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### Review Article

## Nanoparticles: An Overview

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ABSTRACT

#### ARTICLE DETAILS

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Keywords: Ligands, Asialoglycoprotein, Phagocytosis, Galactose/mannose, Biodegradable polymers. In the recent years, the potential use of polymeric nanoparticles as carriers for a wide range of drugs for therapeutic applications has been increased due to their versatility and wide range of properties. Due to limitations in the conventional drug therapy the increased risk of adverse reactions will occur. Nanoparticles provide the action at the desired sites and thus gaining importance now days. With these nanoparticles the specific targeting to various cells or receptors can be achieved. There are various mechanisms exists which are responsible for cellular internalization and cellular uptake.

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#### INTRODUCTION

Nanoparticles can be defined as the colloidal particles having size ranging from 10 to 1000 nm. The advantages of nanotechnology is to provide the safe and the effective medicine (Nanomedicine) is set to substantially influence the landscape of both pharmaceutical and biotechnology industries<sup>[1]</sup>.

They have application in various fields of life sciences such as separation technologies, histological studies, clinical diagnostic assays and drug delivery systems (DDS). The application of the nanoparticle for the DDS is of particular interest because they have some advantages such as easy purification and sterilization, drug targeting possibilities, and sustained release [2] However, thev action have some disadvantages such as they can be easilv recognized by the reticuloendothelial system (RES) within seconds or minutes after injection, which is due to phagocytosis by macrophages present in the liver and spleen. The surface modification of nanoparticles by albumin or hydrophilic poly(ethylene oxide) chains have reduced the recognition of nanaoparticles and thus they are available for the more prolonged circulation, increased half-life in blood and a reduced rate of uptake by the liver .Especially, active targeting of carriers to specific cells using antibodies, or sugars has been attempted [3].

\*Author for Correspondence: Email: archana.dhyani89@gmail.com A large number of drugs can be delivered using nanoparticulate carriers via a number of routes. These include many hydrophilic drugs, hydrophobic drugs as well as for proteins, vaccines, biological macromolecules, etc. They can be formulated for targeted delivery to the lymphatic system, brain, arterial walls, lungs, liver, spleen, or made for long-term systemic circulation <sup>[4]</sup>.

#### Method of Preparation of Nanoparticles

The various methods can be used for preparation of nanoparticles. For example, drugs can be entrapped in the polymer matrix, encapsulated in a nanoparticle core, surrounded by a shell- like polymer membrane, chemically conjugated to the polymer, or either it may be bound to the particle's surface by adsorption.

## a) Emulsification solvent evaporation technique

One of the methods used for the preparation of nanoparticles is emulsification solvent evaporation technique.It is basically used for encapsulating hydrophobic drugs, but shows the poor results for incorporation of bioactive agents of a hydrophilic nature. The solvent evaporation is carried out the polymer and the compound are dissolved in an organic solvent such as chloroform, ethyl acetate, or methylene chloride and then it is emulsified in an aqueous phase containing a stabilizer (e.g., PVA). Just after formation of the nanoemulsion the solvent diffuses to the external phase until saturation. The solvent molecules that reach the water-air

interphase evaporate, which leads to continuous diffusion of the solvent molecules from the inner droplets of the emulsion to the external phase; simultaneously, the precipitation of the polymer leads to the formation of nanospheres.



Figure 1: Type of biodegradable nanoparticles: According to the structural organization biodegradable nanoparticles are classified as nanocapsule, and nanosphere

In many cases, the induction of nanosized polymer droplets can be done by sonication or homogenization. The organic solvent is then evaporated and the nanoparticles are usually collected by centrifugation and lyophilization <sup>[5]</sup>.

The small changes in this method are used for encapsulating hydrophilic compounds and proteins, which can be done by, the double, or multiple emulsion technique. As the name signifies firstly, a hydrophilic drug and a dissolve in water.The primary stabilizer emulsion is prepared by dispersing the aqueous phase into an organic solvent containing a dissolved polymer. This is then emulsified in an aqueous outer phase also containing stabilizer. The nanoparticles can be achieved by the solvent evaporation method. One of the main problems associated for the encapsulation of a hydrophilic molecule like a protein or peptidedrug is the rapid diffusion of the molecule into aqueous phase during the outer the emulsification. This can result in poor encapsulation efficiency, i.e. drug loading <sup>[6]</sup>.

## b) Emulsification Diffusion method

Another method which can be used for preparation of nanoparticles is the emulsification diffusion method. The method utilizes a partially

water-soluble solvent like acetone or propylene carbonate. The polymer and the drug are dissolved in the solvent and it is emulsified in the aqueous phase containing the stabilizer. The role of stabilizer prevents the aggregation of emulsion droplets by adsorbing of the surface of the droplets. Addition of water to the emulsion, allow the diffusion of the solvent into the water. The solution is stirred leading to the nanoprecipitation of the particles. Further, it can be collected by centrifugation, or the solvent can be removed effectively by dialysis. The main problem with this method is that the water soluble drugs tend to leak out from the polymer phase during diffusion steps. So, in order to avoid this problem the dispersing medium changed from aqueous medium to medium chain triglycerides and a small amount of surfactant is added into it. The nanpoarticles are collected from the oily suspension by centrifugation<sup>[7]</sup>.

## c) Nanoprecipitation method

Nanoparticles can be synthesized by the nanoprecipitation method. In this method. polymer and drug are dissolved in acetone, ethanol, or methanol and incorporated under magnetic stirring into an aqueous solution of the surfactant. The organic solvent diffuses instantaneously to the external aqueous phase, followed by precipitation of the polymer and drug. After formation of the nanoparticles, the solvent is eliminated and the suspension concentrated under reduced pressure. The advantage of this method is that no surfactant is employed; however, the method is limited to drugs that are highly soluble in a polar solvent [8].

## d) Salting-out method

The salting-out process is another method for the preparation of nanoparticles.This technique is based on the precipitation of a hydrophobic polymer, is useful for the encapsulation of either hydrophilic or hydrophobic drugs because a variety of solvents, including polar (e.g., acetone or methanol) and non-polar (methylene chloride or chloroform) solvents can be chosen for dissolving the drug <sup>[7-8]</sup>.

## Polymeric nanoparticles

Controlled release systems generally refer to technologies or the sytems in which the drugs are released at predetermined and/or tuneable rates, or in response to some external stimuli and triggers. Polymers can be used as a controlled release system since they have unique physicochemical, synthetic, biocompatibility, and degradation properties.

Process	Examples of Materials	Solvent	Stabilizer	Remarks	References
Polymerization	PECA	-	Pluronic F68	Monomers are polymerized in an aqueous solution. Drug is	68
	PLGA/PLA/ PCL	Acetone	Pluronic F-68	incorporated either by being dissolved in the polymerization medium or by adsorption onto the nanoparticles after polymerization completed.	69
Nanoprecipitation	PLGA	Acetonitrile	-	Polymer and drug dissolved in acetone and added in aqueous	70
	PLA-PEG- PLA	DCM	PVA	solution containing Pluronic F-68.The acetone is evaporated under reduced pressure	71
Solvent evaporation	PLGA	DCM	-	Polymer and the compound in an organic solvent is emulsified in	72
	PLA	Acetone	PVA	an aqueous phase containing a stabilizer and the organic solvent is evaporated.	73
Salting out	PLA	Acetone/MC	Pluronic F68	W/O emulsion is formed containing polymer,acetone, stabilizer,drug. Water is added until volume is sufficient to allow for diffusion of acetone into the water.	74
Solvent displacement	SB-PVA-G- PLA	Acetone/ethy l acetate	Poloxamer188	Drug was dissolved in a mixture of two organic solvents (2 : 1) and then the polymer was added and completely dissolved in this organic phase. The polymeric drug solution is added in	75
				aqueous phase containing surfactant, and water is added under moderate magnetic stirring	

Table 1: Overview of Nanoparticles for drug delivery: Synthesis and material [68-75]

PACA - poly(aminocyanoacrylate), PLGA - poly(lacticcoglycolicacid), PLA - poly(lacticacid), PEG - poly(ethyleneglycol), PHDCA - poly(hexadecylcyanoacrylate), PCL - poly(caprolactone), SB-PVA-g-PLGA - sulfobutylated poly(vinyl alchol)grafted PLGA, DCM – dichloromethane, MC - methylene chloride, PVP - polyvinylpyrrolidone

Additionally, polymeric nanoparticles also have advantages over lipidic carriers such as liposomes. For example some of the limitations of liposomes include: their propensity to burst release cargo in vivo, a lack of compatibility with various active agents, a limited drug loading oxidation volume, the of liposomal phospholipids, and poor shelf-life stability.In contrast, polymeric drug delivery systems are comparably stable in vivo, having high drug loading capacities, and can employ both controlled or triggered release of drugs.Due to these properties, polymeric nanomaterials are well preferred than the lipid nanoparticles [8,9].

#### **Biodegradable polymers**

Nanoparticles can be synthesized from the biodegradable as well as non-biodegradable polymers.A wide range of the natural and the synthetic polymers can be used for the preparation.Today, the most commonly used polymers for controlled drug release applications include poly(D,L-lactide-co-glycolide) (PLGA), poly(lactic acid) (PLA), poly(glutamic acid) poly(caprolactone) (PGA), (PCL), N-(2hydroxypropyl)-methacrylate copolymers poly(amino acids). (HPMA), and In particular, PLGA, PGA and PLA have been widely used in an impressive number of controlled release products, particularly due to their favourable biocompatibility and biodegradability properties <sup>[10]</sup>.

PLGA has been the most common polymer used to make biodegradable nanoparticles, however, these are clearly not the optimal carrier for all drug delivery applications. For each application, one must evaluate the properties of the system (drug and particle) .For example,for poly(butyl cyanoacrylate) emulsion double solvent evaporation technique have been successful in delivering drugs to the brain <sup>[11]</sup>. Other cyanoacrylate-based nanoparticles such as polyalkylcyanoacrylate (PACA) and polyethylcyanoacrylate (PECA), have also been prepared. The cyanoacrylates may be considered as the promising drug delivery systems due to their various properties like mucoadhesive properties and ability to entrap biologically compounds. These polymers active are biodegradable, biocompatible, as well as compatible with a wide range of compatible drugs. Further these polymers having a faster degradation rate than PLGA, which in some cases may be more desirable. PECA nanoparticles have been prepared by emulsion polymerization technique <sup>[10,11]</sup>.



Figure 2: Chemical structures of various biodegradable polymers

The nanoparticles can also be prepared from the functionalized PLGA polymers.Jung et al., synthesized nanoparticles made of a branched, poly(2-sulfobutyl-vinyl alcohol)-g- PLGA . The purpose of using sulfobutyl groups attached to the hydrophilic backbone was to provide a higher affinity to proteins by electrostatic interactions.In another case,a carboxylic end group of PLGA was conjugated to a hydroxyl group of doxorubicin and formulated into nanoparticles. This modification produced a sustained release of the drug that was

approximately six times longer than with unconjugated drug<sup>[12]</sup>.

The main advantages of the controlled release systems were principally they enable the potential use of molecules that had short halflives as therapeutics so that their availability in the systemic circulation can be increased, to enhance patient compliance, to improve drug efficacy and reduce side effects by delivering agents locally, and to simplify dosing in cases where prolonged drug exposure was necessary <sup>[13]</sup>. For example, Zoladex, a PLGA copolymer impregnated with Goserelin acetate can be used for treatment of breast and prostate cancers and was approved by the FDA in 1998. It is basically injected subcutaneously so that the active agent released slowly into could be systemic circulation and reach its target sites <sup>[14]</sup>. In the same year, Lupron Depot, launched a PLGA microsphere formulation of leuprolide acetate, which was used the to treat advanced prostate cancers later it was approved by FDA <sup>[15]</sup>. Gliadel, a biodegradable Polifeprosan 20 carmustine-embedded wafer for the treatment of gliomas that became the first new treatment for gliomas in 20 years on its approval in 1996 <sup>[16]</sup> and Atridox, a polylactide and N-methyl-2pyrrolidone (NMP) polymer blend containing doxycycline hyclate for subgingival delivery, which was FDA approved in 1998 to treat periodontal disease <sup>[17]</sup>.

Another controlled release formulations of include Sandostatin LAR, a PLGA slow release formulation of octreotide acetate for tumor control in neuroendocrine disorders approved by FDA in 1998.Trelstar Depot, a PLGA based microparticle formulation of triptorelin pamoatea can be used for prostate cancer and other indications, was FDA approved in 2000 <sup>[18]</sup>. The ability to manufacture the polymeric formulations leads to the growing interest in the nanotechnology.

The molecular weight and concentration of the polymer will also affect the nanoparticles. The molecular weight of the polymer has opposite effects of nanoparticles and encapsulation efficiency <sup>[10]</sup>. Smaller size nanoparticles, approximately 100nm, can be prepared with lower molecular weight polymer; however, it reduces the drug encapsulation efficiency. On the other hand, increase in polymer concentration increases encapsulation efficiency and the size of the nanoparticles. When considering a particular polymeric drug delivery application, particle size

and the encapsulation efficiency are two of the most important characteristics of nanoparticles. Another, important characteristics of nanoparticles is zeta potential. It represents the particle stability <sup>[11]</sup>.

### Surface Modifications of Nanoparticles

Phagocytosis plays a critical and important physiological role in the defense mechanism.It protects the body against the infectious agents (most bacteria and some viruses) as well exogenous inert particles-including drug delivery nanoparticles.

Mechanism of opsonization and phagocytosis Phagocytosis occurs primarily in specialized cells, also called professional phagocytes and non-professional phagocytes: macrophages, monocytes, neutrophils and dendritic cell. These are professional phagocytes <sup>[19]</sup>. Other types of cells(fibroblasts, epithelial and endothelial cells), referred to as para and non-professional phagocytes, which shows,lower extent of phagocytosis <sup>[20]</sup>. The phagocytic pathway of entry into cells can be described using three distinct steps: recognition by opsonization in the bloodstream; adhesion of the opsonized particles to the macrophages; ingestion of the particle.

Opsonization is the process of attaching of the foreign nanoparticles by proteins called opsonins, so that the it can be recognise by macrophages.Major opsonins include immunoglubulins (IgG and M) as well as complement components (C3, C4, C5),<sup>[21,6]</sup> in blood serum proteins addition to other (including laminin, fibronectin, **C**-reactive protein, type-I collagen) <sup>[22]</sup>.

The opsonized particles then attach to the macrophage surfaces through the specific receptor-ligand interactions .The major and best-studied receptors for this purpose include the Fc receptors (FcR) and the complement receptors (CR). FcRs bind to the constant fragment of particle-adsorbed immunoglobulins, the best understood interaction involving IgG and FcR; CRs mostly bind to C3fragments <sup>[19,22]</sup>. Other receptors, involving in the phagocytosis are the mannose/ fructose and scavenger receptors, while new opsono-receptors like CD44 are still being discovered <sup>[23]</sup>.

However, in order to avoid opsonization, several methods of surface modifications have been developed so that polymeric colloidal particles can not recognized by the Reticuloendothelial systems(RES).So, the nanoparticles can be coated

with hydrophilic molecules in order to hide the hydrophobicity as the body considers the hydrophobic particles as the foreign and thus the can easily eliminates from the blood RES stream. The most common moiety for surface modification is the hydrophilic and non-ionic polymer polyethylene glycol (PEG). It has been largely demonstrated that the "PEGylation" increases their blood circulation and half-life by several orders of magnitude. Moreover, PEG exhibits excellent biocompatibility. an Poloxamer, poloxamines or Chitosan have also been studied for surface modification [24,10]. These groups can block the electrostatic and hydrophobic interactions that help opsonins to bind to particle surfaces.



Figure 3: Schematic representation of the endosomal pathway

On the other hand,the other application of surface modification is the targeting of tumors or organs of nanoparticles to increase selective cellular binding and internalization through receptor-mediated endocytosis <sup>[25]</sup>. Ligands need to be optimally conjugated on nanoparticles to maintain their affinity for receptors binding. As a sufficient PEG coating is essential for avoiding recognition by the RES, ligands should be extended away from the nanoparticle surfaces to avoid shielding by the PEG chains <sup>[26]</sup>.

Surface charges of nanoparticles also have an important influence on their interaction with cells and on their uptake. Positively charged nanoparticles seem to allow higher extent of internalization, apparently as a result of the ionic interactions established between positively charged particles and negatively charged cell membranes <sup>[27,28]</sup>. Moreover, positively charged nanoparticles seem to be able to escape from lysosomes after being internalized and exhibit

perinuclear localization, whereas the negatively and neutrally charged nanoparticles prefers to colocalize with lysosomes <sup>[29]</sup>. PLGA nanoparticles have negative charges which can be shifted to neutral or positive charges by surface modification, for example by PEGylation of the PLGA polymer [<sup>30]</sup> or chitosan coating <sup>[31]</sup>, respectively.

# Localisation of Nanoparticles: By Passive or Active Targeting

### Passive targeting

The majority of these nanoparticles exhibit prolonged circulation times in vivo and thus accumulate at particular sites simply due to blood hemodynamic forces and diffusive mechanisms. The advantages of utilizing passive targeting of nanoparticles in the field of oncology due to its widely reported "enhanced permeation and retention" (EPR) effect. Maeda et al., in the 1980's demonstrated the principle of passive targeting of colloidal particles to tumors. In their initial studies, significantly higher concentrations of the cytotoxic drug neocarzinostatin was discovered in tumor tissue postal administration of the polymer-drug conjugate poly(styrenecomaleic acid)-neocarzinostatin (SMANCS), in comparison to control experiments where the drug was administered in its free form <sup>[32]</sup>. This led Maeda et al. to postulate that the enhanced accumulation of the colloidal particles in the tumor was attributed to the structural features of the tumor vasculature, an observation, which was termed the EPR effect [33]. The EPR effect has observed with a wide been range of macromolecular agents such as proteins; including immunoglobulin G (IgG),drug-polymer conjugates, micelles, liposomes, polymeric nanoparticles and many other types of nanoparticles [34,35].

Current, the observations of EPR are the main premise for the design of tumor specific nanomedicines for drug delivery or imaging applications, however there are a number of factors that need to be considered [36,37]. The majority of passively targeted nanoparticles should be surface modified ,that is ,it's coated with PEG polymer for biocompatibility; to avoid the endocytic uptake by cancer cells within the tumour. This problem which has been referred to by some as the "PEG dilemma" [38,39] has been suggested to hamper efficient drug delivery in tumors as passively targeted nanoparticles end up releasing their therapeutic pay load into the tumor milieu rather than within cancer cells. However, in the case of cytotoxic drugs-many

have been shown to have longer elimination halflives in tumors than the normal tissue. Therefore, the delivery of higher amounts of drugs to tumors can lead to longer durations of drug exposure at higher concentrations and enhanced efficacy.For example, docetaxel (Dtxl) has an elimination half-life of 2.2–4.5 h in normal tissue and 22 h in tumours, demonstrating long tumor site retention relative to non-tumoral tissues <sup>[40]</sup>.

### Active targeting

Active targeting is based on the affinity of the ligands to direct the binding of nanoparticles to antigens, differently over expressed on the plasma membrane of diseased cells or to the extra-cellular matrix proteins that are differentially over expressed in the diseased tissue.

Targeting molecules can be either antibody or non-antibody ligands: The advantages of the non-antibody ligands, like peptides, sugars or vitamins, they are readily available, inexpensive to manufacture and easy to handle <sup>[41,42]</sup>. Actively targeted nanoparticles can be utilized in applications where drug release is either extracellular or intracellular. Therapies that act on intracellular sites of action are most effectively delivered with targeted nanoparticles [43] The various mechanisms for the internalization actively targeted NPs can be clathrin-dependent endocvtosis pathways. caveolin-assisted. adhesion cell molecule directed, or lipid raft associated mechanisms, leading to endosome formation, which ultimately leads to lysosomes. For hydrophobic small molecule drugs that can readily permeate through the lipid bilayer of the endosomal membrane, drug release within the endosome will result in permeation within the intracellular compartments. For delivery of bioactive macromolecules such as nucleic acids (DNA, siRNA, miRNA) or charged hydrophilic small molecules that are relatively impermeable to the endosomal membrane, the nanoparticles need to escape the endosome prior to fusion with lysosomes if NPs are to reach their desired subcellular compartments [44].

Antibodies, have a higher specificity and a wide range of binding affinities as compared to the non –antibody ligands. Ligand mediated targeting is also beneficial in the case of vascular endothelial targeting for both oncology and cardiovascular applications as well as other various diseases, and the identification of high affinity ligands for this purpose is an active area of research <sup>[42]</sup>. Typically, the ligand receptor binding involves the exploitation of a binding interaction of either a ligand at that site for an introduced receptor (targeting receptor) or of a receptor at that site for an introduced ligand (targeting ligand). The feasibility of using carbohydrate ligands to target protein receptors at sites of localization, termed 'glycotargeting', was first demonstrated in 1971 [<sup>45,46]</sup>. During this year,the potential of using carbohydrates to create or actively-targeted drug delivery system has been made clear. However, despite 30 years of research, there is no general therapeutic system on the market, and many challenges still exist.



Figure 4: Schematic representation of active and passive targeting

The asialoglycoprotein receptor (ASGP-R) which is particularly presents on mammilain hepatocytes can be utilize for active targeting by natural and synthetic ligands.By using its utilizing this receptors can provides an unique means for the development of liver-specific carriers, such as liposomes, recombinant lipoproteins, and polymers for drug or gene delivery to the liver, especially to hepatocytes [47]. These receptors recognize the ligands with terminal galactose or N-acetylgalactosamine residues, and endocytose the ligands for an intracellular degradation process. The use of its natural ligand, i.e. asialofetuin, [48] or synthetic ligands with galactosylated or lactosylated

residues, such as galactosylated cholesterol, glycolipids, or galactosylated polymers has achieved significant targeting efficacy to the liver <sup>[49]</sup>. There are several examples of successful targeted therapy for acute liver injury with asialofetuin-labeled and vitamin E-associated liposomes <sup>[50]</sup>.

#### Asialoglycoprotein receptor (ASGP-R)

Asialoglycoprotein receptor (ASGP-R), also called hepatic lectin, is responsible for the clearance of glycoproteins with desialylated galactose or acetylgalactosamine residues from the circulation by receptor-mediated endocytosis. It also involves the clearance of lipoproteins, and apoptotic cells. It is an integral transmembrane glycoprotein heteroligomer with an apparent molecular mass of 41 kD, which is composed of two structurally different subunits, H1 and H2, in human hepatocytes. H1 is the major species of the receptor and is seven times more abundant than H2. Both subunits are similar in molecular weight, and share 57% sequence homology [51]. Subunit H1 and H2 has an amino-terminal cytoplasmic tails, transmembrane domains that function as internal signal sequences, and carboxyl terminal extracellular domains, which contain N-linked oligosaccharide binding sites. galactose-binding extracellular domain The belongs to the long-form subfamily with three conserved intramolecular disulphide bonds. It isnable to bind terminal non-reducing galactose residues and N-acetylgalactosamine residues of desialylated tri or tetraantennary N-linked glycans <sup>[52]</sup>. Chronic ethanol exposure leads to impairment of receptors in rat hepatocytes due to hyperphosphorylation of ASGP-R<sup>[53]</sup>.

#### Carbohydrate recognition domain (CRD)

The specificity of the receptor for D-galactose or D-mannose is accomplished by specific hydrogen bonding of the 3 and 4-hydroxyl groups with carboxylate and amide side-chains. Therefore, mutation of the amino acid sequence in the carbohydrate recognition domain (CRD) results in a conversion of its specificity. The structure also provides a direct confirmation for the conversion of the ligand-binding site of mannose-binding protein to an ASGP-R-like specificity <sup>[54]</sup>.

#### Internalization of the ligands by ASGP-R

The internalization of the receptor-ligand complex occurs once the ligands bind to the extracellular domains of the receptors.

Identity	Ligand	Target	Nanoparticle	Active Pharmaceutical Ingredient (API)	Indication	Status	Reference
BIND-014	Small molecule	PSMA	Polymeric	Docetaxel	Solid tumours	Phase I	[76]
CALAA-01	Transferrin	Transferrin receptor	Polymeric	siRNA	Solid tumours	Phase I	[77]
MCC-465	Antibody fragment	Tumour antigen	Liposome	Doxorubicin	Metastatic stomach Cancer	Phase I	[78]
MBP-426	Transferrin	Transferrin receptor	Liposome	Oxaliplatin	Gastric, esophageal, gastroesophageal adenocarcinoma	Phase Ib/II	[79]
SGT53-01	Antibody fragment	Transferrin receptor	Liposome	p53 gene	Solid tumours	Phase Ib	[80]

 Table 2: Targeted Nanoparticles in clinical development [76-80]

PSMA: prostate specific membrane antigen.

The carbohydrate recognition domain binds to the specific carbohydrate residues in the extracellular space, and after endocytosis, the ligands are released into endosomes (lower pH). This process is pH dependent <sup>[55]</sup>. The acidification in endosomes leads to segregation of ligand from the receptor, with receptor molecules recycling back to the plasma membrane. Two subunits of the receptor are endocytosed at different average rates, and ligand binding increases the turnover rates of both subunits <sup>[56]</sup>.

Nanometer particles, generated from poly ( $\lambda$ benzyl L-glutamine) (PBLG) or poly(lactic acid) (PLA), were conjugated with poly(vinyl benzyllactonamide) (PVLA) as the carbohydratecarrying polystyrene (PS). The particles were loaded with colchicine, cytochalasin B and taxol for testing their feasibility in delivering the drugs to primary hepatocytes in vitro. It has been shown by confocal microscopy that the drugloaded particles coated with sugar-carrying polymers were internalized by the hepatocytes after one hour of incubation, and that the internalization process occurs via a receptormediated mechanism <sup>[49]</sup>.

## The Biophysicochemical Characteristics of Nanoparticles

The physicochemical properties of nanoparticles, such as size, geometry/shape,surface charge, surface chemistry, hydrophobicity, roughness, rigidity, and degree of composition , can affect in the differential uptake and/or targeting to certain organs, tissues or cells <sup>[57]</sup>.

#### Influence of Nanoparticles size

One of the parameters affecting the cellular uptake rate of nanoparticles is its size as it influences their internalization mechanism, and thus affects the in vivo circulation half-life. The two major endocytic mechanisms by which cells take up particles and macromolecules, and these are referred to as phagocytosis and pinocytosis (or fluid-phase uptake) <sup>[58]</sup>. The phagocytosis mechanisms are responsible for internalization of large particles(41mm), which are present only phagocytic cells, such as macrophages, on neutrophils, or dendritic cells. Therefore, pinocytosis is more relevant to nanoparticle cellular uptake and can occur either via adsorptive pinocytosis (non-specific adsorption of nanoparticle or macromolecules to the cell membrane followed by internalization) or via receptor-mediated endocytosis (RME, which describes the interaction of nanoparticles and macromolecules with receptors, followed by internalization) [59,60] Pinocvtic their

mechanisms of uptake can be further divided into caveolae mediated endocytosis or clathrinmediated endocytosis, as well as clathrinindependent or caveolin-independent endocytosis (smaller nanoparticles can be internalized through a number of these pathways) <sup>[61-63]</sup>. Cellular internalization of nanoparticles is majorly dependent on the size of the nanoparticles, and in general, particles in the 40–50 nm range exhibit maximal uptake in vitro <sup>[62]</sup>.

The accepted size range for the development of nanoparticles, is 10-100 nm for in vivo applications which relates to their in vivo clearance and biodistribution patterns. The main problem with the large nanoparticles is their interactions with opsonins. the The nanoparticles, smaller than approximately 5.5 nm have been shown to be rapidly cleared by glomerular filtration in the kidneys.

## Influence of nanoparticles shape

The majority of nanoparticles developed for drug delivery have a spherical shape. In some cases, it was found that spherical nanoparticles had a higher and faster rate of endocytosis compared to rods or disks shaped nanoparticles.

Recent studies have shown that particle shape may be an important factor in the rate of nanoparticle cellular internalization. This is mainly due to the fact that nanoparticle shapes that can accommodate cellular membrane wrapping processes become more effective at cellular uptake <sup>[64]</sup>.

Nanoparticles, shape is also an important factor for the biodistribution and circulation of nanoparticles, in vivo. Geng and Decuzzi et al. have reported that non-spherical particles with longitudinal lengths reaching cellular diameters and discoidal shapes can exhibit long circulation times than spherical particles <sup>[65]</sup>.

## Influence of Nanoparticles surface charge

Surface charges of nanoparticles also have an important influence on their interaction with cells and on their uptake. Positively charged nanoparticles have higher extent of internalization, apparently as a result of the ionic interactions established between positively charged particles and negatively charged cell membranes <sup>[58]</sup>. Moreover, positively charged nanoparticles seem to be able to escape from lysosomes after being internalized and exhibit perinuclear localization, whereas the negatively and neutrally charged nanoparticles prefer to colocalize with lysosomes [66].

### Influence of Nanoparticles hydrophobicity

Hydrophobic surfaces of nanoparticles can be easily binds with opsonins so in order to avoid this interaction by surface modification with hydrophilic polymers.In addition, surface effects such as smooth versus rough surfaces also influence the degree of nanoparticles, surface binding to cells <sup>[67]</sup>.

### Influence of nanoparticles PEGylation

The body recognizes hydrophobic particles as foreign. The reticulo-endothelial system (RES) eliminates these from the blood stream and takes them up in the liver or the spleen. This process is one of the most important biological barriers to nanoparticles-based controlled drug delivery <sup>[10]</sup>. The binding of opsonin proteins present in the blood serum to inject nanoparticles leads to the attachment of opsonized particles to macrophages and subsequently to their internalization by phagocytosis <sup>[25]</sup>.

In order to avoid these problems, several methods of surface modifications have been developed to produce nanoparticles not recognized by the RES. Nanoparticles can be coated with molecules that hide the hydrophobicity by providing a hydrophilic layer at the surface. The most common moiety for surface modification is the hydrophilic and nonionic polymer polyethylene glycol (PEG). It has been largely demonstrated that the "PEGylation" increases their blood circulation half-life by several orders of magnitude [25]. Moreover, PEG exhibits an excellent biocompatibility. PEG is a highly hydrophilic polymer that ensures prolonged in vivo half-lives.

Indeed, uncoated NPs have been observed to be rapidly cleared by the macrophages.The density and thickness of this PEG masking layer have also been found to affect opsonization and distribution of injected nanoparticles, and should be studied with more high-throughput and combinatorial approaches that can reproducible manner, together with a comprehensive study that combinatorially investigates the interrelation of nanoparticles,PEG lengths and densities leading to reduced clearance.

Another application of surface modification is the targeting of tumors or organs to increase selective cellular binding and internalization through receptor-mediated endocytosis. Targeting ligands are often grafted at the nanoparticles surface via a linkage on PEG chains <sup>[26]</sup>. Ligands need to be optimally conjugated on nanoparticles to maintain their affinity for receptors binding. As a sufficient PEG coating is essential for avoiding recognition by the RES, ligands should be extended away from the nanoparticle surfaces to avoid shielding by the PEG chains.

Applications of Nanoparticulate Delivery Systems

Tumor targeting using nanoparticulate delivery systems

The rationale of using nanoparticles for tumor targeting is based on:

- 1. Nanoparticles will be able to deliver a concentrate dose of drug in the vicinity of the tumor targets via the enhanced permeability and retention effect or active targeting by ligands on the surface of nanoparticles;
- 2. Nanoparticles will reduce the drug exposure of health tissues by limiting drug distribution to target organ [81].

## Long circulating nanoparticles

To be successful as a drug delivery system, nanoparticles must be able to target tumors which are localized outside mononuclear phagocytic system -rich organs. In the past decade, a great deal of work has been devoted to developing so-called "stealth particles or PEGvlated nanoparticles, which are invisible to macrophages or phagocytes. А maior breakthrough in the field came when the use of hydrophilic polymers (such as polyethylene glycol, poloxamines, poloxamers, and polysaccharides) to efficiently coat conventional nanoparticle surface produced an opposing effect to the uptake by the MPS. These coatings provide a dynamic "cloud" of hydrophilic and neutral chains at the particle surface which repel plasma proteins. As a result, those coated nanoparticles become invisible to MPS, therefore, remained in circulation for a longer period of the time.Extensive efforts have been devoted to achieving "active targeting" of nanoparticles in order to deliver drugs to the right targets, based on molecular recognition processes such as ligand-receptor or antigen-antibody interaction. Considering that fact that folate receptors are over expressed on the surface of some human malignant cells and the cell adhesion molecules such as selectins and integrins are involved in metastatic events, nanoparticles bearing specific ligands such as folate may be used to target

ovarian carcinoma while specific peptides or carbohydrates may be used to target integrins and selectins <sup>[82,83]</sup>.

Nanoparticles for oral delivery of peptides and proteins

Significant advances in biotechnology and biochemistry have led to the discovery of a large number of bioactive molecules and vaccines based on peptides and proteins. Development of suitable carriers remains a challenge due to the fact that bioavailability of these molecules is limited by the epithelial barriers of the gastrointestinal tract and their susceptibility to gastrointestinal degradation digestive bv enzymes. Polymeric nanoparticles allow encapsulation of bioactive molecules and protec enzymatic and hydrolytic them against degradation. For instance, it has been found that insulin-loaded nanoparticles have preserved insulin activity and produced blood glucose reduction in diabetic rats for up to 14 days following the oral administration [84].

# Targeting of nanoparticles to epithelial cells in the GI tract using ligands

Targeting strategies to improve the interaction of nanoparticles with adsorptive enterocytes and M-cells of Pever's patches in the GI tract can be classified into those utilizing specific binding to ligands or receptors and those based on nonspecific adsorptive mechanism. The surface of enterocytes and M cells display cell-specific carbohydrates, which may serve as binding sites to colloidal drug carriers containing appropriate ligands. Certain glycoproteins and lectins bind selectively to this type of surface structure by specific receptor-mediated mechanism. Different lectins, such as bean lectin and tomato lectin, have been studied to enhance oral peptide adsorption .Vitamin B12 absorption from the gut under physiological conditions occurs via receptor-mediated endocytosis. The ability to increase oral bioavailability of various peptides (e.g., granulocyte colony stimulating factor, erythropoietin) and particles by covalent coupling to vitamin B-12 has been studied [85].

## Nanoparticles for gene delivery

Nanoparticles loaded with plasmid DNA could also serve as an efficient sustained release gene delivery system due to their rapid escape from the degradative endo-lysosomal compartment to the cytoplasmic compartment. Hedley et al., reported that following their intracellular uptake and endolysosomal escape, nanoparticles could release DNA at a sustained rate resulting in sustained gene expression. This gene delivery strategy could be applied to facilitate bone healing by using PLGA nanoparticles containing therapeutic genes such as bone morphogenic protein <sup>[86]</sup>.

Nanoparticles for drug delivery into the brain Strategies for nanoparticle targeting to the brain rely on the presence of nanoparticle interaction specific receptor-mediated with transport systems in the BBB (blood brain barrier). For polysorbate example 80/LDL, transferrin receptor binding antibody (such as OX26), lactoferrin, cell penetrating peptides and melanotransferrin have been shown capable of delivery of a self non transportable drug into the brain via the chimeric construct that can undergo receptor-mediated transcytosis.It has been reported poly(butylcyanoacrylate) nanoparticles was able to deliver hexapeptide dalargin, doxorubicin and other agents into the brain which is significant because of the great difficulty for drugs to cross the BBB . Despite some reported success with polysorbate 80 coated NPs, this system does have many shortcomings including desorption of polysorbate coating, rapid NP degradation and toxicity caused by presence of high concentration of polysorbate 80 [87]

## CONCLUSIONS

Polymeric nanoparticles have therapeutic potential at both research and clinical levels. In prepare order to successfully and biofunctionalise nanoparticles for a given biomedical application, a wide range of physical, chemical, biological and physiological factors and conditions must be taken into account. However, by tuning the nature of the core, shell and ligands, these factors can be taken advantage of to provide the desired, biocompatibility and biofunctionality, making nanocrystals suitable for a very wide range of applications in diagnostics and therapy for numerous medications.By utilizing carbohydrate ligand receptors binding can also be beneficial for the for the future prospects of the many therapeutic applications.

We have confidence that with a well characterized system including: safe, effective, and specific targeting ligands, biocompatible, biodegradable and bioeliminable materials, and appropriate choice of therapeutics and disease models, targeted polymeric nanoparticles could yield more effective treatments of important human diseases.

#### REFERENCES

- [1] Takami A.,Masanori B,Mitsuru A.,Preparation of nanoparticles by self – organisation of polymers consisting of hydrophobic and hydrophilic segments:Potential applications,Polymer ,2007,48,6729-6747.
- [2] Hagan S.A,Coombes A.G.A Garnett, M.C,Dunn S.E;Davies M.C,Illum L.,Davis, S.S., Polylactide}poly(ethylene glycol) copolymers as drug delivery systems,Langmuir,1996,12,2153-61.
- [3] Fabienne Danhier,Eduardo Ansorena Joana,M. Silva.,Régis Coco,Aude Le, Breton,VéroniquePréat;PLGA based nanoparticles:An Overview of biomedical applications,J.Control.Release,2012,161,50 5-522.
- [4] Burgess P.,Hutt,P.B.,Farokhzad O.C.,Langer R.,Minick S.,Zale S.,Reshaping the future of Nanopharmaceuticals,Nat. Biotechnol., 2010, 28, 1267–70.
- [5] Torche A-M, Ex vivo and in situ PLGA microspheres uptake by pig ileal Peyer's patch segment., Int J Pharm 2000,201,15–27.
- [6] Majeti N.V., Kumar Ravi, Kumar Neeraj,Domb. A.J., Arora Meenakshi,Pharmaceutical polymeric controlled drug delivery systems,Adv.in polymer Sci.,2012,160,47-108.
- [7] Kwon H-Y., Preparation of PLGA nanoparticles containing estrogen by emulsification–diffusion method, Colloids Surf. Release, 2001,182,123–30.
- [8] York A.W.,.Kirkland S.E.,McCormick C.L.,Adv,Drug.Delivery,2008,60,1018-1036.
- [9] Bae Y, Kataoka S.E., Adv.Drug.Delievery, Rev.2009,61,768-84.
- [10] Kumari A.,YadavS.K.,,Yadav S.C.,Biodegradable polymeric nanoparticles based drug delivery systems,Colloids and Surfaces B:Biointerfaces,2010,75,1-18.
- [11] Fabienne Danhier,EduardoAnsorena, Joana M. Silva,Régis Coco, Aude Le Breton,Véronique Préat,PLGA based nanoparticles:An Overview of biomedical applications,J.Control.Release,2012,161,50 5-522.
- [12] Behan N.,Bukinshow C.Clarke.,n-butyl cyanoacrylate nanoparticles:A mechanistic study of polymerization and particle formation ,Biomaterials,2001,22,1335-44.
- [13] West C.P.,Lumsden M.A.,Lawson S.,Willaimson J.,Baird D.T.,Shrinkage of 125

uterine fibroids during therapy with goserelin (Zoladex): a luteinizing hormonereleasing hormone agonist administered as a monthly subcutaneous depot,Fertil.Steril.,1987,48,45-51.

- [14] Sethi R., Sanfilippo N; Six-month depot formulation of leuprorelin acetate in the treatment of prostate cancer, Clin Interv Aging.,2009, 4, 259–267.
- [15] Karan Gulati, Moom Sinn Aw, Dusan Losic, Nanoengineered drug-releasing Ti wires as an alternative for local delivery of chemotherapeutics in the brain, Int J Nanomedicine, 2012, 7, 2069–2076.
- [16] Johnson L.R, Stoller N.H, Rationale for the use of Atridox therapy for managing periodontal patients. Compend Contin Educ Dent.,1999,2,19-25,quiz 35.
- [17] Anthony L.,Freda P.U.,From somatostatin to octreotide LAR: evolution of a somatostatin analogue, Curr Med Res Opin., 2009 ,25,2989-99.
- [18] David E.,Crawford Jason,Phillips M.,Sixmonth gonadotropin releasing hormone (GnRH) agonist depots provide efficacy, safety, convenience, and comfort, Cancer Manag Res., 2011, 3, 201–209.
- [19] Aderem A.,Underhill D.,Mechanisms of phagocytosis in macrophages, Annu.Rev., Immunol, 1999,17,593-623.
- [20] Rabinovitch M.,Professional and nonprofessional phagocytes-An introduction,Trends Cell Biol,1995,5,85-87.
- [21] Vonarburg A.,Passirani C.,Sualnier P.,Benoit J.,Parameters influencing the stealthinessof colloidal drug delivery systems,Biomaterials,2006,27,4356-4373.
- [22] Groves E.,Dart A., Covarelli V.,Caron E.,Molecular mechanisms of phagocytic uptake in mammalian cells,Cell. Mol. Life. Sci., 65,2008,1957-1976.
- [23] VachonE.,MartinR.,PlumbJ.,KwokV.,Vandivi rR.,GlogauerM.,KapusA.,WangX,ChowC.,Gri nstinS.,Daney G ,CD44 is aphagocytic receptor, Blood,2006,107,4149-4158.
- [24] HansM.l,LowmanA.M.,Biodegradable nanoparticles for drug delievery and targeting, Curr. Opin, Solid State Matter,Sci,2002,6,319-327.
- [25] OwensD.E.,Peppas N.A., Opsonization, biodistribution and pharmacokinetics of polymeric nanoparticles, Int.J.Pharm., 2006,307,93-102.
- [26] BetarcourtT.,ByrneJ.D.,SunaryoN.,Crowder S.W,KadapakkamM,PatelS.,CasciatoS.,Peppa sL.,Pegylation strategies for active

targeting of PLA/PLGA nanoparticles, J.Biomed, Mater Res., 2009, 91, 263-276.

- [27] Foged C.,Brodin B.,Frokjaer S.,Sundblad S.,Particle size and surface charge affect particle uptake by human dendritic cells in an in vitro model, Int. J. Pharm, 2005,298, 315–322.
- [28] Vasir J.K.,Labhasetwar V., Quantification of the force of nanoparticle–cell membrane interactions and its influence on intracellular trafficking of nanoparticles,Biomaterials, 2008,29 4244– 4252.
- [29] Yue Z.G.,Wei W,Lv P.P.,Yue H.,Wang L .Y.,Su Z.G.,MaG.H., Surface charge affects cellular uptake and intracellular trafficking of chitosan-based nanoparticles, Biomacromolecules, 2011,12, 2440–2446.
- [30] Danhier F.,Feron O.,Preat V.,To exploit the tumor microenvironment: passive and active tumor targeting of nanocarriers for anti-cancer drug delivery, J. Control. Release, 2010,148,135–146.
- [31] Tahara K.,Sakai T.,Yamamoto H.,Takeuchi H.,Hirashima N.,Kawashima Y.,Improved cellular uptake of chitosan-modified PLGA nanospheres by A549 cells, Int. J. Pharm. ,2009,382,198–204.
- [32] Maeda H.,Tumour selective delievey of Macromolecular drugs via EPR,Bioconjugate Chem,2010,21,797-802.
- [33] Matsumura Y.,Maeda H.,A new concept for macromolecular therapeutics in cancer chemotherapy ,Cancer Research,1986,46,6387-6392.
- [34] Maeda H.,Bhorate G.Y.,Daruwalla J.,Polymeric drugs for efficient tumour targeted drug delievery based on EPR effect,Eur.J.Pharm Biopharm,2009,71,409-419.
- [35] TorchilinV.P.,Passive and active targeting,Handb.Exp.Pharmacol,2010,197,3 -53.
- [36] Akinori Nagamitsu,Khaled Greish,Hiroshi Maeda,Elevating Blood Pressure as a stategy to increase tumour targeted delievery of Macromolecular drugs SMANCS, Japanese J.Clin.Oncol., 2009, 39,756-766.
- [37] ChratinaA.,MasseyK.A.,SchnitzerJ.E.,WileyIn terdiscip.Rev.,Nanomedicine.Nanobiotechn ol.,2011,3,421-27.
- [38] Romberg B.,Hennik W.E.,Storm G.,Effective delievery of PEGylated siRNA, Pharm.,Res.,2008,25,55-71.

- [39] Hatakeyama H.,AkitaH.,HarashimaH.,Drug Delievery in Oncology,Adv.Drug Deliev.Rev.,63,152-160.
- [40] ZamboniW.C.,StrychorS.,JosephE.,PariseR.A ,,EgorinM.J.,EisemannJ.L;Tumour,tissueand plasma pharmacokinetic studies and antitumours responses studies of Docetaxel,Cancer,Chemother.Pharmacol.,2 008,62.417-26.
- [41] WinterP.M.,MoraswkiA.M.,CaruthersS.D.,Fu hrhopR.W.,ZhangH.,WillaimsT.A.,AllenJ.S.,L acy E.K.,Robertson J.D.,Lanza G.M.,Wickline S.A.,Molecular imaging of angiogenesis in early stage artherosclerosis with aipha(v)bety(3)-integrin targeted nanoparticles,Circulation,2003,108,2270-2274.
- [42] Pasaqualini R.,Koivumen E.,Rusolethi E.,Aipha(v) integrin as receptor for tumour targeting by circulating ligands,Nature Biotechnology,1997,131,542-46.
- [43] Sugano M., EquilmezN.K., Yokota S.J.,ChenF.A.,HardingJ.,HuangS.K.,BanketK.B ,Antibody Targeting of doxorubicin –loaded liposomes suppresses the growth and metastatic spread of established human lung tumour xenografts in severe combined immunodeficient mice ,Cancer, Res., 2000,60,6942-6949.
- [44] Bareford L.M.,Swaan P.W.,Nanomedicine,Adv.Drug.Deliev.Rev.20 07,59,748-58.
- [45] Monsigny M.,Roche A.C.,Midoux P.,Mayer R;Glcoconjugates as carrier for specific delievery of therapeutic drugs and genes,Adv.Drug Deliev,Rev,1994,14,1-24.
- [46] Rogers J.C.,Kornfeld S.,Hepatic uptake of proteins coupled to fetuin glycopeptides,Biochem Biophys Res Commun,1971,45,622-672.
- [47] WuJ.,ZernM.A.,Hepatic stellate cells,a target for treatment of liver fibrosis,J.Gastroenterol,35,2000,665-72.
- [48] BilderM.C.,CescatoR.,NenoP.,SpiessM.,High affinity to subunit h1 of the asialoglycoprotein receptor in the absence of subunit H2, Eur.J.Biochem,230,1997,207-212.
- [49] Cho C.S.,Kabayashi A.,Takei R.,Ishihara A.,Maruyama A.,Akaike T.,Receptor – mediated cell modulated delivery to hepatocytes using nanoparticles coated with carbohydrates carrying polymers,Biomaterials,22,2001,45-51.
- [50] WuJ.,LiuP.,ZhuL.,Maddukuri S.,ZernM.A., Increased liver uptake of liposomes and

improved targeting efficacy by labelling with asialofeutin in rodents,Hepatology,1998,27,772-778.

- [51] Sorenson A.L.T.,Clausen H.,Wanderl H.Hans.,Carbohydrate clearance receptorin transfusion medicines,Biochimica Biophysica,2012,1797-1808.
- [52] McAbee D.D.,Jiang X.,Copper and zinc ionsdifferntially block asialoglycoprotein receptor-mediated endocytosisin isolated rat hepatocytes,J.Biol Chem,1999,274,14750-14758.
- [53] McVickerB.L.,TumaD.J.,CaseyC.A.,Hyperpho sphorylation of the asialoglycoprotein receptor in isolated rat hepatocytes following ethanol administration,Biochem Pharmacol,2000,60,343-51.
- [54] MeierM.,BiderM.D.,MalashkevichV.N.,Spiess M.,BurkhardP.,Cystal structure of the carbohydrate recognition domain of H1 Subunit of asialoglycoprotein receptor,J.Mol.Biol.,2000,300,857-65.
- [55] Wragg S,Drickamer K,Identification of amino acid residues that determine pH dependence of ligand bindng to the asialoglycoprotein receptor during endocytosis,J.Biol.Chem.1994,274,35400-406.
- [56] Fukeena T.,Wu G.Y.,WuC.H.,Liver selective nucleic acid targeting using asialoglcoprotein receptor,Gene Ther.Reg.,2000,1,79-93.
- [57] GrislainL.,CouvreurP.,LenaertsV.,RolandM., DeprezdecampeneereD,SpeiserP,pharmaco kinetics and distribution of a biodegradable drug carrier ,Int.J.Pharm.,1983,15,335-345.
- [58] Zhao F.,ZhaoY.,Liu Y.,Chang X.,Chang C.,Cellularuptake,IntracellularTrafficking and cytotoxicity of nanomaterials,Small,2011,7,1322-1337.
- [59] Mukherjee S.,Ghosh R.N.,Maxfield F.R.;Cellbased, High content screen for receptor internalization,Recycling and Intracellular Trafficking,Endocytosis,Physiol.Rev.,1997,7 7,759-803.
- [60] Wang J.,ByrneJ.D.,Napier M.E.,Desimone J.M.,More effective nanomedicine through particle design,Small,2011,7,1919-1931.
- [61] Verma A.,Stellai F.,Effet of surface properties on nanoparticles cellinteractions,Small,2010,6,12-21.
- [62] Gratton S.E.,Ropp P.A.,Pohlhaus P.D.,Luft J.C.,MaddenV.J.,Derisome J.E.,The effect of particle design on cellular internalization pathways,Proc.Natl.Acad.Sci.USA,2008,105, 11613-618.

- [63] He C.,HuY.,Yin L.,Tang C.,Yin C.,Effects of particle size and surface charge on cellular uptake and biodistribution of polymeric nanoparticles,Biomaterial,2010,31,3657-66.
- [64] Devika C.,Ghazani A.,ChanW.,Size and shape dependence of nanoparticles on cellular uptake,Nano lett,2006,6,662-68.
- [65] GengY.,Dalhaimer P.,Tsai R.,Tewari M.,Minko T.,Discher D.E.,Shape effects of filaments versus spherical particlesin flow and drug delivery,Nanotechnol., 2007,2,249-255.
- [66] Foged C.,Brodin B.,Frokjaer S.,Sundbard A.,Particle size and surface charge affect particle uptake by human dendritic cells in in vitro model,Int.J.Pharm.2005,298,315-322.
- [67] Vonaburg A.,Passirani C.,Saulnier P.,Benoit J.P.,Particle size and surface charge affect particle uptake by human dendritic cells in an in an vitro model, Int.J.Pharm.,2006,47,4356-73.
- [68] Puglisi G.,Fresta M.,Giammona G.,Ventura C.A.,Influence of the preparation conditions on poly(ethylcyanoacrylate) nanocapsule formation,Int. J. Pharm. ,1995,125, 283-287.
- [69] Verger ML-L, Preparation and characterization of nanoparticles containing an antihypertensive agent. Eur J Pharm Biopharm Control Release, 1998,46,137–43.
- [70] Govender T.,PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug. J Control Release 1999,57,171–85.
- [71] Matsumoto J.,Preparation of nanoparticles consisted of poly(L-lactide)-poly(ethylene glycol)-poly(L-lactide) and their evolution in vehicle for the improvement of the delivery of drugs to the ocular vitro. Int J Pharm 1999,185,93–101.
- [72] Cheng Y-H, Illum L, Davis S.S., A poly(D,Llactide-co-glycolide) microsphere depot system for delivery of haloperidol. J Control Release 1998,55,203–12.
- [73] Leroux J-C.,Biodegradable nanoparticlesfrom sustained release formulations to improved site specific drug delivery. J Control Release 1996,39,339–50.
- [74] Fishbein I .,Nanoparticulate delivery system of a tyrphostin for the treatment of restenosis. J Control Release 2000,65,221– 9.

- [75] Jung T., Breitenbach A.,Kissel T., Sulfobutylated poly(vinyl alcohol graftpoly(lactide-co-glycolide)s facilitate the preparation of small negatively charged biodegradable nanospheres, J. Control Release,2000,67,157-69.
- [76] Davis M.E.,The first targeted delivery of siRNA in humans via a self-assembling, cyclodextrin polymer-based nanoparticle: from concept to clinic, Mol Pharmaceutics.,2009,6,659-68.
- [77] Sarris A.H.,Hagemeister F.,Romaguera J.,Rodriguez M.A.,McLaughlin P.,Tsimberidou A.M., Liposomal vincristine in relapsed non-Hodgkin's lymphomas: early results of an ongo-ing phase II trial, Ann Oncol. ,2000,11, 69-72.
- [78] Lim W.T., Tan E.H, Toh C.K., Hee S.W., Leong S.S., Ang P.C., Phase I pharmacokinetic study of a weekly liposomal paclitaxel formulation (Genexol-PM) in patients with solid tumors, Ann Oncol, 2010, 21, 382-8.
- [79] Petrelli F.,Borgonovo K.,Barni S., Targeted delivery for breast cancer therapy: the history of nanoparticle-albumin-bound paclitaxel, Expert Opin Pharmacother.,2010,11, 1413-32.
- [80] Sankhala K.K.,Mita A.C.,Adinin R.,Wood L.,Beeram M.,Bullock S., A phase I pharmacokinetic (PK) study of MBP-426, a novel liposome encapsulated oxaliplatin, J Clin Oncol.,2009,27 S2535.
- [81] Panyam J.,Williams D.,Dash A.,Leslie-Pelecky D.,Labhasetwar V.,Solid-state solubility influences encapsulation and release of hydrophobic drugs from PLGA/PLA nanoparticles. J Pharm Sci ,93,2004, 1804-14.
- [82] Couvreur P., Kante B., Lenaerts V., Scailteur V., Roland M., Speiser P., Tissue distribution of antitumor drugs associated with polyalkylcyanoacrylate nanoparticles. J Pharm Sci 69,1980, 199-202.
- [83] Krishna R., Mayer L.,Multidrug resistance (MDR) in cancer-mechanisms, reversal using modulators of MDR and the role of MDR modulators in influencing the pharmacokinetics of anticancer drugs. Eur. J. Cancer Sci , 11,2000, 265-283.
- [84] Damge C.,Michel C.,Aprahamian M.,Couvreur P.,Devissaguet J.P.,Nanocapsules as carriers for oral peptide delivery. J. Control. Release ,13,1990, 233-239.
- [85] Haltner E.,Easson J.,Lehr C.,Lectins and bacterial invasion factors for controlling

endo- and transcytosis of bioadhesive drug carrier systems. Eur. J. Pharm. Biopharm ,44,1997, 3-13.

- [86] Hedley M.,Curley J.,Urban R.,Microspheres containing plasmid-encoded antigens elicit cytotoxic T-cell responses. Nat Med ,4,1998, 365-368.
- [87] Kreuter J.,Influence of the surface properties on nanoparticle-mediated transport of drugs to the brain. J Nanosci Nanotechnol ,4,2004, 484-8.