

PHARMACEUTICAL TECHNOLOGY

Pharmaceutical Nanotechnology

Dr. N.K. Jain
Professor of Pharmaceutics
Dept. of Pharmaceutical Science
Dr. Hari Singh Gour University
Sagar-470 003

(17-09-2007)

CONTENTS

[Introduction](#)

[Nano-Definitions](#)

[Opportunities and Scope](#)

[Pharmaceutical Nanotechnology based Systems](#)

[Characterization of Pharmaceutical Nanotools](#)

[Engineering of Pharmaceutical Nanosystems](#)

[Applications of Pharmaceutical Nanotools](#)

[Challenges to Pharmaceutical Nanotechnology](#)

[Future Prospects of Pharmaceutical Nanotechnology](#)

[Conclusion](#)

Introduction

Size reduction is a fundamental unit operation having important applications in pharmacy. It helps in improving solubility and **bioavailability**, reducing toxicity, enhancing release and providing better formulation opportunities for drugs. In most of the cases, size reduction is limited to micron size range, for example, various pharmaceutical dosage forms like powder, **emulsion**, **suspension** etc. Drugs in the nanometer size range enhance performance in a variety of dosage forms. Major advantages of nanosizing include (i) increased surface area, (ii) enhanced solubility, (iii) increased rate of **dissolution**, (iv) increased oral **bioavailability**, (v) more rapid onset of therapeutic action, (vi) less amount of dose required, (vii) decreased fed/fasted variability, and (viii) decreased patient-to-patient variability.

The word ‘nano’ is derived from Latin word, which means dwarf. Nano size refers to one thousand millionth of a particular unit thus nanometer is one thousand millionth of a metre (i.e. $1\text{nm} = 10^{-9}\text{ m}$).

Nanotechnology is the science that deals with the processes that occur at molecular level and of nanolength scale size. There are numerous examples from nature like DNA, water molecules, virus, red blood corpuscles (RBC) etc., which are of nanodimensions; even our history has numerous examples which prove that we have exploited the advantages of technology in one or other form. Figure 1 depicts various examples from nature and pharmaceuticals which are operated at various dimensions of nanolength scale. The term nanotechnology has been most commonly used in other fields of science like electronic, physics and engineering since many decades. Nanotechnology has shown tremendous progress in these fields. However, biomedical and pharmaceutical fields remain yet to be explored.

Nanotechnology is a multidisciplinary field, convergence of basic sciences and applied disciplines like biophysics, molecular biology, and bioengineering. It has created powerful impact in various fields of medicine including cardiology, ophthalmology, endocrinology, oncology, pulmonology, immunology etc., and to highly specialized areas like gene delivery, brain targeting, tumor targeting, and oral vaccine formulations. Nanotechnology provides intelligent systems, devices and materials for better pharmaceutical applications.

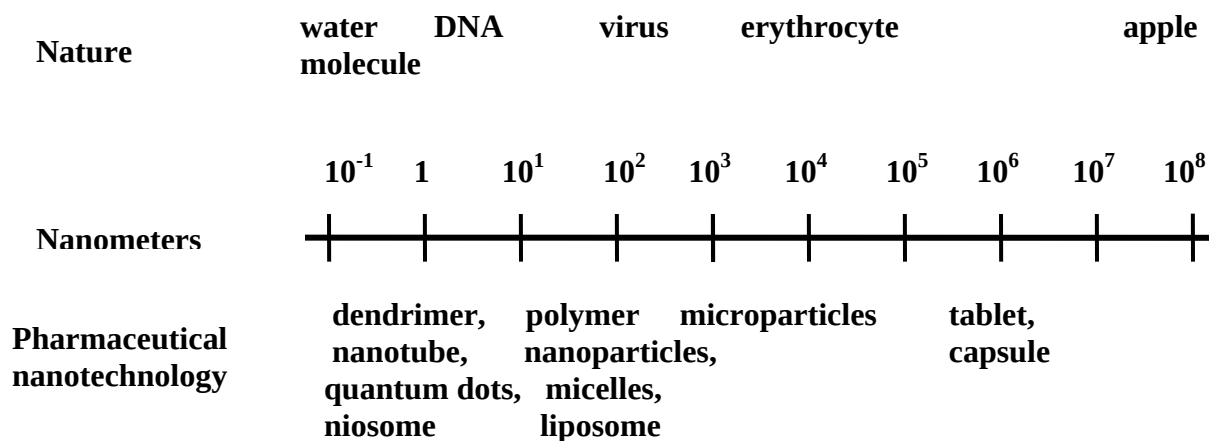


Fig. 1: Dimensions of Nanotechnology

Nano-Definitions

Important nano-definitions are described below:

- **‘Nanoscience’** can be defined as study of phenomenon and manipulation of materials at atomic and molecular scales.
- **‘Nanotechnology’** is related to design characterization, production and applications of structures, devices and systems by controlling shape and size at nanometer scale.
- **‘Pharmaceutical nanotechnology’** embraces applications of nanoscience to pharmacy as nanomaterials, and as devices like drug delivery, diagnostic, imaging and biosensor.
- **‘Nanomedicine’** is defined as submicron size (<1µm) modules, used for treatment, diagnosis, monitoring, and control of biological system.

Pharmaceutical nanotechnology has provided more fine-tuned diagnosis and focused treatment of disease at a molecular level. Pharmaceutical nanotechnology is most innovative and highly specialized field, which will revolutionize the pharmaceutical industry in near future. Pharmaceutical nanotechnology presents revolutionary opportunities to fight against many diseases. It helps in detecting the antigen associated with diseases such as cancer, diabetes mellitus, neurodegenerative diseases, as well as detecting the microorganisms and viruses associated with infections. It is expected that in next 10 years market will be flooded with nanotechnology-devised medicine.

Opportunities and Scope

In view of post GATT (General Agreement of Trade and Tariff) scenario pharmaceutical industries are focusing towards their research on nanotechnology because developing new chemical entity (NCE) is very time consuming and expensive affair and most of drugs will be off patent very soon causing huge revenue loss. Applications of nanotechnology to pharmacy that provide intelligent and smart drug delivery systems is expected to emerge as most important and powerful tool as alternate to conventional dosage form. These nano-intelligent drug delivery systems need little investment while expected to be a high profit making deal due to new patent-protection for current or soon-to-be off-patent drugs. A recent report claimed that 23 major pharmaceutical patents would expire by 2008 leading to revenue loss of US \$ 46 billion and by 2011, US \$ 70-80 billion loss is expected as various drugs go off-patent (Baba, 2007). Therefore most of industrial research interest lies in fact to exploit the newer technology to develop drug delivery system of available drugs in order to reduce or overcome their shortcoming like high toxicity, instability in biological environment, poor bioavailability, and low therapeutic concentration at site of action, which rendered them poor candidates in currently available dosage forms.

Now days most of the industries have realized the potential applications of nanotechnology in pharmacy and are making their efforts in research and development in this area. Recent data depicts that global investment on nanotechnology reached US \$ 12.4 billion in 2006. The data presented below suggests the global interest over nanotechnology investment and related issue (Fig. 2) (Riesch, 2007).

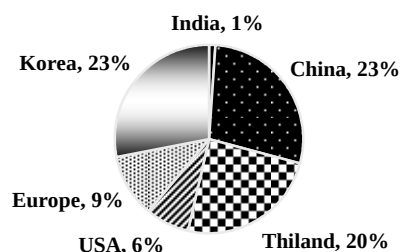


Fig. 2: Global investment on nanotechnology (9 billion US \$, 2005)

Novel drug delivery comprises of a number of features of nanotechnology, which make it a suitable tool to address major issues. The scope of pharmaceutical nanotechnology is very wide from smart material for tissue engineering to intelligent tools for delivery of drugs and diagnostics, and more recently, artificial RBC etc. Current applications of nanotechnology in pharmacy are development of nanomedicine, tissue engineering, nanorobots, advance diagnostic, as carrier of diagnostic and therapeutic modalities and as **biosensor**, **biomarker**, image enhancement device, implant technology, bioactive surfaces etc. A large number of nanosystems, which have been investigated in pharmacy to date, are **liposomes**, **dendrimers**, **metallic nanoparticles**, polymeric nanoparticles, **carbon nanotubes**, **quantum dots**, **nanofibres** etc.

Pharmaceutical Nanotechnology Based Systems

Pharmaceutical nanotechnology provides two basic types of nanotools viz. nanomaterials and nanodevices, which play a key role in realm of pharmaceutical nanotechnology and related fields. **Nanomaterials** are biomaterials used, for example, in orthopedic or dental implants or as scaffolds for tissue-engineered products. Their surface modifications or coatings might greatly enhance the biocompatibility by favoring the interaction of living cells with the biomaterial. These materials can be sub classified into nanocrystalline and nanostructured materials.

Nanocrystalline materials are readily manufactured and can substitute the less performing bulk materials. Raw nanomaterials can be used in drug **encapsulation**, bone replacements, **prostheses** (artificial mechanical devices to replace body parts lost in injury and or by birth e.g. artificial limbs, facial prosthetics and neuroprosthetics etc.), and **implants**.

Nanostructured materials are processed forms of raw nanomaterials that provide special shapes or functionality, for example **quantum dots**, **dendrimers**, **fullerenes** and **carbon nanotubes**. **Nanodevices** are miniature devices in the nanoscale and some of which include nano- and micro-electromechanical systems (NEMS/ MEMS), **microfluidics**(control and manipulation of micro or nanolitre of fluids), and **microarrays**(different kind of biological assay e.g. DNA, protein, cell, and antibody). Examples include **biosensors** and **detectors** to detect trace quantities of bacteria, airborne pathogens, biological hazards, and disease signatures and some intelligent machines like respirocytes (Fig. 3).

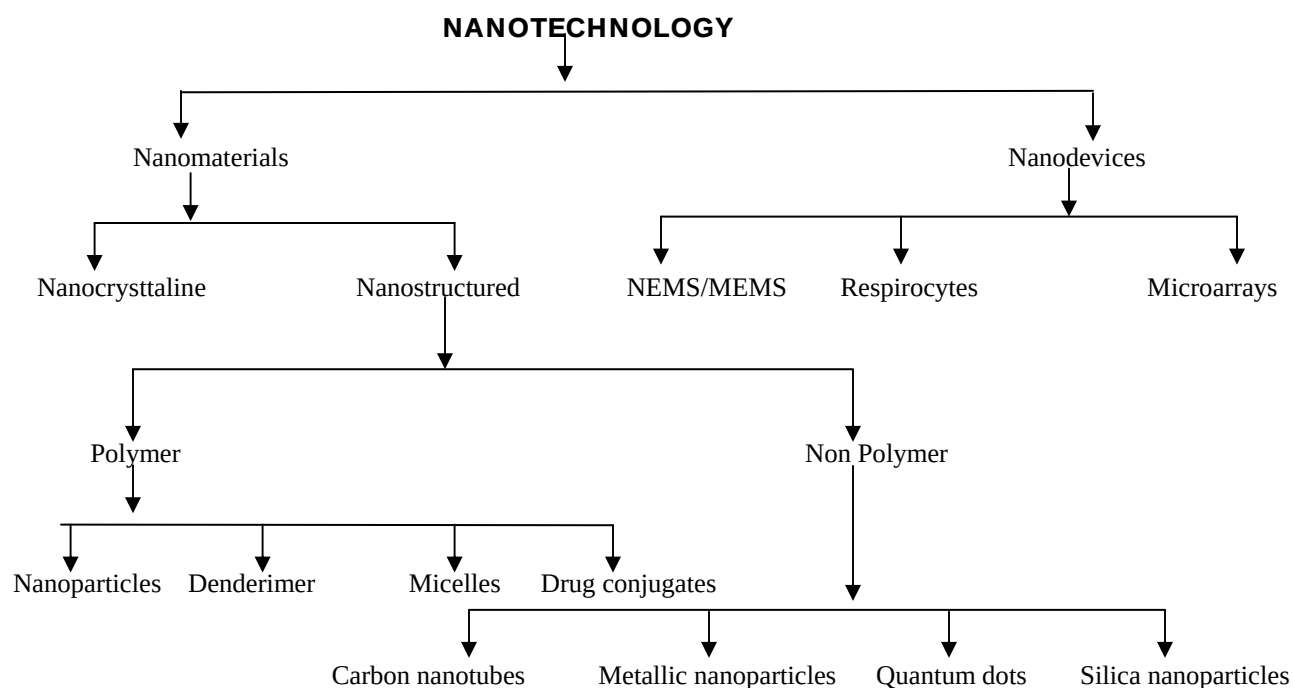


Fig. 3: Schematic diagram of various types of pharmaceutical nanosystems

A brief discussion of various types of pharmaceutical nanosystems is presented below:

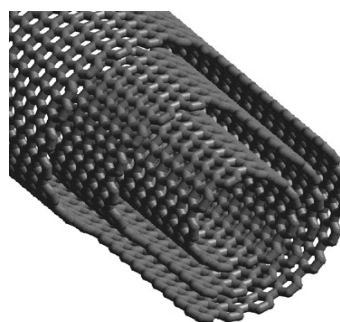
1 Carbon nanotubes: Carbon nanotubes are hexagonal networks of carbon atoms, 1 nm in diameter and 1–100 nm in length, as a layer of graphite rolled up into a cylinder. There are two types of nanotubes: single-walled nanotubes (SWNTs) and multi-walled nanotubes (MWNTs), which differ in the arrangement of their graphene cylinders (Fig.4).



(a)

(a) single walled (SWNTs)

(adopted from www.3dchem.com/images/molecules/Buckytubes2.jpg¹)



(b)

(b) multi walled (MWNTs)

(adopted from [www.msm.cam.ac.uk/.../msps100/cutMWNT.jpg](http://www.msm.cam.ac.uk/msps100/cutMWNT.jpg)²)

Fig. 4: Structure of Carbon nanotubes

These are small macromolecules that are unique for their size, shape, and have remarkable physical properties. Nanotubes offer some distinct advantages over other drug delivery and diagnostic systems due to very interesting physicochemical properties such as ordered structure

with high aspect ratio, ultra-light weight, high mechanical strength, high electrical conductivity, high thermal conductivity, metallic or semi-metallic behavior and high surface area (Sinha and Yeow, 2005).

2 Quantum dots: Quantum dots (QDs) are semiconducting materials consisting of a semiconductor core (CdSe), coated by a shell (e.g., ZnS) to improve optical properties, and a cap enabling improved solubility in aqueous buffers. They are neither atomic nor bulk semiconductors. Their properties originate from their physical size, which ranges from 10–100 Å in radius. Due to their bright fluorescence, narrow emission, broad UV excitation and high photostability QDs have been adopted for *in vitro* bioimaging for real time monitoring or tracking of intracellular process for longer time (Fig. 5). Quantum-dots have a large impact on some important development in different medical areas like diagnostic tools (magnetic resonance imaging, MRI), *in vitro* and *in vivo* detection and analysis of **biomolecules**, **immunoassays**, **DNA hybridization**, development of non-viral vectors for **gene therapy**, transport vehicles for DNA, protein, drugs or cells, time graded fluorescence imaging of tissue, labeling of cells and as therapeutic tools for cancer treatment (Bailey et al., 2004).

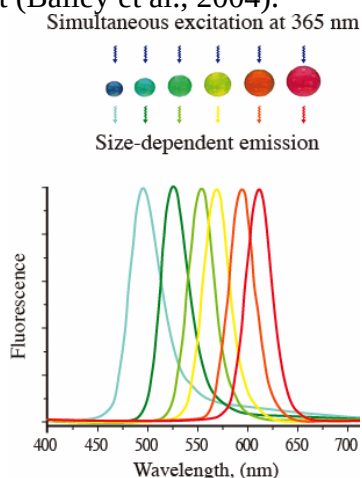


Fig. 5: Size dependent representation of a quantum dots
(adopted from http://www.aist.go.jp/aist_e/aist_today/2006_21/pict/p22_2.png)

3 Dendrimers: Dendrimers are hyperbranched, tree-like structures and have compartmentalized chemical polymer. Dendrimer contain three different regions: core, branches, and surface (Fig. 6). The **macromolecule** constituents radiate in branching form from the central core, creating an internal cavity as well as a sphere of end groups that can be tailored according to requirements. They can be tailored or modified into **biocompatible** compounds with low **cytotoxicity** and high **biopermeability**. They bear promising properties for delivery of bioactives ranging from drugs, vaccines, metal, and genes to desired sites. Their hollow interior provides space to incorporate drugs and other bioactive physically or by various interactions to act as drug delivery vehicles. Most important applications of dendrimers are solubilization, gene therapy, dendrimer based drug delivery, **immunoassay** and MRI contrast agent. Dendrimers is ideal carrier for drug delivery due to advantages like very low size (1-5 nm), feasibility to develop with defined molecular weight, very low polydispersity index (ratio of weight average molecular weight (Mw) to number average molecular weight (Mn) of polymer), good entrapment efficiency and offering surface

for functionalization. They can be modulated for target-specific drug delivery but their toxicity profile renders them not very popular system for use as delivery means (Jain et al, 2001).

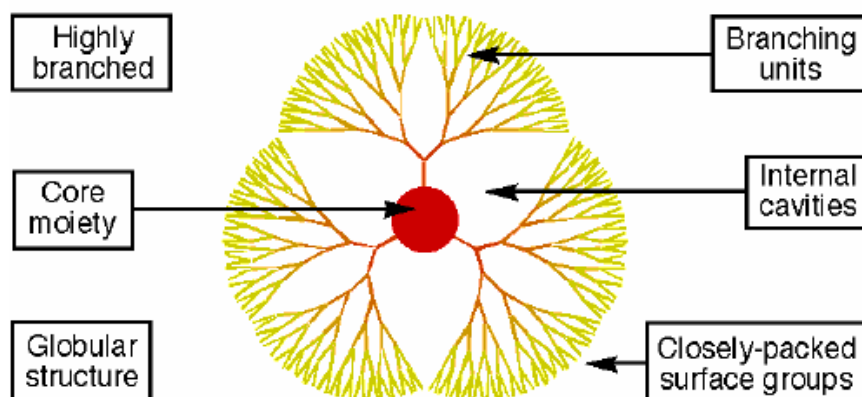


Fig. 6: Schematic representation of a dendrimer showing core, branches, and surface
(adopted from <http://www.ninger.com/dendrimer/01.gif>)

4. Polymeric nanoparticles: Polymeric nanoparticles (Fig. 7) provide an alternative to above-mentioned nanosystems due to some inherent properties like **biocompatibility**, nonimmunogenicity, nontoxicity and **biodegradability**. These are colloidal carrier, 10 nm -1 μ m in size, consisting of synthetic or natural polymers. Polymeric nanoparticles are a broad class comprised of both vesicular systems (nanocapsules) and matrix systems (nanospheres). **Nanocapsules** are systems in which the drug is confined to a cavity surrounded by unique polymeric membrane whereas nanospheres are systems in which the drug is dispersed throughout the polymer matrix. The various natural polymers like gelatin, albumin and alginate are used to prepare the nanoparticles; however they have some inherent disadvantages like poor batch-to-batch reproducibility, prone to degradation and potential antigenicity.

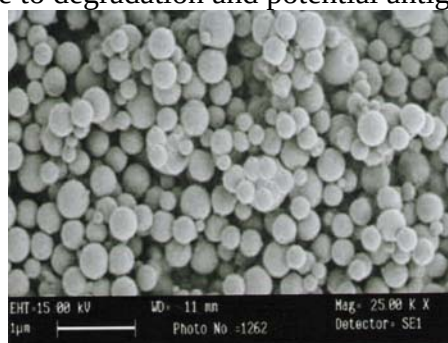


Fig.7. Scanning electron microscopy image of polymer nanoparticles
(Adopted from Senthilkumar *et al.*, J Biomed Nanotech.2007, 1, 52-60)

Synthetic polymers used for nanoparticles preparation may be in the form of preformed polymer e.g. polyesters like polycaprolactone (PCL), poly lactic acid (PLA) or monomers that can be polymerized *in situ* e.g. polyalkyl cyanoacrylate. The candidate drug is dissolved, entrapped, attached or encapsulated throughout or within the polymeric shell/matrix. Depending on the

method of preparation, the release characteristic of the incorporated drug can be controlled. Polymeric nanoparticulate systems are attractive modules for intracellular and site specific delivery. Nanoparticles can be made to reach a target site by virtue of their size and surface modification with a specific recognition ligand. Their surface can be easily modified and functionalized.

5. Metallic nanoparticles: Metallic nanoparticles are emerging as good delivery carrier for drug and **biosensor**. Although nanoparticles of various metals have been made yet silver and gold nanoparticles are of prime importance for biomedical use (Fig. 8). Their surface functionalization is very easy and various ligands have been decorated onto the surface. A large numbers of ligands have been linked to nanoparticles including sugars, peptide, protein and DNA. They have been used for active delivery of bioactive, drug discovery, bioassays, detection, imaging and many other applications due to surface functionalization ability, as an alternative to quantum-dots.

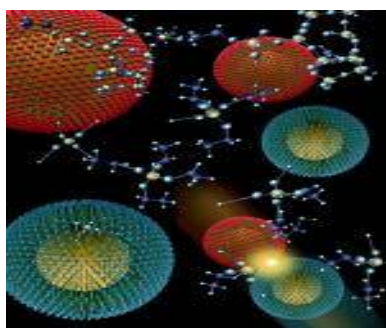


Fig. 8: Surface functionalized gold nanoparticles
(adopted from www.ia.ucsb.edu/pa/display.aspx?pkey=13005)

6 Liposomes: Liposomes have been extensively explored and most developed nanocarriers for novel and targeted drug delivery. These closed vesicles are formed when dry phospholipids are hydrated (Fig. 9). Liposomes are classified into three basic types based on their size and number of bilayers. **Multilamellar vesicles (MLVs)** consist of several lipid bilayers separated from one another by aqueous spaces. These entities are heterogeneous in size, often ranging from a few hundreds to thousands of nanometers in diameter. On the other hand, both **small unilamellar vesicles (SUVs)** and **large unilamellar vesicles (LUVs)** consist of a single bilayer surrounding the entrapped aqueous space. SUVs are less than 100 nm in size whereas LUVs have diameters larger than 100 nm.

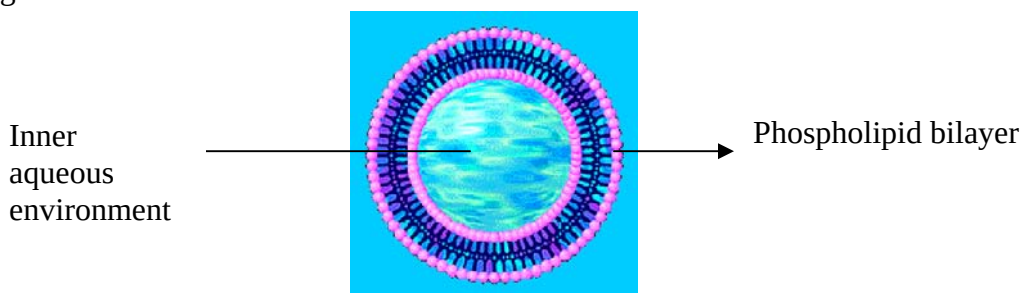


Fig. 9: Structure of Liposomes
(adopted from www.scienceinyoureyes.memphys.sdu.dk/liposome.gif ⁶)

Drug molecules can be either entrapped in the aqueous space or intercalated into the lipid bilayer of liposomes, depending on the physicochemical characteristics of the drug. Liposomes can be prepared with enormous diversity in structure, composition, size, flexibility, and a variety of surface modification approaches proving most intelligent carrier system for both active and passive delivery of bioactives. They have been successfully exploited in cancer therapy, carrier for **antigens**, pulmonary delivery, **leishmaniasis**, ophthalmic drug delivery etc. Some of liposome-based formulations are already in market (Table 1).

Table: 1 Liposomal formulation in market

PRODUCT	STATUS	PAYLOAD	INDICATION
Daunoxome®	Market	Daunorubicin	Cancer
Doxil®/caelyx®	Market	Doxorubicin	Cancer
Moet®	Market	Doxorubicin	Cancer
Ambisome®	Market	Amphotericin B	Fungal infections

7 Polymeric micelles: Amphiphilic block copolymers assemble into nanoscopic supramolecular core-shell structures known as ‘polymeric micelles’. Polymeric micelles are usually of <100 nm and their hydrophilic surface protects their nonspecific uptake by reticuloendothelial system. Micelles are formed in solution as aggregates in which the component molecules (e.g., **amphiphilic** AB-type or ABA-type block copolymers, where A and B are **hydrophobic** and **hydrophilic** components, respectively) are generally arranged in a spheroidal structure with hydrophobic cores shielded from water by a mantle of hydrophilic groups (Fig. 10).



Fig. 10: Structure of block copolymer micelles

(adopted from http://www.gkss.de/templates/images_d/werkstoff/micelle.jpg⁷)

These dynamic systems are used for the systemic delivery of water-insoluble drugs. Drugs or contrast agents may be trapped physically within the hydrophobic cores or can be linked covalently to component molecules of the micelle. Polymeric micelles have proved an excellent novel drug delivery system due to high and versatile loading capacity, stability in physiological conditions, slower rate of dissolution, high accumulation of drug at target site and possibility of functionalization of end group for conjugation of targeting ligands.

8 Polymer drug conjugate: The conjugation of low molecular weight drugs with polymer causes drastic change in pharmacokinetic disposition of drug in whole body and at cellular level. Polymer-drug conjugates are thus designed to increase the over all molecular weight, which

facilitates their retention in cancer cells through enhanced permeation and retention (EPR) effect using **passive delivery** approach.

9 Polyplexes/Lipopolyplexes: These are assemblies, which form spontaneously between nucleic acids and polycations or cationic liposomes (or polycations conjugated to targeting ligands or hydrophilic polymers), and are used in transfection protocols. The shape, size distribution, and transfection capability of these complexes depends on their composition and charge ratio of nucleic acid to that of cationic lipid/polymer. Examples of polycations that have been used in gene transfer/therapy protocols include poly-L-lysine, linear- and branched-poly (ethyleneimine), poly (amidoamine), poly-amino esters, and cationic cyclodextrin.

Table: 2 Brief descriptions of nanosystems (Nahar et al., 2006)

Types of Nanosystems	Size	Characteristics	Applications
Polymeric nanoparticles	10-1000 nm	Biocompatible, biodegradable, offer complete drug protection	Excellent carrier for controlled and sustained delivery of drugs. Stealth and surface modified nanoparticles can be used for active and passive delivery of bioactives.
Nanocrystals Quantum dots	2–9.5 nm	Semi conducting material synthesized with II-VI and III-V column element; Size between 10-100 Å; Bright fluorescence, narrow emission, Broad UV excitation and high photo stability	Long term multiple color imaging of liver cell; DNA hybridization , immunoassay ; receptor mediated endocytosis ; labeling of breast cancer marker Her ₂ surface of cancer cells
Carbon nanotubes	0.5–3 nm diameter and 20–1000 nm length	Third allotropic crystalline form of carbon sheets either single layer (single walled nanotube, SWNT) or multiple layer (multi-walled nanotube, MWNT). These crystals have remarkable strength and unique electrical properties (conducting, semi conducting, or insulating)	Functionalization enhanced solubility, penetration to cell cytoplasm and to nucleus, as carrier for gene delivery, peptide delivery
Dendrimer	<10 nm	Highly branched, nearly monodisperse polymer system produced by controlled polymerization; three main parts core, branch and surface	Long circulatory, controlled delivery of bioactives, targeted delivery of bioactives to macrophages , liver targeting
Metallic nanoparticles	<100 nm	Gold and silver colloids, very small size resulting in high surface area available for functionalization, stable	Drug and gene delivery, highly sensitive diagnostic assays, thermal ablation and radiotherapy enhancement
Polymeric micelles	10-100nm	Block amphiphilic copolymer micelles, high drug entrapment, payload, biostability	Long circulatory, target specific active and passive drug delivery, diagnostic value
Liposome	50-100 nm	Phospholipid vesicles, biocompatible, versatile, good entrapment efficiency, offer easy	Long circulatory, offer passive and active delivery of gene, protein, peptide and various other

		surface functionalization	bioactives
Iron oxide nanocrystal	4–5 nm with a hydrodynamic radius of 15–25 nm	Superparamagnetism	Magnetic resonance imaging (disease detection such as cancer, arthritis , and atherosclerosis); intracellular monitoring
Silica Nanoparticles	10 nm–50 μ m	Silanised and coated with oligonucleotide . Observable by fluorescence method.	Efficient nucleic acid hybridization Detection of DNA Nanobiosensor for trace analysis
Nanoshells		Nanoshells typically have a silicon core that is sealed in an outer metallic core. By manipulating the ratio of wall to core, the shells can be precisely tuned to scatter or absorb very specific wavelengths of light	Gold encased nanoshells have been used to convert light into heat, enabling the destruction of tumors by selective binding to malignant cells.

Characterization of Pharmaceutical Nanotools

1 Structural characterization: Structural characterization is a parameter that plays an important role in determining various attributes of a nanosystem like shape, size, surface morphology, structural arrangement, spatial distribution, density, geometric features etc. Development of electron microscopy tools improves accessibility and feasibility to determine these attributes at nanometer scale. Scanning electron microscopy (SEM) produces the image down to length scales of 10 nm and provides valuable information regarding structural arrangement, spatial distribution as well as surface morphology of nanoparticles. Transmission electron microscopy (TEM) and high resolution TEM are more powerful imaging tools than SEM and give more detailed geometrical features and information like crystal structure, quality, and orientation of nanoparticles. Moreover, scanning tunneling probe such as scanning tunneling microscope (STM), electrical field gradient microscopy (EFM), and scanning thermal microscopy, combined with atomic force microscopy (AFM) have been employed to illustrate structural, electronic, magnetic and thermal properties besides topographical properties of nanosystems.

2 Particle Size Distribution: Particle size distribution (also known as polydispersity index) being an important aspect during the formulations of nanosystems, efforts are made to achieve a system with lowest polydispersity index. Some techniques to determine the particle size distribution are dynamic light scattering, which is used to measure particles ranging from a few nanometers to about 3 μ m, while laser diffraction is used to detect microparticles or possible aggregates of drug nanoparticles.

3 Particle Charge / Zeta Potential: **Zeta potential** is used to determine the charge at particle surface. Zeta potential measurement is made to optimize formulation parameters and to make predictions regarding the storage stability of the **colloidal dispersion**. Currently, the principal technique involved in zeta potential determination is laser doppler anemometry.

4 Crystalline Status: Differential scanning calorimetry, X ray diffraction and other analytical methods are used to assess any possible changes brought about in the physical form of the drug during processing.

5 Toxicity Evaluation : Some important types of acute toxicities caused by nanosystem are enhanced endocytosis (cause inflammation, granuloma formation); oxidative stress (cause cell death due to free radical generation) and alter/modify protein/gene structure (resulting immune response causes autoimmune effect) while long-term toxicities are bioaccumulation, poor biodistribution and ultimate fate of nanosystem in body. These toxicities of nanosystems are evaluated using well defined and established protocols available in literature. Ex vivo toxicity evaluation generally carried out in various cell lines and MTT assay is used to determine the cell viability. In vivo acute and chronic toxicities are determined in various animal models.

Engineering of Pharmaceutical Nanosystems

Most of the nanosystems discussed above are not very efficient in biomedical and pharmaceutical applications due to non-specific uptake by reticulo endothelial system (RES); **opsonization**, aggregation and poor biocompatibility associated with them. However, manipulations in their size and surface by biocompatible polymers, hydrophilic polymers and some site-specific ligands render them efficient delivery vehicle for various drugs and utilized for various biomedical applications. Some examples of such manipulations are discussed below.

1 Functional nanosystems: Modification in properties by incorporation, adsorption or covalent coupling by moieties like polymers and/or ligands to nanoparticles surface is known as **surface functionalization**. Some commonly used tools for surface modification are polymers, carbohydrates, endogenous substances/ligands, peptide, protein, nucleic acid and polysaccharides. These tools make the nanosystems a intelligent tools and confer a large varieties of properties like higher biostability, lower aggregation and high target specificity in comparison to conventional nanosystems. Various nanosystems like polymeric nanoparticles, liposomes, dendrimers, carbon nanotubes, quantum dots etc. have been successfully functionalized for efficient use in biomedical area. Functional polymer nanoparticles, their methods of surface modifications and their pharmaceutical applications have been excessively reviewed by our group (Nahar et al., 2006)

2 Multifunctional nanosystems: Multifunctional nanosystems could be developed in following ways:

1. Multifunctionality imparted to core:

- Simultaneous delivery of two or more therapeutic active moieties,
- Containing contrast enhancer; and
- Containing permeation enhancer

2. Multifunctionality imparted to surface:

- Steric stabilization by PEG(poly ethylene glycol) in order to modify circulation time, and
- Use of targeting moiety

3. Multifunctionality imparted to material:

- By use of thermal sensitive, pH and stimuli sensitive biomaterials.

Applications of Pharmaceutical Nanotools

Miniaturization is often beneficial in pharmaceutical technology. Although it has increased complexity yet it imparts large number of benefits in drug delivery and diagnostic. Miniaturization is helpful in overcoming various physiological, biochemical and pharmaceutical barriers. Pharmaceutical nanotechnology provides wide array of systems or device of nanosize, which offer numerous benefits. Some major advantages are (i) improved **bioavailability**, (ii) reduced toxicity, (iii) sustained and controlled release, (iv) ability to target, (v) do not occlude blood capillaries and traverse easily to most physiological biobarrier and provide effective delivery to brain and intracellular compartment, (vi) protects fragile drugs/proteins from harsh biological environment, (vii) faster, safer and more accurate disease diagnosis, (viii) more accurate, less invasive surgery, (ix) inexpensive, and (x) large-scale production is feasible. However some shortcomings in pharmaceutical applications of nanotechnology are (i) high aggregation in biological system due to high surface energy, (ii) poor solubility and poor biocompatibility in case of carbon nanotubes, (iii) quickly scavenged by RES system of body resulting in low biological half life, (iv) poor target and site specificity, (v) high immunogenicity or foreignness, (vi) undefined and unpredictable safety issue, and (vii) acute and chronic toxicity.

Inspite of the above shortcomings, there are various pharmaceutical and biomedical areas where pharmaceutical nanosystems have achieved remarkable breakthrough and realized their market applications. Some important applications areas are discussed here:

1 As nanomaterials for tissue engineering: Nanotechnology offered numerous smart materials that are used for tissue repair and replacement, implant coatings, tissue regeneration scaffolds, structural implant materials, bone repair, bioresorbable materials, some implantable devices (sensory aids, retina implants etc.), surgical aids, operating tools, and smart instruments.

Table: 3 Applications of nanosystems in tissue regeneration, growth and repair

Nanosystems	Property	Applications
Nanoengineered prosthetics	Increased miniaturisation; increased prosthetic strength & weight reduction; improved biocompatibility	Retinal, auditory, spinal and cranial implants
Cellular manipulation	Manipulation of cellular systems	Persuasion of lost nerve tissue to grow; growth of body parts

2 As drug carrier system: Conventional drug delivery systems or dosage forms suffer from many limitations such as lack of target specificity, high rate of drug metabolism, cytotoxicity, high dose requirement, poor patient compliance etc. Nanotechnology enabled drug delivery system with optimized physical, chemical and biological properties can serve as effective delivery tools for currently available bioactives. Some nanobased drug delivery tools are polymeric **nanoparticles**, **liposome**, **dendrimer**, **polymeric micelles**, **polymer-drug conjugates**, antibody- drug conjugates, which can broadly be classify as (i) sustained and controlled delivery system, (ii) stimuli sensitive delivery system, (iii) functional system for delivery of bioactives, (iii) multifunctional system for combined delivery of therapeutics, biosensing and diagnostic, and (iv) site specific targeting (intracellular, cellular, tissue) (Vasir et al., 2005).

(i) **Cancer treatment:** Nanotechnology can have a revolutionary impact on cancer diagnosis and therapy. Available therapies commonly employed in cancer treatment include surgery, **chemotherapy**, **immunotherapy**, and **radiotherapy**. Nanotechnology offers tremendous opportunities to aid and improve these conventional therapies by virtue of its nanotools. Some nanotools that have played key role in cancer therapy are listed below (Table 4).

Table: 4 Applications of various nanosystems in cancer therapy

Nanosystem	Applications in cancer therapeutics
Carbon nanotubes	DNA mutation detection, disease protein biomarker detection
Dendrimers	Controlled release drug delivery, image contrast agents
Nanocrystals	Improved formulation for poorly-soluble drugs
Nanoparticles	MRI and ultrasound image contrast agents, targeted drug delivery, permeation enhancers, reporters of apoptosis , angiogenesis , etc.
Nanoshells	Tumor-specific imaging, deep tissue thermal ablation
Nanowires	Disease protein biomarker detection, DNA mutation detection, gene expression detection
Quantum dots	Optical detection of genes and proteins in animal models and cell assays, tumor and lymph node visualization.

Targeting and localized delivery are the key challenges in cancer therapy. These challenges can be overcome by virtue of development of functional and multifunctional system for passive and active delivery. The approaches are basically attributed to the pathophysiology of diseased sites like leaky vasculature of the cancer tissues (Ferrari, 2005). The nanocarriers can alter the **biodistribution** and **pharmacokinetic** parameters of the anticancer drug significantly compared to free drug due to nano size of the carrier. These nanotools identify biomarker or detect mutation in cancer cell and treat the abnormal cells by (i) **thermotherapy** by photo-thermal ablation therapy using silica nanoshells, carbon nanotubes; magnetic field-induced **thermotherapy** using magnetic nanoparticles; photodynamic therapy by quantum dots as photosensitizers and carriers, (ii) **chemotherapy** by nano-structured polymer nanoparticles, dendrimers and nanoshells, and (iii) **radiotherapy** by carbon nanotubes, dendrimers for boron neutron capture therapy.

(ii) **Implantable delivery systems:** Nanotechnology is opening up new opportunities in implantable delivery systems by virtue of its size, controlled and approximately zero order release which otherwise may cause toxicity when compared to intravenous administration (due to first order drug kinetics). Some pharmaceutical novel nano drug vascular carriers like liposome, ethosome and transferrinosome and some implant chips have been envisaged recently, which may help in minimizing peak plasma levels and reduce the risk of adverse reactions, allow for more predictable and extended duration of action, reduce the frequency of re-dosing and improve patient acceptance and compliance.

(iii) **Site specific drug delivery:** Several approaches are now being tested for better site-specific delivery using liposomes, polymeric micelles, dendrimers, iron oxide, proteins using manipulation in passive and active uptake of drug. The tumor targeting of drugs with passive delivery using enhanced permeation and retention (EPR) effect is thought to be one intelligent

approach using these carrier system taking the advantages of leaky vasculature of tumor. Some surface modification approaches using various site-specific ligands via covalent binding or adsorption with carrier system enhanced their site specificity and make them intelligent tools for active delivery. The conjugations of these carriers with ligands provide them site specificity at various levels. In the chemotherapy of tuberculosis with active delivery to lung cells is reported to have improved drug bioavailability, reduction in dose frequency and overcoming the non-adherence problem encountered in the control of TB.

(iv) **Gene therapy:** In gene therapy, a normal gene is inserted in place of an abnormal disease-causing gene using a carrier molecule. Conventional uses of viral vectors are associated with adverse immunologic, inflammatory reactions and diseases in the host. Nanotechnology enabled delivery systems have currently emerged as potential vector and are shown to be effective and promising tool in systemic gene treatment. Various polymer based nanoparticles like chitosan, gelatin and poly-l-lysine and modified silica nanoparticles have been reported to have increased transfection efficiency and decreased cytotoxicity. It is well established that nanotechnology provides viable option as ideal vector in gene delivery

3 Molecular Diagnostics : Molecular imaging is the nanoscience of representing, characterizing, and quantifying subcellular biological processes in intact organisms. These processes include gene expression, protein-protein interaction, signal transduction, cellular metabolism, and both intracellular and intercellular trafficking. Some nanoparticles, which have inherent diagnostic properties, are quantum dots, iron oxide nanocrystal and metallic nanoparticles. They have been successfully utilized in various magnetic resonance imaging, optical imaging, ultrasonic imaging and nuclear imaging (Wickline and Lanza, 2002). Some other applications of nanoparticles in diagnostics are as specific labeling of cells and tissues, useful for long-term imaging, useful for multi-color multiplexing, suitable for dynamic imaging of sub-cellular structures and may be used for **fluorescence resonance energy transfer (FRET)**-based analysis and **magnetic resonance imaging (MRI)**. FRET-based analysis and MRI are two main diagnostic techniques developed for molecular level diagnostics. Traditional MRI contrast agents (paramagnetic and superparamagnetic materials) are now being replaced by various novel nanosystems like dendrimer, quantum dots, carbon nanotubes and magnetic nanoparticles. They are proved very efficient contrast agent in providing stable, intense, clearer image of object due to high intensity photostability, resolution, resistance to photo bleaching. Some approved nanoparticles used as imaging and as drug carriers are listed below (Table 5).

Table: 5 Approved nanoparticles as imaging agents and drug carriers

Modality	Compound	Status	Use
Imaging Agents Endorem®	superparamagnetic iron oxide nanoparticle	Market	MRI agent
Gadomer®	Dendrimer-based MRI agents	Phase III clinical trial	MRI agent-cardiovascular
Drug delivery Abraxane®	Albumin nanoparticle containing paclitaxel	Market	Breast cancer

4 Biosensor and biolabels: A number of analytical tools have been developed with application of this smart and potential technology. These tools are employed for determination of various pathological proteins and physiological-biochemical indicator associated with disease or disrupted metabolic conditions of body. Various nanoenabled technologies, techniques and their analytical applications are listed below (Table 6).

Table: 6 Nanoenabled technologies, techniques and their analytical applications

Technology	Technique	Application
Bioarrays and Biosensors	Nanofabrication	Nano-Objects Detection
DNA Chips	Lab on Chip Nanotubes	Electrochemical Detection
Protein-Chips	Pill on Chip Nanowires	Optical Detection
Glyco-Chips	Nanofluidics Nanoparticles	Mechanical Detection
Cell-Chips	Nanostructured Surfaces	Electrical Detection

A **biosensor** is generally defined as a measurement system that consists of a probe with a sensitive biological recognition element, or bioreceptor, a physicochemical detector component, and a transducer in between to amplify and transduce these signals in to measurable form. A nanobiosensor or nanosensor is a **biosensor** that has dimensions on the nanometre size scale. Applications of various nanosystems as biosensor and biolabels are given below (Table: 7).

Table: 7 Applications of various nanosystems as biosensor and biolabels (Kubik et al., 2005)

Nanosystem	Applications
Gold Nanoparticles	For ssDNA detection; in immunohistochemistry to identify protein-protein interaction
Iron oxide nanocrystal	Monitor gene expression; detect the pathogens such as cancer, brain inflammation, arthritis and atherosclerosis
Nanopores	Sensing single DNA molecules by nanopores
Cantilever array	Diagnosis of diabetes mellitus, for detection of bacteria, fungi, viruses; for cancer diagnosis
Carbon nanotube	Blood glucose monitoring; sensors for DNA detection
Nanowire	Electrical detection of single viruses and biomolecules
Nanoparticle-based biodetection	Detection of pathogenic biomarkers, Ultra-sensitive detection of single bacteria

Nanosensors could provide the tools to investigate important biological processes at the cellular level *in vivo*. The basic functions of nanosensors are to understand living cell, to monitor cell as biomarker, as sensor and fluorescent biological labels (Kubik et al., 2005). Biosensors are currently used in the areas of target identification, validation, assay development, and absorption, distribution, metabolism, excretion and toxicity determination (Jain, 2005).

5 Drug discovery: Pharmaceutical nanotechnology is playing crucial role in drug discovery that rely on better understanding of mechanism of the drug action and identification of biomarker associated with specific disease. Nanotechnology help identification and validation of target by identifying the protein present on the cell surface or target surface. Nanotechnology will enhance the drug discovery process, through miniaturization, automation, speed and reliability of assays. For example single walled nanotubes are successfully used to identify surface protein of pathogen. QDs are used to track individual glycine receptors and to analyze their dynamics in the neuronal membrane of living cells, for periods ranging from milliseconds to minutes. Similarly, gold nanoparticles, nanobodies (smallest, available, intact, antigen-binding fragments) produced by Ablynx (Ghent, Belgium,) are some commonly used nanomaterials in diagnostics (Jain, 2005).

6 Miscellaneous Applications: Various other applications of nanosystems in biomedical and pharmaceutical fields are (i) biodetection of pathogens in humans, (ii) separation and purification of molecules and cells, (iii) detoxifying agents etc. One of future proposed nanomachine known as respirocyts is the nano-on-board minicomputer which can be used to simultaneous detection of disease causing marker/antigen/marker, to view the diseased site and to deliver the therapeutic agent to that site.

Challenges to Pharmaceutical Nanotechnology

Pharmaceutical nanotechnology has provided fine-tuned diagnosis and focused treatment of disease. However some ethical, scientific, social and regulatory issues posing various challenges in practical realization of pharmaceutical nanotechnology. Some major health risk associated with such devices includes cytotoxicity, translocation to undesired cells, acute and chronic toxicity; some unknown, unpredictable and undefined safety issues, environmental impacts of nanomaterials and non-biocompatibility. Some ethical issues are altered gene expression, ultimate fate and altered or permanent anomaly in cell behavior/ response on short/long term exposure. There are no specific FDA directives to regulate pharmaceutical nanotechnology based products and related issues. Altogether these challenges cause urgent need to regulate these nanotechnology based products and delivery devices. The characterization, safety and environment impact are three main elements that need to be regulated. Though regulatory agencies like FDA, EPA (Environment Protection Agency) and nuclear protection agency etc. are regulating the major health risk associated with nanomaterials, yet lack of adequate and conclusive research on the health risks of nano-based substances demand the need for a dialogue on regulatory adequacy, inadequacy, or possible alternatives more urgent. US-FDA kept nanotechnology as an element under evaluation in its critical path initiative. FDA regulates most pharmaceutical nanotechnology based products as “combination products” (i.e., drug-device, drug-biologic, and device-biologic products). Some FDA approved nanotechnology based products, which have entered the market are liposome, nanoparticles, monoclonal antibody based product, polymer drug conjugate, polymer–protein conjugate and some polymeric drugs. Well-tuned, coordinated and sincere effort of government, industries, academia and researchers over guidelines for regulation must be drawn in order to utilize the benefit of nano-based technology without hampering its development.

Future Prospects of Pharmaceutical Nanotechnology

Pharmaceutical nanotechnology is an emerging field that could potentially make a major impact on human health. Nanomaterials promise to revolutionize medicine and are increasingly used in drug delivery or tissue engineering applications. Newly developed hybrid systems seem promising for future applications in human. Functional and multifunctional approaches have tremendous potential in temporal and spatial controlled delivery of bioactives. A modular approach to construct delivery systems that combine targeting, imaging and therapeutic functionalities into nanoplateforms is emerging as intelligent concept. These multifunctional nanoplateforms would localize to target cells, enable diagnostics and subsequently deliver therapeutics with great precision. But such approaches to nanodevice construction are inherently complex. One very interesting and novel future strategy is to devise a nanomachine, which can detect and attack pathogen simultaneously, detect the change in molecular event during diseased state, and also monitor the efficacy of treatment. However such intelligent machine (also known as nanorobots which can serve as mini onboard computer in human body) is very far reaching concept. In short, recent development, market realization of various pharmaceutical nanotools and global interest shown by scientists, governments and industries ensure that there is tremendous potential and scope of nanobased drug delivery system in near future. There is no doubt to presume that in next ten years market will be flooded with nano-enabled delivery devices and materials.

Conclusion

Pharmaceutical nanotechnology offers new tools, opportunities and scope, which are expected to have a great impact on many areas in disease diagnostics and therapeutics. Pharmaceutical nanotechnology has emerged as a discipline having enormous potential as carrier for spatial and temporal delivery of bioactives and diagnostics and provides smart materials for tissue engineering. Pharmaceutical nanotechnology is now well-established as specialized area for drug delivery, diagnostics, prognostic and treatment of diseases through its nanoengineered tools. Few nanotechnology based products and delivery systems are already in market. Pharmaceutical nanotechnology provides opportunities to improve materials, medical devices and help to develop new technologies where existing and more conventional technologies may be reaching their limits. Pharmaceutical nanotechnology raises new hope to pharmaceutical industries by providing new cutting age patentable technologies in view of revenue loss caused due to off-patent drugs. Scientific societies, industries and governments all over world are looking with great anticipation and contributing their best to clutch the potential of this technology. This technology has the potential to make significant contributions to disease detection, diagnosis, therapy, and prevention. Pharmaceutical nanotechnology could have a profound influence on disease prevention efforts because it offers innovative tools for understanding the cell as well as the differences between normal and abnormal cells. It could provide insights into the molecular basis of disease. However going towards bottom size increases the unknown health risk. However, some suggested initiative must be taken in order to exploit the advantage of this very fascinating and ever growing potential technology. Some of these are (i) identifying, defining and characterizing model nanomaterials, (ii) developing toxicity testing protocol, (iii) detecting and monitoring exposure level, (iv) assessing the impact of environment, and (v) developing the biocompatible hybrid system. We still lack sufficient data and guidelines regarding safe use of these nanotechnology based devices and materials. There are several confounding unresolved issues, which warrant the application in its full bloom. Pharmaceutical nanotechnology is still in

infancy. Some concerning issues like safety, toxicity hazards, bioethical issues, physiological and pharmaceutical challenges get to be resolved by the scientists.

References:

1. Baba R .Patent and Nanomedicine. Nanomedicine (2007) 2(3), 351-374.
2. Bailey, R. E., Smith, A.M., Nie S. Quantum dots in biology and medicine. Physica E (2004),25, 1–12.
3. Ferrari, M. Cancer nanotechnology: opportunities and challenges. Nature Reviews /Cancer. (2005) 5,161-171.
4. Jain, K.K. The role of nanobiotechnology in drug discovery. Drug Discovery Today (2005)10(21).1435-1442.
5. Jain S., Jain N.K. Liposomes as drug carrier, In: Jain NK, editor. Controlled and novel drug delivery. 2nd ed. CBS publisher, New Delhi, 2002. p.304-52.
6. Khopde AJ, Jain, NK. Dendrimer as potential delivery system for bioactive In: Jain NK, editor. Advances in controlled and novel drug delivery. CBS publisher, New Delhi, 2001 p. 361-80.
7. Kubik, T. Bogunia-Kubik K., Sugisaka. M. Nanotechnology on Duty in Medical Applications. Current Pharmaceutical Biotechnology. (2005) 6, 17-33.
8. Nahar M, Dutta T, Murugesan S, Asthana A, Mishra D, Rajkumar V, Tare M, Saraf S, Jain NK. Functional polymeric nanoparticles: an efficient and promising tool for active delivery of bioactives. Crit Rev Ther Drug Carrier Syst. (2006) 23(4), 259-318.
9. Reisch MS. Nano goes big time. Chem. Eng. News (2007), 85(4), 22-25.
10. Senthilkumar M., Subramanian G., Ranjitkumar A.,Nahar, M., Mishra, P. Jain N.K. PEGylated Poly (Lactide-co-Glycolide) (PLGA) nanoparticulate delivery of Docetaxel: synthesis of diblock copolymers, optimization of preparation variables on formulation characteristics and in vitro release studies. J Biomed Nanotech. (2007) 1, 52-60.
11. Sinha, N., Yeow J.T.W. Carbon Nanotubes for Biomedical Applications. IEEE transactions on nanobioscience. 2005 4(2) 180-195.
12. Vasir, J. K. Reddy M.K. and Labhasetwar V. D. Nanosystems in Drug Targeting: Opportunities and Challenges. Current Nanoscience. (2005), 1, 47-64.
13. Wickline S.A., Lanza G.M. Molecular Imaging, Targeted Therapeutics, and Nanoscience. J Cellular Biochem Supp (2002)39, 90–97.
14. [www.3dchem.com/ imagesofmolecules /Buckytubes2.jpg](http://www.3dchem.com/imagesofmolecules/Buckytubes2.jpg)
15. [www.msm.cam.ac.uk/ .../msps100/cutMWNT.jpg](http://www.msm.cam.ac.uk/.../msps100/cutMWNT.jpg)
16. http://www.aist.go.jp/aist_e/aist_today/2006_21/pict/p22_2.png
17. <http://www.ninger.com/dendrimer/01.gif>
18. www.ia.ucsb.edu/pa/display.aspx?pkey=1300
19. [www.scienceinyoureyes.memphys.sdu.dk/ liposome.gif](http://www.scienceinyoureyes.memphys.sdu.dk/liposome.gif)
20. http://www.gkss.de/templates/images_d/werkstoff/micelle.jpg

Further Readings

1. Advances in Controlled and Novel Drug Delivery. Jain NK, editor.. CBS publisher New Delhi, 2001,
2. Controlled novel drug delivery. Jain NK, editor.2nd ed. CBS publisher New Delhi, 2002.
3. Cancer Nanotechnology: Nanomaterial for Cancer Diagnosis and Therapy. Hari Singh Nalwa and Thomas Webster(editors),, American Scientific Publishers, USA,2007
4. Springer Handbook of Nanotechnology. B. Bhoosan editor. Springer,2004
5. Nanoparticles: From Theory to Application. Edited by Gunter Schmid, Wiley-Vch Verlag GmbH & Co. KGaA, Weinheim, 2004