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Review Article

Targeted Drug Delivery Systems: A Prospective Review

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ABSTRACT

Targeted drug delivery, also known as smart drug delivery, is a method of treatment that involves the increase in medicament in one or few body parts in comparison to others. Two strategies are widely used for drug targeting to the desired organ/tissue: passive targeting and active targeting.

Drug delivery vehicles transport the drug either within or in the vicinity of target. An ideal drug delivery vehicle is supposed to cross even stubborn sites such as a blood brain barrier. Recently, Nano-medicine has emerged as the medical application of nanotechnology. Since nanoparticles are very small in size, nano-drug delivery can allow for the delivery of drugs with poor solubility in water and also aid in avoiding the first pass metabolism of liver. Nanotechnology derived drug delivery can cause the drug to remain in blood circulation for a long time, thereby leading to lesser fluctuations in plasma levels and therefore, minimal side effects. These include polymer-drug conjugates and nano-particulate systems such as liposomes, quantum dots, dendrimers, etc. There are several other approaches as well. These also include the strategies wherein the therapeutic agents are coupled with "targeting ligands" that possess the ability to recognize antigens associated with tumors.

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INTRODUCTION

In conventional drug delivery systems such as oral ingestion or intravascular injection, the medication is distributed throughout the body by means of systemic blood circulation. For most therapeutic agents, only a small portion of the medication reaches the affected organ or tissue, such as in chemotherapy where roughly 99% of the drugs administered do not reach the tumour site. Targeted drug delivery sue to deliver medication in the tissues of interest while reducing the relative concentration of the medication in the remaining tissues [1].

For example, by avoiding the host's defence mechanisms and inhibiting non-specific distribution in the liver and spleen, a system can reach the intended site of action in higher concentrations. Targeted delivery is believed to improve efficacy while reducing side-effects.

The concept of targeted drugs was first proposed on 1906 by scientist Ehrlich. As a theoretical concept it became most popular and found to be and strong alternative for effective and site specific treatment, but still the 'magic bullet' continues to be a challenge to implement it clinically [1].

The major challenges that are matter of concern behind the success of targeted drug delivery systems are, finding the proper target for a particular disease state; finding a drug that effectively treats this disease; and finding a suitable drug carrier system to deliver the drug to specific sites while avoiding the immunogenic and nonspecific interactions that efficiently clear foreign material from the body.

What is drug targeting?

The therapeutic response of a drug depends upon the interaction of drug molecules with cell on cell membrane related biological events at receptor sites in concentration dependent manner.

Selective and effective localization of the pharmacologically-active moiety at pre-identified target(s) in therapeutic concentration, while restricting its access to non-target(s) normal cellular linings, thus minimizing toxic effects and maximizing the therapeutic index.

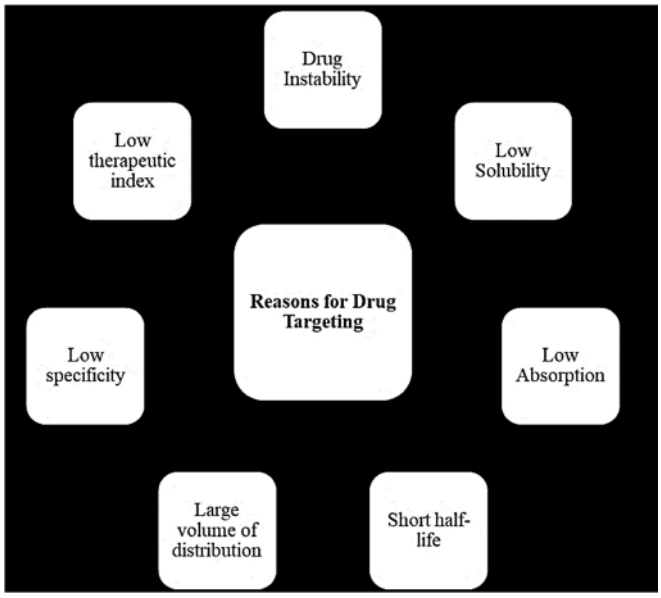

Figure. 1: Different reason or need for Drug targeting

Common Approaches of Targeted Drug Delivery [2]

The basic approaches for targeting the drug to specific site based on different research outcomes may be categorized broadly in to followings, though there are number of effective and successful strategies used in drug targeting.

I. Controlling the distribution of drug by incorporating it in a carrier system
II. Altering the structure of the drug at molecular level
III. Controlling the input of the drug into bioenvironment to ensure a programmed and desirable biodistribution

Properties of ideal targeted drug delivery:

I. It should be nontoxic, biodegradable, biocompatible and physicochemical stable in-vivo and in-vitro
II. It should be capable to deliver the drug to target cells or tissue or organ and should have uniform capillary distribution.
III. It should release the dug in a controlled and predictable manner for a suitable period of time.
IV. It should efficiently maintain the drug concentration at the targeted site within the therapeutic window for prolong period of time
V. Minimal drug losses due to leakage of the carrier system should be ensured.
VI. Carrier used should be biodegradable or and get readily eliminated from the body without showing any toxic interaction.
VII. Its preparation should be easy or reasonably simple, reproductive and cost effective.

Important Properties Influencing Drug Targeting

The general properties influencing drug targeting can be divided into three broad categories, like properties related to Drug, Carrier and In Vivo Environment, which is briefed in below table 1.

Table 1: Properties Influencing Drug Targeting

Properties related to	Important Properties/ Characteristics
Drug	Concentration, Particulate location and Distribution Molecular Weight, Physiochemical properties Drug Carrier Interaction
Carrier	Type and Amount of Excipients Surface Characteristics, Size Density
In Vivo Environment	pH, Polarity, Ionic Strength, Surface Tension, Viscosity, Temperature, Enzyme Electric Field

Strategies of Drug Targeting [2]

A. **Passive Targeting:** Drug delivery systems which are targeted to systemic circulation are characterized as Passive delivery systems. The ability of some colloid to be taken up by the Reticulo Endothelial Systems (RES) especially in liver and spleen made them ideal substrate for passive hepatic targeting of drugs.

B. **Inverse Targeting:** In this type of targeting attempts are made to avoid passive uptake of colloidal carrier by RES and hence the process is referred to as inverse targeting. To achieve inverse targeting, RES normal function is suppressed by pre injecting large amount of blank colloidal carriers or macromolecules like dextran sulphate. This approach leads to saturation of RES and suppression of defence mechanism. This type of targeting is an effective approach to target drug(s) to non-RES organs.

C. **Active targeting:** In this approach carrier system bearing drug reaches to specific site on the basis of modification made on its surface rather than natural uptake by RES. Surface modification technique include coating of surface with either a bioadhesive, non-ionic surfactant or specific cell or tissue antibodies (i.e. monoclonal antibodies) or by albumin protein.

   Active targeting can be affected at different levels:

   i. First order targeting (organ compartmentalization) - Restricted distribution of the drug carrier system to the capillary bed of a pre-determined target site, organ or tissue.
   ii. Second order targeting (cellular targeting) - The selective drug delivery to a specific cell type such as tumour cells (& not to the normal cells)
   iii. Third order targeting (intercellular organelles targeting) - Drug delivery specifically to the intracellular organelles of the target cells

D. **Ligand-mediated Targeting:** In his approach ligands are used as carrier surface group(s), which can selectively direct the carrier to the pre-specified site(s) housing the appropriate receptor units to serve as 'homing device' to the carrier/drug. Most of the carrier systems are colloidal in nature & can be specifically functionalized using various biologically-relevant molecular ligands including antibodies, polypeptides, oligosaccharides, viral proteins & fusogenic residues. The ligands confer recognition & specificity

upon drug carrier & endow them with an ability to approach the respective target selectivity & deliver the drug

Table 2: Examples of Ligands

Ligands	Target	Tumour target
Folate	Folate receptor	Overexpression of folate receptor
Transferrin	Transferrin receptor	Overexpression of transferrin receptor
Galactosamine	Galactosamine receptors on hepatocytes	Hepatoma

E. **Physical Targeting:** This approach was found exceptional for tumour targeting as well as cytosolic delivery of entrapped drug or genetic material. Characteristics of environment changes like pH, temperature, light intensity, electric field, and ionic strength.

Table 3: Physical Targeting Methods

Physical Targeting	Formulation System	Mechanism for Drug Delivery
Heat	Liposome	Change in Permeability
Magnetic Modulation	Magnetically Responsive Microspheres Containing Iron oxide	Magnetic Field can retard fluid Flow of particles.
Ultrasound	Polymers	Change in Permeability
Electrical Pulse	Gels	Change in Permeability
Light	Photo responsive Hydro Gels Containing AzoDerivatives	Change in Diffusion Channels, Activated by Specific Wavelength

F. **Dual Targeting:** In this targeting approach carrier molecule itself have their own therapeutic activity and thus increase the therapeutic effect of drug. For example, a carrier molecule having its own antiviral activity can be loaded with antiviral drug and the net synergistic effect of drug conjugate was observed.

G. **Double Targeting:** When temporal and spatial methodologies are combined to target a carrier system, then targeting may be called double targeting. Spatial placement relates to targeting drugs to specific organs tissues, cells or even subs cellular compartment .whereas temporal delivery refers to controlling the rate of drug delivery to target site.

H. **Combination Targeting:** These targeting systems are equipped with carriers, polymers and homing devices of molecular specificity that could provide a direct approach to target site.

Advantages of drug targeting:

i. Drug administration protocols may be simplified.
ii. Toxicity is reduced by delivering a drug to its target site, thereby reducing harmful systemic effects.
iii. Drug can be administered in a smaller dose to produce the desire effect.
iv. Avoidance of hepatic first pass metabolism.
v. Enhancement of the absorption of target molecules such as peptides and particulates.
vi. Dose is less compared to conventional drug delivery system.
vii. No peak and valley plasma concentration.
viii. Selective targeting to infections cells that compare to normal cells.

Disadvantages of drug targeting:

i. Rapid clearance of targeted systems.
ii. Immune reactions against intravenous administered carrier systems.
iii. Insufficient localization of targeted systems into tumour cells.
iv. Diffusion and redistribution of released drugs.
v. Requires highly sophisticated technology for the formulation.
vi. Requires skill for manufacturing storage, administration.
vii. Drug deposition at the target site may produce toxicity symptoms.
viii. Difficult to maintain stability of dosage form. E.g.: Resealed erythrocytes have to be stored at 4°C.
ix. Drug loading is usually law. E.g. As in micelles. Therefore it is difficult to predict/ fix the dosage regimen.

CARRIERS FOR TARGETING DRUGS [3]

LIPOSOMES [3, 4]

Liposome was first discovered in the early 1965 by Alec D. Bangham which is derived from the Greek word, where lipo means “fatty” constitution and soma means “structure”. Liposome are relatively small in size and it ranges from 50 nm to several micrometres in diameter. These are spherical vesicle in which aqueous core is entirely enclosed by one or more phospholipid bilayers. It having the unique ability to entrap both lipophilic and hydrophilic compounds. The hydrophobic or lipophilic molecules are inserted into the bilayer membrane, whereas hydrophilic molecules can be entrapped in the aqueous centre. Because of their biocompatibility, biodegradability, low toxicity, and aptitude

to trap both hydrophilic and lipophilic drugs and simplify site-specific drug delivery to tumour tissues, liposomes have increased rate both as an investigational system and commercially as a drug delivery system. Many studies have been conducted on liposomes with the goal of decreasing drug toxicity and/ or targeting specific cells.

Advantages:

i. Suitable for delivery of hydrophobic (e.g. amphotericin B) hydrophilic (e.g. cytrabine) and amphipathic agents.
ii. Liposome increases efficacy and therapeutic index of drug (actinomycin-D)
iii. Liposome increase stability via encapsulation
iv. Suitable for targeted drug delivery
v. Suitable to give localized action in particular tissue
vi. Suitable to administer via various routes
vii. Liposomes help to reduce the exposure of sensitive tissue to toxic drug.

Disadvantages:

i. Once administrated, liposome cannot be removed.
ii. Possibility of dumping, due to faulty administration.
iii. Leakage of encapsulated drug during storage.
iv. Low solubility
v. Production cost is high.

Classification [4]:

Based on structural parameters:

i. MLV: multilamellar large vesicles .0.5 µm. They have several bilayer
ii. OLV: oligo lamellar vesicles 0.1-1µm. Made up of 2-10 bilayer of lipid surrounding a large internal volume.
iii. UV: unilamellar vesicles (all size range)
iv. SUV: small unilamellar vesicle composed of single lipid bilayer with diameter ranging from 30-70 nm
v. MUV: medium unilamellar vesicle
vi. LUV: large unilamellar vesicle > 100 µm
vii. GUV: giant unilamellar vesicle >1µm
viii. MV: Multivesicular vesicle >1µm

Based on method of preparation:

i. REV: single or oligo lamellar vesicles made by reverse phase evaporation method
ii. MLV-REV: multilamellar vesicle made by reverse phase evaporation method
iii. SPLV: stable plurilamellar vesicle
iv. FATMLV: Frozen and thawed MLV
v. VET: vesicle prepared by extraction method
vi. DRV: dehydration-rehydration method

Based on composition and application:

i. Conventional Liposomes (CL): Neutral or negatively charged phospholipid and cholesterol
ii. Fusogenic Liposomes (RSVE): Reconstituted Sendai virus envelopes
iii. pH sensitive Liposomes: Phospholipid such as PE or DOPE with either CHEMS or OA
iv. Cationic Liposomes: Cationic lipids with DOPE
v. Long Circulatory (stealth) Liposomes (LCL): Liposome that persist for prolong period of time in the blood stream
vi. Immuno-Liposomes: immune liposome have specific antibody on their surface to enhance target site binding.

PREPARATION OF LIPOSOMES [2, 3, 4]

There are many ways of preparing liposomes. Some of the important methods are:

1. Hydration of lipids in presence of solvent

2. Ultrasonication

3. French Pressure cell

4. Solvent injection method

a) Ether injection method

b) Ethanol injection

5. Detergent removal Detergent can be removed by

a) Dialysis

b) Column chromatography

c) Bio-beads

6. Reverse phase evaporation technique

7. High pressure extrusion

8. Miscellaneous methods

a) Removal of Chaotropic ion

b) Freeze-Thawing

Characterisation of liposomes:

Broadly classified into three categories:

I. Physical characterisation: evaluates parameters including size, Shape, surface features, lamellarity, phase behaviour and drug release profile.
II. Chemical characterisation: includes those studies which establish the purity, potency of various lipophilic constituents.
III. Biological characterisation: establishes the safety and suitability of formulation for therapeutic application.

Table 4: Characterisation of Liposomes [4]

Parameters	Technique
Vesicle shape	Electron microscopy
Lamellarity	Freeze fracture electron microscopy, p-31, Nuclear magnetic resonance spectroscopy.
Vesicle size and distribution	Light microscopy, fluorescent microscopy, Electron microscopy, laser scattering photon correlation spectroscopy, gel permeation technique
Surface morphology and size of Vesicles	Cryo- transmission electron microscopy.
Encapsulation efficiency	Mini column centrifugation method, protamine aggregation method
Phase response and transitional behaviour	Freeze fracture electron microscopy, differential scanning calorimetry
Drug release	In vitro diffusion cell

Applications of Liposomes [3, 4]:

Cancer chemotherapy:

Liposome are successfully used to entrap anticancer drugs. This increases circulation life time, protect from metabolic degradation.

Liposome as carrier of drug in oral treatment:

Steroids used for arthritis can be incorporated into large MLVs.

Alteration in blood glucose levels in diabetic animals was obtained by oral administration of liposome encapsulated insulin.

Liposome for topical application:

Drug like triamcinolone, methotrexate, benzocaine, corticosteroids etc. Can be successfully incorporated as topical liposome.

Liposome for pulmonary delivery:

Inhalation devises like nebulizers are used to produce an aerosol of droplets containing liposome.

VIROSOMES [4]

Virosomes are immuno modulating liposomes consisting of surface glycoprotein of influenza virus (immune stimulating reconstituted influenza

virosome) muramyl dipeptide etc. Virosomes must be target oriented and their fusogenic characteristics could be exploited in genome grafting and cellular micro injection.

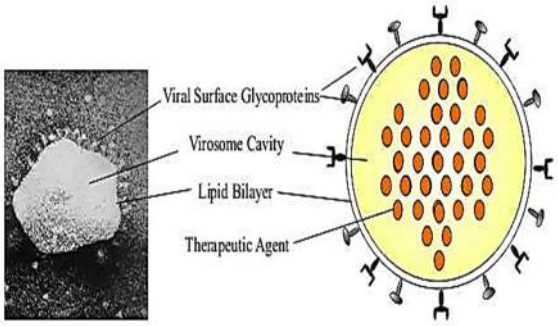

Figure 2: Virosomes for delivery of drug candidates at targeted sites

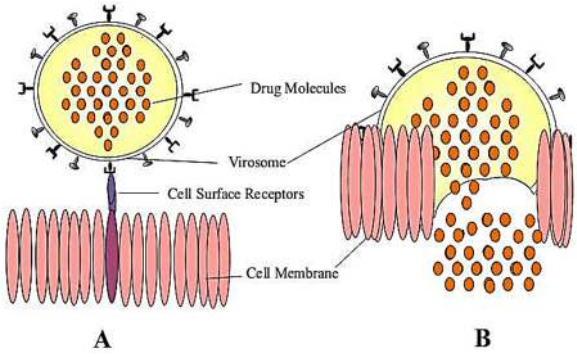

Figure 3: (A.) Interaction of the virosomes with cell surface receptors. (**B.)** Release of the encapsulated drug molecules in the target cell.

NIOSOMES [3, 4]

Niosomes are one of the novel drug delivery system of encapsulating the medicament in a vesicular system. The vesicle composed of a bilayer of non-ionic surfactants and hence the name niosomes. The niosomes are very small, and microscopic in size (in nanometric scale). Although being structurally similar to liposomes, they have several advantages over them.

Advantages of Niosomes:

a. The vesicles may act as a depot, releasing the drug in a controlled manner.
b. They are osmotically active and stable, and also they increase the stability of entrapped drug.
c. They improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells. The surfactants used are biodegradable, biocompatible and non-immunogenic.
d. They improve oral bioavailability of poorly absorbed drugs and enhance skin penetration of drugs. They can be made to reach the site of action by oral, parenteral as well as topical routes.
e. Handling and storage of surfactants requires no special conditions.
f. Due to the unique infrastructure consisting of hydrophilic, amphiphilic and lipophilic moieties together they, as a result can accommodate drug molecules with a wide range of solubilities.

g. Niosomal dispersion in an aqueous phase can be emulsified in a non-aqueous phase to regulate the delivery rate of drug and administer normal vesicle in external non-aqueous phase.

Disadvantages of Niosomes

a. Physical instability of the noisome vesicles is major disadvantage of the noisome drug delivery system. Aggregation: Aggregation of the noisome vesicles can be another disadvantage to be considered. Fusion: Fusion of the niosomal vesicles to form loose aggregates or to fuse into larger vesicles will affect the uniformity of the size of the noisome vesicles.
b. Leaking of entrapped drug: leakage of the entrapped drugs from the polymer system will affect the intended properties of the niosomes.
c. Hydrolysis of encapsulated drugs which limiting the shelf life of the dispersion.

***Types of Niosomes*:**

The niosomes are classified as a function of the number of bilayer (e.g. MLV, SUV) or as a function of size. (E.g. LUV, SUV) or as a function of the method of preparation (e.g. REV, DRV).

1. *Multilamellar vesicles (MLV):* It consists of a number of bilayer surrounding the aqueous lipid compartment separately. The approximate size of these vesicles is 0.5-10 µm diameter. Multilamellar vesicles are the most widely used niosomes. These vesicles are highly suited as drug carrier for lipophilic compounds.

2. *Large unilamellar vesicles (LUV):* Niosomes of this type have a high aqueous/lipid compartment ratio, so that larger volumes of bio-active materials can be entrapped with a very economical use of membrane lipids.

3. *Small unilamellar vesicles (SUV):* These small unilamellar vesicles are mostly prepared from multilamellar vesicles by sonication method, French press extrusion electrostatic stabilization is the inclusion of dicetyl phosphate in 5(6)-carboxyfluorescein (CF) loaded Span

60 based niosomes.

Applications of Niosomes

Some of the applications of niosomes in various diseases are either proven or research are still being carried out:

Drug Targeting:

Niosomes are often used for target drugs to the reticulo-endothelial system. The reticulo- endothelial system (RES) preferentially takes up niosome vesicles. The uptake of niosomes is controlled by circulating serum factors called opsonins. These opsonins mark the niosome for

clearance. Such localization of drugs is utilized to treat tumours in animals known to metastasize to the liver and spleen. This localization of drugs can also be used for treating parasitic infections of the liver.

Niosomes can also be utilized for targeting drugs to organs other than the RES. A carrier system (such as antibodies) can be attached to niosomes (as immunoglobulins bind readily to the lipid surface of the niosome) to target them to specific organs. Many cells also possess the intrinsic ability recognize and bind specific carbohydrate determinants, and this can be exploited by niosomes to direct carrier system to particular cells.

Anti-neoplastic Treatment:

Most antineoplastic drugs cause severe side effects. Niosomes can alter the metabolism, prolong circulation and half-life of the drug, thus decreasing the side effects of the drugs.

Niosomal entrapment of Doxorubicin and Methotrexate (in two separate studies) showed beneficial effects over the unentrapped drugs, such as decreased rate of proliferation of the tumour and higher plasma levels accompanied by slower elimination.

Leishmaniasis:

Leishmaniasis is a disease in which a parasite of the genus Leishmania invades the cells of the liver and spleen. Commonly prescribed drugs for the treatment are derivatives of antimony (antimonials), which in higher concentrations can cause cardiac, liver and kidney damage. Use of niosomes in tests conducted showed that it was possible to administer higher levels of the drug without the triggering of the side effects, and thus allowed greater efficacy in treatment. Delivery of Peptide Drugs:

Oral peptide drug delivery has long been faced with a challenge of bypassing the enzymes which would breakdown the peptide. Use of niosomes to successfully protect the peptides from gastrointestinal peptide breakdown is being investigated. In an in vitro study conducted by Yoshida et al, oral delivery of a vasopressin derivative entrapped in niosomes showed that entrapment of the drug significantly increased the stability of the peptide.

Use in Studying Immune Response:

Due to their immunological selectivity, low toxicity and greater stability; niosomes are being used to study the nature of the immune response provoked by antigens.

Niosomes as Carriers for Haemoglobin:

Niosomes can be used as carriers for haemoglobin within the blood. The niosomal vesicle is permeable to oxygen and hence can act as a carrier for haemoglobin in anaemic patients.

UFASOMES [4]

These are bilayer structures formed by using single chain unsaturated fatty acids. Pharmacosomes: The term pharmacosome comprises of two main parts Pharmacon (active principle) and some carriers postulated that amphipathic drug can self-assemble to form vesicle and these vesicles are termed as pharmacosomes. Drug covalently bound to lipid may exist in a colloidal dispersion as ultrafine, micelles or hexagonal aggregates which are known as pharmacosomes.

CUBOSOMES [4]

Cubosomes are liquid crystalline phase forming small cubic particles suitable for injection.

TRANSFEROSOMES [5]

A transferosomes, in functional terms, may be described as lipid droplets of such deformability that permits its easy penetration through the pores much smaller than the droplets size. Transferosomes is a supramolecular entity that can pass through a permeability barrier and there by transport material from the other site. • These are more elastic than standard liposomes.

NANOPARTICLES [5, 6]

Rolland et. al., (1989) designed a site specific drug delivery system consisting of poly metacryclic nanoparticles. The main goal in designing nanoparticles as a delivery system is to control size of particle, surface characteristics and discharge of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen.

***Advantages of Nanoparticles*:**

1. Increases the stability of any volatile pharmaceutical agents, easily and cheaply fabricated in large quantities by a multitude of methods.
2. They offer a significant improvement over traditional oral and intravenous methods of administration in terms of efficiency and effectiveness.
3. Delivers a higher concentration of pharmaceutical agent to a desired location.
4. The choice of polymer and the ability to modify drug release from polymeric nanoparticles have made them ideal candidates for cancer therapy, delivery of vaccines, contraceptives and delivery of targeted antibiotics.
5. Polymeric nanoparticles can be easily incorporated into other activities related to drug delivery, such as tissue engineering.

Disadvantages of Nanoparticles:

1. Small size & large surface area can lead to particle aggregation.

2. Physical handling of nano particles is difficult in liquid and dry forms.
3. Limited drug loading.
4. Toxic metabolites may form. etc.

Preparation of Nanoparticles **[6]**

Nanoparticles can be prepared from a variety of materials such as polysaccharides, proteins and Synthetic polymers. Selection of matrix materials depends on many factors including:

a) Size of nanoparticles required
b) Inherent properties of the drug, e.g., stability
c) Surface characteristics such as charge and permeability
d) Degree of biodegradability, biocompatibility and toxicity
e) Drug release profile desired
f) Antigenicity of the final product.

Different techniques like polymerization, preformed polymers or ionic gelation etc are used.

 Preparation of nanoparticles from dispersion of preformed polymer:

Dispersion of drug in preformed polymers is a common technique used to prepare biodegradable nanoparticles from poly (lactic acid) (PLA), poly (D, L-glycolide) (PLG), poly (D, L-lactide-co-glycolide) (PLGA). These can be accomplished by different methods described below.

a) Solvent evaporation

b) Nano precipitation

c) Emulsification/solvent diffusion

d) Salting out

e) Dialysis

f) Supercritical fluid technology (SCF)

 Preparation of nanoparticles from polymerization of monomers

a) Emulsion

b) Mini emulsion

c) Micro emulsion

d) Interfacial polymerization

e) Controlled/Living radical polymerization

 Ionic gelation or coacervation of hydrophilic polymers

Table 5: Techniques for Physicochemical Characterization of Nanoparticles [6]

Parameters	Technique
Particle size and morphology	Transmission electronic microscopy, scanning (electron, force, tunneling) microscopy, freeze-fracture electron microscopy, photon correlation spectroscopy
Drug content in vitro drug release	Ultra centrifugation followed by quantitative analysis of in vitro release characteristics under physiologic and sink conditions.
Molecular weight crystallinity	Gel permeation chromatography, X-ray diffraction, differential scanning calorimetry
Surface charge Surface hydrophobicity	Zeta potential measurement, hydrophobic interaction chromatography, contact angle measurement
Surface chemical analysis	Secondary ion mass spectrometry, X-ray photoelectron spectroscopy, nuclear magnetic resonance, Fourier transform Infrared spectroscopy
Protein adsorption	Two- dimensional polyacrylamide gel electrophoresis

***Applications of Nano Particulate Drug Delivery Systems* [6]:**

i. *Vaccine adjuvant. DNA delivery.*
ii. *Ocular delivery.*
iii. *Internalization:* Internalization within mammalian cells can be achieved by surface functionalized carbon nanotubes
iv. *Vaccine delivery:* Conjugation with peptides may be used as vaccine delivery structures
v. *Gene delivery:* with the advancement in molecular dynamics simulations, the flow of water molecules through surface-functionalized carbon nanotubes has been modelled in such a way so that they can be conveniently utilized as small molecule transporters in transporting DNA, indicating potential use as a gene delivery tool.
vi. *Transport of peptides, nucleic acids and other drug molecules*: Incorporation of carboxylic or ammonium groups to carbon nanotubes enhances their solubility which makes them more suitable for the transport of peptides, nucleic acids and other drug molecules.
vii. *Reduced toxicity and increases the efficacy*.
viii. *Cancer therapy:* This technology is being evaluated for cancer therapy. Nanoshells are tuned to absorb infrared rays when exposed from a source outside the body and get heated and cause destruction of the tissue. This has

been studied in both in vitro and in vivo experiments on various cell lines.

ix. *Diagnostic purposes:* They are useful for diagnostic purposes in whole blood immunoassays e.g. coupling of gold nanoshells to antibodies to detect immunoglobulins in plasma and whole blood. etc.

NANO TUBES [5]

They are hollow cylinder made of carbon, atoms which can be filled and sealed for potential drug delivery.

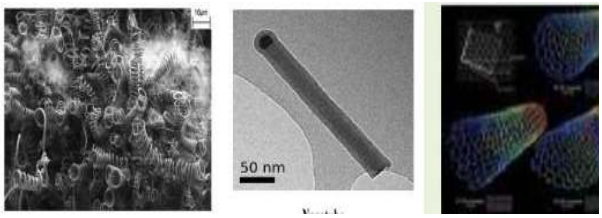

Figure 4: Nanotubes as drug delivery carriers

***Application*:** Cellular scale needle for attaching drug molecule(s) to cancer cells. As an electrode in thermo cells.

NANOCRYSTALS [5]

Nanocrystal is any Nano material with at least one dimension ≤ 100nm and that is single crystalline. More properly, any material with a dimension of less than 1 micrometre, i.e., 1000 nanometers, should be referred to as a nanoparticle, not a Nanocrystal. For example, any particle which exhibits regions of crystallinity should be termed nanoparticle or nanocluster based on dimensions.

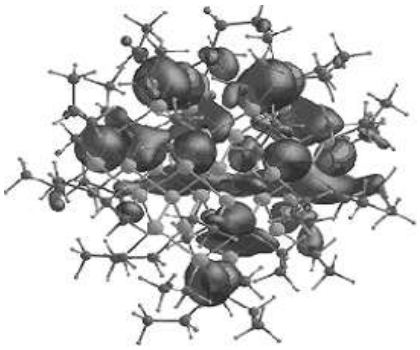

Figure 5: Nanocrystals as drug carriers

NANO WIRES [5]

The nanowire pinpoint damage from injury and stroke, localize the cause of seizures, and detect the presence of tumours and other brain abnormalities.

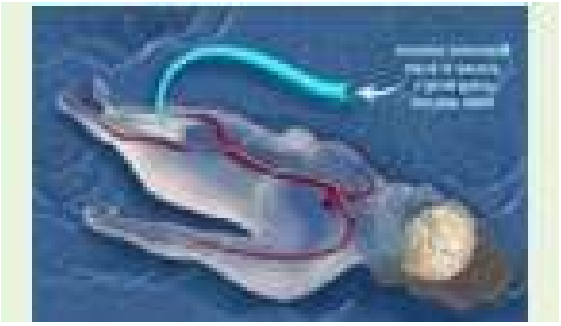

Figure 6: Nanowires

Application: Technique has potential as a treatment for Parkinson's and similar diseases.

NANOSHELLS:

Nanoshells are hollow silica spheres covered with gold. Scientists can attach antibodies to their surfaces, enabling the

shells to target certain shells such as cancer cells.

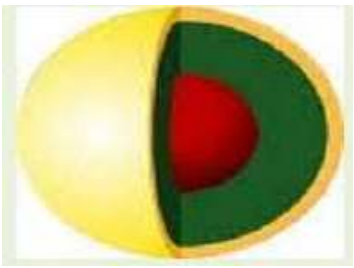

Figure 7: Nanoshells

Application: Technique has potential for targeting anticancer drugs to the targeted cancer cells.

NANOBOTS

Nanorobotics is the technology of creating machines or robots at or close to the microscopic scale of a nanometer (10−9meters). More specifically, nanorobotics refers to the still largely hypothetical nanotechnology engineering discipline of designing and building nanorobots, devices ranging in size from 0.1-10 micrometers and constructed of nano scale or molecular components.

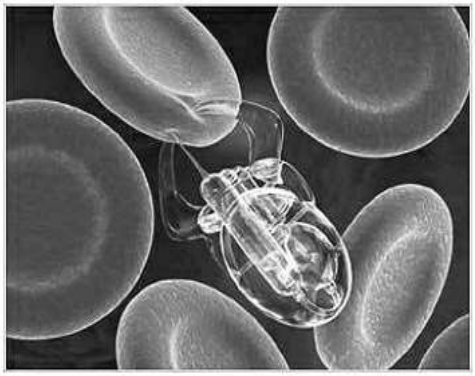

Figure 8: Nanobots

QUANTUM DOTS

Quantum dots are miniscule semiconductor particles that can serve as sign posts of certain types of cells or molecules in the body.

Figure 9: Quantom dots as drug delivery carriers

Application: Technique has potential for targeting cancerous drug.

NANO PORES:

Engineered into particles, they are holes that are so tiny that DNA molecules can pass through them one strand at a time, allowing for highly precise and efficient DNA sequencing.

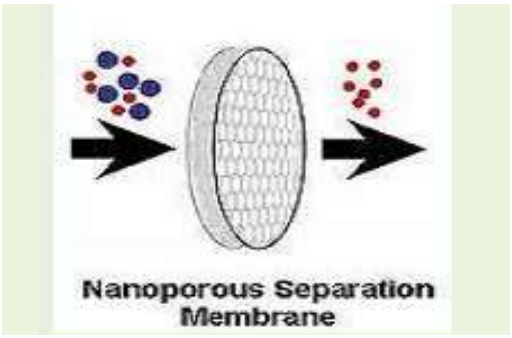

Figure 10: Nanopores as drug delivery carriers for target specific drug delivery

Application: Potential in genetic engineering and bio technology.

GOLD NANOPARTICLES:

Particle Scientist uses gold nanoparticle to develop ultrasensitive detection system for DNA and protein markers associated with many forms of cancer, including breast prostate cancer.

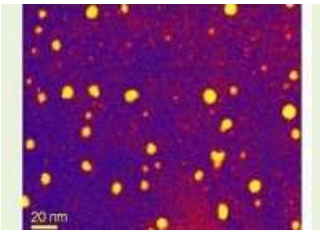

Figure 11: Gold nanoparticles as drug delivery carriers

Application: In cancer Treatment and Genetic engineering.

DENDRIMERS:

Dendrimers precisely defined, synthetic nanoparticles that are approximately 510 nm in diameter. They are made up of layers of polymer surrounding a control core. The dendrimers surface contains many different sites to which drugs may be attach.

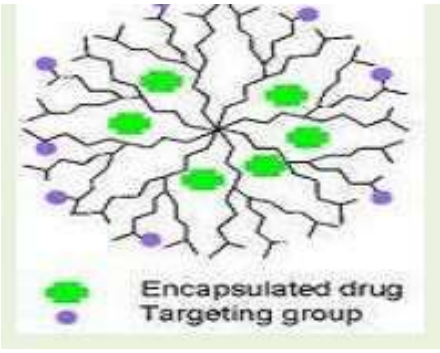

Figure 12: Dendrimers for encapsulation of drug to be targeted pr delivered at the site

Application: In gene transfection, medical imaging

MONOCLONAL ANTIBODIES [7, 8]:

An antibody is a protein used by immune system to identify and neutralize foreign objects like bacteria and viruses. Each antibody recognizes a specific antigen unique to its target. The high specificity of antibodies makes them an excellent tool for detecting and quantifying a broad array of targets, from drugs to serum proteins to microorganisms. With in-vitro assays, antibodies can be used to precipitate soluble antigens, agglutinate (clump) cells, opsonize and kill bacteria with the assistance of complement, and neutralize drugs, toxins, and viruses.

Monoclonal antibodies (mAB) are single type of immunoglobulin that are identical and are directed against a specific epitope (antigen, antigenic determinant) and are produced by B- cell clones of a single parent or a single hybridoma cell line. A hybridoma cell line is formed by the fusion of one B-cell lymphocyte with a myeloma cell. Some myeloma cell synthesize single mAB antibodies naturally. Derivation from a single B-cell clones and subsequent targeting of a single epitope is what differentiates monoclonal antibodies from polyclonal antibodies. Polyclonal antibodies are antibodies that are derived

from different cell lines. They differ in amino acid sequences.

History and Development [7]

- Paul Enrlich at the beginning of 20th century coined the term "magic bullets" and postulated that, if a compound could be made that selectively targets a disease-causing organism, then a toxin for that organism could be delivered along with the agent of selectivity.
- In the 1970s, the B-cell cancer multiple myeloma was known. It was understood that these cancerous B-cells all produce a single type of antibody (a paraprotein).
- In 1975, Kohler and Milstein provided the most outstanding proof of the clonal selection theory by fusion of normal and malignant cells (Hybridoma technology) for which they received Nobel Prize in 1984.
- In 1986, first monoclonal antibody was licenced by FDA. Orthoclone OKT3 (muromonab-CD3) which was approved for use in preventing kidney transplant rejection.

***Advantages of Monoclonal antibodies*:**

a) Though expensive, monoclonal antibodies are cheaper to develop than conventional drugs because it is based on tested technology.
b) Side effects can be treated and reduced by using mice-human hybrid cells or by using fractions of antibodies.
c) They bind to specific diseased or damaged cells needing treatment.
d) They treat a wide range of conditions.

***Disadvantages of Monoclonal antibodies*:**

a) Time consuming project - anywhere between 6 -9 months.
b) Very expensive and needs considerable effort to produce them.
c) Small peptide and fragment antigens may not be good antigens-monoclonal antibody may not recognize the original antigen.
d) Hybridoma culture may be subject to contamination.
e) System is only well developed for limited animal and not for other animals.
f) More than 99% of the cells do not survive during the fusion process – reducing the range of useful antibodies that can be produced against an antigen
g) It is possibility of generating immunogenicity.

***Application of Monoclonal antibodies*[8,9]**

1. ***Diagnostic Applications:***

Monoclonal antibodies have revolutionized the laboratory diagnosis of various diseases. For this purpose, MAbs may be employed as diagnostic reagents for

biochemical analysis or as tools for diagnostic imaging of diseases.

(A) MAbs in Biochemical Analysis:

Diagnostic tests based on the use of MAbs as reagents are routinely used in radioimmunoassay (RIA) and enzyme-linked immunosorbent assays (ELISA) in the laboratory. These assays measure the circulating concentrations of hormones (insulin, human chorionic gonadotropin, growth hormone, progesterone, thyroxine, triiodothyronine, thyroid stimulating hormone, gastrin, renin), and several other tissue and cell products (blood group antigens, blood clotting factors, interferon's, interleukins, histocompatibility antigens, tumour markers). In recent years, a number of diagnostic kits using MAbs have become commercially available. For instance, it is now possible to do the early diagnosis of the following conditions/diseases.

Pregnancy:

Pregnancy by detecting the urinary levels of human chorionic gonadotropin.

Cancers:

Cancers estimation of plasma carcinoembryonic antigen in colorectal cancer, and prostate specific antigen for prostate cancer. Besides diagnosis, estimation of tumor markers is also useful for the prognosis of cancers. That is a gradual fall in a specific tumor marker is observed with a reduction in tumor size, following treatment.

Hormonal disorders:

Hormonal disorders analysis of thyroxine, triiodothyronine and thyroid stimulating hormone for thyroid disorders.

Infectious diseases:

Infectious diseases by detecting the circulatory levels of antigens specific to the infectious agent e.g., antigens of Neisseria gonorrhoea and herpes simplex virus for the diagnosis of sexually transmitted diseases.

(B) MAbs in Diagnostic Imaging:

Radiolabeled—MAbs are used in the diagnostic imaging of diseases, and this technique is referred to as immunoscintigraphy. The radioisotopes commonly used for labelling MAb are iodine—131 and technetium—99. The MAb tagged with radioisotope are injected intravenously into the patients.

These MAbs localize at specific sites (say a tumor) which can be detected by imaging the radioactivity. In recent years, single photon emission computed tomography (SPECT) cameras are used to give a more sensitive three dimensional appearance of the spots localized by radiolabeled—MAbs.

Immunoscintigraphy is a better diagnostic tool than the other imaging techniques such as CT scan, ultrasound scan and magnetic resonance. For instance, immunoscintigraphy can differentiate between cancerous and non-cancerous growth, since radiolabeled—MAbs are tumor specific. This is not possible with other imaging techniques. Monoclonal antibodies are successfully used in the diagnostic imaging of cardiovascular diseases, cancers and sites of bacterial infections [9].

2. Therapeutic Applications:

A. Cardiovascular diseases: Myocardial infarction:

The cardiac protein myosin gets exposed wherever myocardial necrosis (death of cardiac cells) occurs. Antimyosin MAb labelled with radioisotope indium chloride (111 In) is used for detecting myosin and thus the site of myocardial infarction. Imaging of radiolabeled MAb, is usually done after 24-48 hours of intravenous administration.

This is carried out either by planner gamma camera or single photon emission computed tomography (SPECT). It is possible to detect the location and the degree of damage to the heart by using radiolabeled antimyosin MAb. Thus, this technique is useful for the diagnosis of heart attacks.

B. Deep vein thrombosis (DVT):

DVT refers to the formation of blood clots (thrombus) within the blood veins, primarily in the lower extremities. For the detection of DVT, radioisotope labelled MAb directed against fibrin or platelets can be used. The imaging is usually done after 4 hours of injection. Fibrin specific MAbs are successfully used for the detection of clots in thigh, pelvis, calf and knee regions. Atherosclerosis:

Thickening and loss of elasticity of arterial walls is referred to as atherosclerosis. Atherosclerotic plaques cause diseases of coronary and peripheral arteries. Atherosclerosis has been implicated in the development of heart diseases. MAb tagged with a radiolabel directed against activated platelets can be used to localize the atherosclerotic lesions by imaging technique [11].

CONCLUSION

Targeted drug delivery is now developing fast due to its potential to deliver drugs at specific sites. This causes injection of a lower amount of dose as well as a significant decrease in side effects that were more pronounced earlier because of the inefficacy of any drug delivery system to deliver drugs at the specific site of action. The application of nanotechnology in drug delivery has particularly enhanced the

delivery of drugs. There are numerous nanoparticles that have been approved for clinical use and, although they are still in their development stages, they hold the key to the future of drug-targeting. Several other approaches have also been developed with similar results. They all outline the bright future of targeted drug delivery.

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