dielectric core covered by a thin gold shell. As novel nanostructures, they possess a remarkable set of optical, chemical and physical properties, which make them ideal candidates for enhancing cancer detection, cancer treatment, cellular imaging and medical bio sensing. Gold Nano shells are unique in that they combine many ideal features in a single particle. As a direct result of Nano scale resonance phenomena, gold Nano shells have very large optical absorption and scattering cross - sections, which render them highly suitable as contrast agents for imaging.

**Quantum dots**

A quantum dot is a semiconductor nanostructure that confines the motion of conduction band electrons, valence band holes, or excitons (bound pairs of conduction band electrons and valence band holes) in all



three spatial directions. The confinement can be due to electrostatic potentials (generated by external electrodes, doping strain, impurities), the presence of an interface between different semiconductor materials (e.g. in core-shell Nano crystal systems), the presence of the semiconductor surface (e.g. semiconductor Nano crystal), or a combination of these. Quantum dots are particularly significant for optical applications due to their theoretically high quantum yield. The ability to tune the size of quantum dots is advantageous for many applications and it is one of the most promising candidates for use in solid-state quantum computation and diagnosis, drug delivery, Tissue engineering, catalysis, filtration and also textiles technologies<sup>18</sup>.

### Ferro fluids

Ferro fluids (known also as magnetic fluids) are a special category of smart nanomaterials, In particular magnetically controllable Nano fluids. These types of Nano fluids are colloids of magnetic nanoparticles, such as Fe<sub>3</sub>O<sub>4</sub>,  $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>, CoFe<sub>2</sub>O<sub>4</sub>, Co, Fe or Fe-C, stably dispersed in a carrier liquid. Consequently, these nanomaterial's manifest simultaneously fluid and magnetic properties.

Macroscopically, the introduction of magnetic forces into the fundamental hydrodynamic equations for the quasihomogeneous magnetizable liquid medium gives rise to the magneto hydrodynamics of magnetic Nano fluids (Ferro fluids), known also as Ferro hydrodynamics and opens up an entire field of new phenomena and promising applications.

### CONCLUSION

Nanotechnology has been attracting significant attention from the media as well as government funding in recent years. Surveyed company representatives point to the strategic relevance of nanotechnology as an innovation driver and success factor in their enterprises. Today, there are estimates 305 Nano life science company's across the globe. The United states accounts for the majority of these company's (52%), followed by Germany (21%).

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