Review Article

Inhaled Nanopartcles: A Review

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ABSTRACT
For the past few decades, there has been a considerable research interest in the area of drug delivery using particulate delivery systems. Nanoparticles have been used as a physical approach to improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules. They have been used in vivo to protect the drug entity in the systemic circulation, restrict access of the drug to the chosen sites and to deliver the drug at a controlled and sustained rate to the site of action. Various polymers have been used in the formulation of nanoparticles for drug delivery research to increase therapeutic benefit, while minimizing side effects. Here, we review various application of nanoparticulate delivery system, preparation of nanoparticles, drug loading of nanoparticulate system, drug release, various inhalation techniques and devices, evaluation method of inhaled nanoparticles, company directory.

Keywords: Nanoparticles, Delivery system, Drug release, Inhalation devices.

INTRODUCTION

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. In recent years, biodegradable polymeric nanoparticles, particularly those coated with hydrophilic polymer such as polyethylene glycol (PEG) known as long-circulating particles, have been used as potential drug delivery devices because of their ability to circulate for a prolonged period time target a particular organ, as carriers of DNA in gene therapy, and their ability to deliver proteins, peptides and genes. The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. Though liposomes have been used as potential carriers with unique advantages including protecting drugs from degradation, targeting to site of action and reduction toxicity or side effects, their applications are limited due to inherent problems such as low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage stability. On the other hand, polymeric nanoparticles offer some specific advantages over liposomes. For instance, they help to increase the stability of drugs/proteins and possess useful controlled release properties[1-3, 31].

CHARACTERIZATION OF NANOPARTICLES

The main important feature of nanoparticles is their size which enables them to traverse blood capillaries and may help to reduce irritant reactions at injection sites. The important methods for the physicochemical characterization of nanoparticles are listed in Table 1. These methods mainly are similar to other materials or to larger particles.

Table 1: Physicochemical characterization method for nanoparticles

Parameter	Method
Particle size	Photon correlation spectroscopy, Scanning electron microscopy, Transmission electron microscopy, Scanning probe microscopy, Fraunhofer diffraction
Molecular weight	Molecular Gel chromatography, mass spectroscopy, Viscosimetry, Analytical ultracentrifuge
Crystallinity	X-ray diffraction, Differential scanning calorimetry
Polymer structure	Nuclear magnetic resonance
Density	Helium compression pycnometry
Surface charge	Electrophoresis, Laser Doppler anemometry

A special difficulty in nanoparticle characterization represents particle sizing. The method of choice for size and size distribution determination is photon correlation spectroscopy (PCS) based on laser light scattering because this method provides a rapid size analysis. However, this method is strongly influenced and skewed by larger particles present in the sample. Therefore, a confirmation of the particle size measurement by another method is advisable. Electron microscopy is especially optimal for this purpose. However, it has to be considered that for scanning electron microscopy a thin conducting layer has to be sputtered onto the sample. Since the thickness of this layer is not certain, errors in particle sizes may result. Another excellent method for particle sizing is transmission electron microscopy. This method, however, is very laborious and enables only the analysis of small sample fractions. The same applies to scanning probe microscopy. Transmission electron microscopy after freeze-fracturing also enables the identification of nanocapsules. If this method is used for sizing it has to be taken into account that particles are not only fractured across the equator but also close to the poles of the particles which again skews the particle size analysis. Four methods exist for the molecular weight determination of nanoparticles: gel permeation chromatography, mass spectroscopy, viscosimetry, and analytical ultracentrifuge. The results obtained with these methods may differ. Mass spectroscopy is mainly useful for smaller molecular weight nanoparticles such as the polyalkyl cyanoacrylate nanoparticles [32]. For gel permeation chromatography and viscosimetry. The nanoparticles have to be soluble in a useful solvent. Analytical ultracentrifuge analysis is very laborious and requires considerable expertise which is not available in most labs. None of these methods is very helpful for the molecular weight determination of nanoparticles made of cross-linked polymers which, therefore, is often impossible. Nuclear magnetic resonance (NMR) was used to answer the question if components that were present during polymerization were covalently linked to the polymer. It was shown by this technique after repeated dissolution and precipitation steps that dextran present during polymerization of polyalkyl cyanoacrylate polymerization was not covalently linked to the cyanoacrylate polymer in significant amounts. It was claimed, however, that methylated PEG was covalently linked to the cyanoacrylate after being present during polymerization. However, this was not proven by repeated dissolution and precipitation. Hence it may be possible that the PEG chain is just entangled with the polycyanoacrylate chain network[3, 4].

APPLICATIONS OF NANOPARTICULATE DELIVERY SYSTEMS

1. **Tumor targeting using nanoparticulate delivery systems:**
   The rationale of using nanoparticles for tumor targeting is based on:
   a) 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,
   b) Nanoparticles will reduce the drug exposure of health tissues by limiting drug distribution to target organ.

Verdun et al demonstrated in mice treated with doxorubicin incorporated into poly isohexyl cyanoacrylate nanopsheres that higher concentrations of doxorubicin manifested in the liver, spleen and lungs

than in mice treated with free doxorubicin. Studies show that the polymeric composition of nanoparticles such as type, hydrophobicity and biodegradation profile of the polymer along with the associated drug's molecular weight, its localization in the nanospheres and mode of incorporation technique, adsorption or incorporation, have a great influence on the drug distribution pattern in vivo. The exact underlying mechanism is not fully understood but the biodistribution of nanoparticles is rapid, within ½ hour to 3 hours, and it likely involves mononuclear phagocytic system (MPS) and endocytosis/phagocytosis process. Recently Bibby et al reported the bio-distribution and pharmacokinetics (PK) of a cyclic RGDdoxorubicin- nanoparticle formulation in tumor bearing mice[33]. Their bio-distribution studies revealed decreasing drug concentrations over time in the heart, lung, kidney and plasma and accumulating drug concentrations in the liver, spleen and tumor. The majority injected dose appeared in the liver (56%) and only 1.6% in the tumor at 48 hrs post injection, confirming that nanoparticles have a great tendency to be captured by liver. This indicates the greatest challenge of using nanoparticles for tumor targeting is to avoid particle uptake by MPS in liver and spleen. Such propensity of MPS for endocytosis/phagocytosis of nanoparticles provides an opportunity to effectively deliver therapeutic agents to these cells. This bio-distribution can be of benefit for the chemotherapeutic treatment of MPS-rich organs/tissues localized tumors like hepatocarcinoma, hepatic metastasis arising from digestive tract or gynaecological cancers, brochopulmonary tumors, primitive tumors and metastasis, small cell tumors, myeloma and leukemia. It has been proved that using doxorubicin loaded conventional nanoparticles was effective against hepatic metastasis model in mice. Histological examination showed a considerable accumulation of nanoparticles in the lysosomal vesicles of Kupffer cells, whereas nanoparticles could not be clearly identified in tumoral cells. Thus Kupffer cells, after a massive uptake of nanoparticles by phagocytosis, were able to induce the release of doxorubicin, leading to a gradient of drug concentration, favorable for a prolonged diffusion of the free and still active drug towards the neighboring metastatic cells. When conventional nanoparticles are used as carriers in chemotherapy, some cytotoxicity against the Kupffer cells can be expected, which would result in deficiency of Kupffer cells and naturally lead to reduced liver uptake and decreased therapeutic effect with intervals of less than 2 weeks administration. Moreover, conventional nanoparticles can also target bone marrow (MPS tissue), which is an important but unfavorable site of action for most anticancer drugs because chemotherapy with such carriers may increase myelosuppresive effect. Therefore, the ability of conventional nanoparticles to enhance anticancer drugs efficacy is limited to targeting tumors at the level of MPS-rich organs. Also, directing anticancer drug-loaded nanoparticles to other tumoral sites is not feasible if a rapid clearance of nanoparticles occurs shortly after intravenous administration[5].

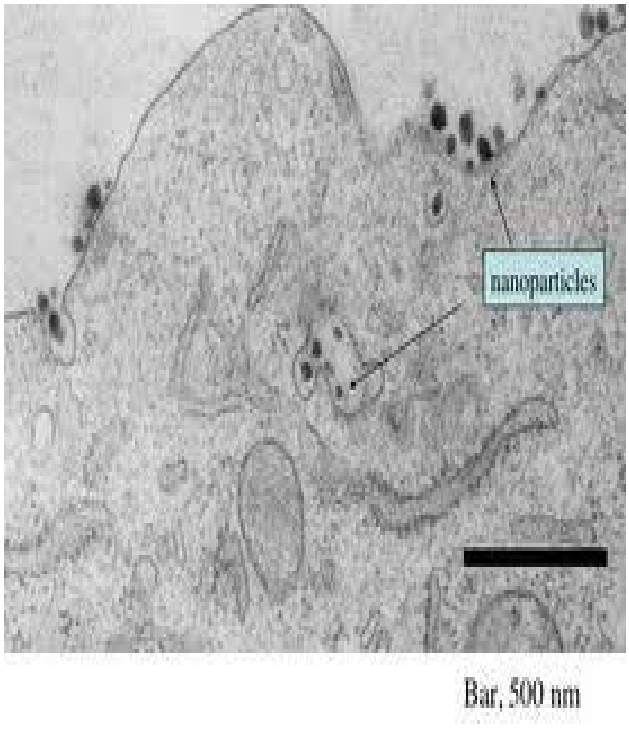

Fig. 1: Tumor targeting using nanoparticulate delivery systems

2. Long circulating nanoparticles:

To be successful as a drug delivery system, nanoparticles must be able to target tumors which are localized outside MPS-rich organs. A major breakthrough in the field came when the use of hydrophilic polymers (such as polyethylene glycol, poloxamines, poloxamers, and polysaccharides) to efficiently coat conventional nanoparticles 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 the circulation for a longer period of time. Hydrophilic polymers can be introduced at the surface in two ways, either by adsorption of surfactants or by use of block or branched copolymers. Studies show nanoparticles containing a coat of PEG also be able to selectively extravasate in pathological sites such as tumors or inflamed regions with a leaky vasculature [34]. As a result, such long-circulating nanoparticles have increased the potential to directly target tumors located outside MPS-rich regions. The size of the colloidal carriers as well as their surface characteristics are the critical to the biological fate of nanoparticles. A size less than 100 nm and a hydrophilic surface are essential in achieving the reduction of opsonisation reactions and subsequent clearance by macrophages. Coating conventional nanoparticles with surfactants or PEG to obtain a long-circulating carrier has now been used as a standard strategy for drug targeting in vivo. 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[5, 6].

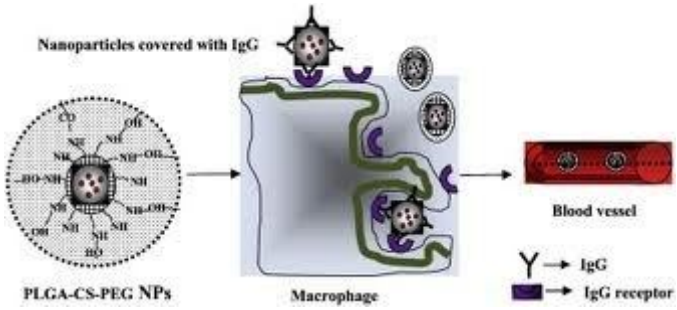

Fig. 2: Long circulating nanoparticles

3. Reversion of multidrug resistance in tumor cells:

Anticancer drugs, even if they are located in the tumor interstitium, can turn out to be of limited efficacy against numerous solid tumor types, because cancer cells are able to develop mechanisms of resistance. These mechanisms allow tumors to evade chemotherapy. Multidrug resistance (MDR) is one of the most serious problems in chemotherapy. MDR occurs mainly due to the over expression of the plasma membrane p-glycoprotein (Pgp), which is capable of extruding various positively charged xenobiotics, including some anticancer drugs, out of cells. In order to restore the tumoral cells' sensitivity to anticancer drugs by circumventing Pgp-mediated MDR, several strategies including the use of colloidal carriers have been applied [35]. The rationale behind the association of drugs with colloidal carriers, such as nanoparticles, against drug resistance derives from the fact that Pgp probably recognizes the drug to be effluxed out of the tumoral cells only when this drug is present in the plasma membrane, and not when it is located in the cytoplasm or lysosomes after endocytosis[6, 7].

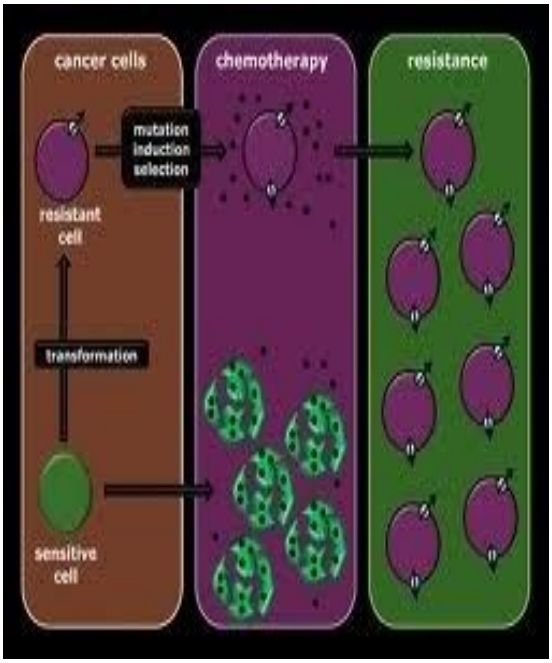

Fig. 3: Reversion of multidrug resistance in tumor cells

4. 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 by digestive enzymes. Polymeric nanoparticles allow encapsulation of bioactive molecules and protect them against enzymatic and hydrolytic 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. The surface area of human mucosa extends to 200 times that of skin. The gastrointestinal tract provides a variety of physiological and morphological barriers against protein or peptide delivery, e.g; (a) proteolytic enzymes in the gut lumen like pepsin, trypsin and chymotrypsin; (b) proteolytic enzymes at the brush border membrane (endopeptidases); (c) bacterial gut flora; and (d) mucus layer and epithelial cell lining itself. The histological architecture of the mucosa is designed to efficiently prevent uptake of particulate matter from the environment. One important strategy to overcome the gastrointestinal barrier is to deliver the drug in a colloidal carrier system, such as nanoparticles, which is capable of enhancing the interaction mechanisms of the drug delivery system and the epithelia cells in the GI tract[8].

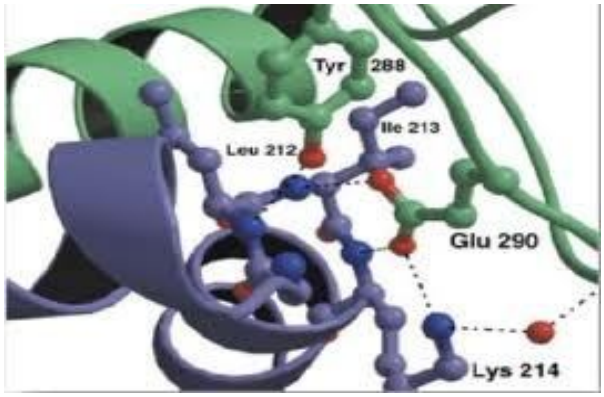

Fig. 4: Nanoparticles for oral delivery of peptides and proteins

5. 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 Peyer'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 [39]. Different lectins, such as bean lectin and tomato lectin, have been studied to enhance oral peptide adsorption. Vitamin B-12 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. For this intrinsic process, mucoprotein is required, which is prepared by the mucus membrane in the stomach and binds specifically to cobalamin. The mucoprotein completely reaches the ileum where resorption is mediated by specific receptors[9].

6. **Absorption enhancement using non-specific interactions:**
In general, the gastrointestinal absorption of macromolecules and particulate materials involves either paracellular route or endocytotic pathway. The paracellular route of absorption of nanoparticles utilises less than 1% of mucosal surface area. Using polymers such as chitosan 68, starch 69 or poly (acrylate) 70 can increase the paracellular permeability of macromolecules. Endocytotic pathway for absorption of nanoparticles is either by receptor-mediated endocytosis, that is, active targeting, or adsorptive endocytosis which does not need any ligands. This process is initiated by an unspecific physical adsorption of material to the cell surface by electrostatic forces such as hydrogen bonding or hydrophobic interactions. Adsorptive endocytosis depends primarily on the size and surface properties of the material. If the surface charge of the nanoparticles is positive or uncharged, it will provide an affinity to adsorptive enterocytes though hydrophobic, whereas if it is negatively charged and hydrophilic, it shows greater affinity to adsorptive enterocytes and Mcells. This shows that a combination of size, surface charge and hydrophilicity play a major role in affinity. This is demonstrated with poly (styrene) nanoparticles and when it is carboxylated[10, 39].

7. **Nanoparticles for gene delivery:**
Polynucleotide vaccines work by delivering genes encoding relevant antigens to host cells where they are expressed, producing the antigenic protein within the vicinity of professional antigen presenting cells to initiate immune response. Such vaccines produce both humoral and cell-mediated immunity because intracellular production of protein, as opposed to extracellular deposition, stimulates both arms of the immune system. The key ingredient of polynucleotide vaccines, DNA, can be produced cheaply and has much better storage and handling properties than the ingredients of the majority of protein-based vaccines. Hence, polynucleotide vaccines are set to supersede many conventional vaccines particularly for immunotherapy [37]. However, there are several issues related to the delivery of polynucleotides which limit their application. These issues include efficient delivery of the polynucleotide to the target cell population and its localization to the nucleus of these cells, and ensuring that the integrity of the polynucleotide is maintained during delivery to the target site. 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[11].

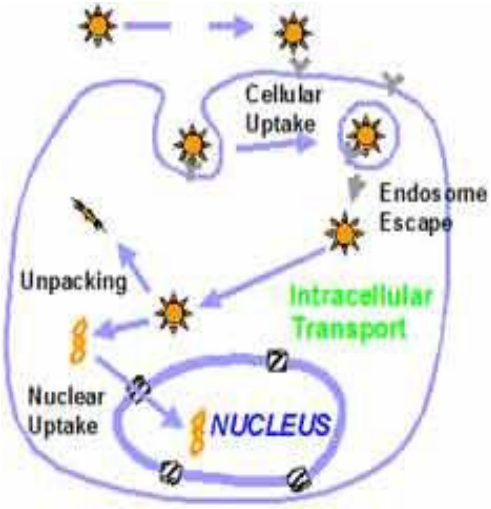

Fig. 5: Nanoparticles for gene delivery

8. Nanoparticles for drug delivery into the brain:

The blood-brain barrier (BBB) is the most important factor limiting the development of new drugs for the central nervous system. The BBB is characterized by relatively impermeable endothelial cells with tight junctions, enzymatic activity and active efflux transport systems. It effectively prevents the passage of water-soluble molecules from the blood circulation into the CNS, and can also reduce the brain concentration of lipid-soluble molecules by the function of enzymes or efflux pumps. Consequently, the BBB only permits selective transport of molecules that are essential for brain function. Strategies for nanoparticle targeting to the brain rely on the presence of and nanoparticle interaction with specific receptor-mediated transport systems in the BBB. For example polysorbate 80/LDL, transferrin receptor binding antibody (such as OX26), lactoferrin, cellpenetrating 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. OX26 MAbs (anti-transferrin receptor MAbs), the most studied BBB targeting antibody, have been used to enhance the BBB penetration of lipsosomes. However, recently, Ji *et al*. demonstrated that brain uptake of lactoferrin, an iron-binding glycoprotein belonging to the transferrin (Tf) family, is twice that of OX26 and transferrrin *in vivo*. It is possible soon we will see these BBB specific molecules used for targeting nanoparticles to the brain[12, 36].

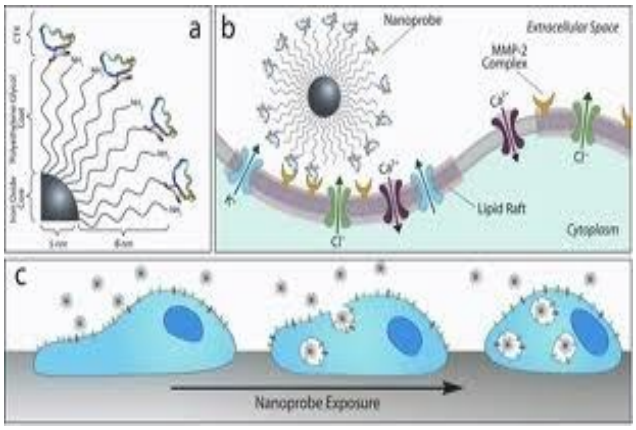

Fig. 6: Nanoparticles for drug delivery into the brain

PREPARATION OF NANOPARTICLES

Nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic polymers. The selection of matrix materials is dependent on many factors including: (a) size of nanoparticles required; (b) inherent properties of the drug, e.g., aqueous solubility and stability; (c) surface characteristics such as charge and permeability; (d) degree of biodegradability, biocompatibility and

toxicity; (e) Drug release profile desired; and (f) Antigenicity of the final product.
Nanoparticles have been prepared most frequency by three methods:
(1) Dispersion of preformed polymers,
(2) Polymerization of monomers and
(3) Ionic gelation or coacervation of hydrophilic polymers.

1. **Dispersion of preformed polymers:**
Dispersion of 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) and poly cyanoacrylate (PCA). This technique can be used in various ways as described below.

   a) **Solvent evaporation method:**
   In this method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate which is also used as the solvent for dissolving the hydrophobic drug. The mixture of polymer and drug solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form oil in water (o/w) emulsion. After the formation of stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring. Particle size was found to be influenced by the type and concentrations of stabilizer, homogenizer speed and polymer concentration. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed[13].

   b) **Spontaneous emulsification or solvent diffusion method:**
   This is a modified version of solvent evaporation method. In this method, the water miscible solvent along with a small amount of the water immiscible organic solvent is used as an oil phase. Due to the spontaneous diffusion of solvents an interfacial turbulence is created between the two phases leading to the formation of small particles. As the concentration of water miscible solvent increases, a decrease in the size of particle can be achieved. Both solvent evaporation and solvent diffusion methods can be used for hydrophobic or hydrophilic drugs. In the case of hydrophilic drug, a multiple w/o/w emulsion needs to be formed with the drug dissolved in the internal aqueous phase[14].

2. **Polymerization method:**
In this method, monomers are polymerized to form nanoparticles in an aqueous solution. Drug is incorporated either by being dissolved in the polymerization medium or by adsorption onto the nanoparticles after polymerization completed. The nanoparticle suspension is then purified to remove various stabilizers and surfactants employed for polymerization by ultracentrifugation and re-suspending the particles in an isotonic surfactant-free medium. This technique has been reported for making polybutylcyanoacrylate or poly alkylcyanoacrylate nanoparticles. Nanocapsule formation and their particle size depend on the concentration of the surfactants and stabilizers used[15, 46].

3. **Coacervation or ionic gelation method:**
Much research has been focused on the preparation of nanoparticles using biodegradable hydrophilic polymers such as chitosan, gelatin and sodium alginate. Calvo and co-workers developed a method for preparing hydrophilic chitosan nanoparticles by ionic gelation. The method involves a mixture of two aqueous phases, of which one is the polymer chitosan, a di-block co-polymer ethylene oxide or propylene oxide (PEO-PPO) and the other is a polyanion sodium tripolyphosphate. In this method, positively charged amino group of chitosan interacts with negative charged tripolyphosphate to form

coacervates with a size in the range of nanometer. Coacervates are formed as a result of electrostatic interaction between two aqueous phases, whereas, ionic gelation involves the material undergoing transition from liquid to gel due to ionic interaction conditions at room temperature[16].

4. **Supercritical fluid method:**
The supercritical fluid technology has been investigated to prepare biodegradable micro- and nanoparticles because supercritical fluids are environmentally safe. A supercritical fluid can be generally defined as a solvent at a temperature above its critical temperature, at which the fluid remains a single phase regardless of pressure. Supercritical CO_2 (SC CO_2) is the most widely used supercritical fluid because of its mild critical conditions (*T*c = 31.1 °C, *P*c = 73.8 bars), nontoxicity, non-flammability, and low price. The most common processing techniques involving supercritical fluids are supercritical anti-solvent (SAS) and rapid expansion of critical solution (RESS). The process of SAS employs a liquid solvent, e.g. methanol, which is completely miscible with the supercritical fluid (SC CO2), to dissolve the solute to be micronized; at the process conditions, because the solute is insoluble in the supercritical fluid, the extract of the liquid solvent by supercritical fluid leads to the instantaneous precipitation of the solute, resulting the formation of nanoparticles. Thote and Gupta (2005) reported the use of a modified SAS method for formation of hydrophilic drug dexamethasone phosphate drug nanoparticles for microencapsulation purpose [45]. RESS differs from the SAS process in that its solute is dissolved in a supercritical fluid (such as supercritical methanol) and then the solution is rapidly expanded through a small nozzle into a region lower pressure, Thus the solvent power of supercritical fluids dramatically decreases and the solute eventually precipitates. This technique is clean because the precipitate is basically solvent free. RESS and its modified process have been used for the product of polymeric nanoparticles. Supercritical fluid technology technique, although environmentally friendly and suitable for mass production, requires specially designed equipment and is more expensive[17].

DRUG LOADING

Ideally, a successful nanoparticulate system should have a high drug-loading capacity thereby reduce the quantity of matrix materials for administration. Drug loading can be done by two methods:

- Incorporating at the time of nanoparticles production (incorporation method)
- Absorbing the drug after formation of nanoparticles by incubating the carrier with a concentrated drug solution (adsorption /absorption technique).

Drug loading and entrapment efficiency very much depend on the solid-state drug solubility in matrix material or polymer (solid dissolution or dispersion), which is related to the polymer composition, the molecular weight, the drug polymer interaction and the presence of end functional groups (ester or carboxyl). The PEG moiety has no or little effect on drug loading. The macromolecule or protein shows greatest loading efficiency when it is loaded at or near its isoelectric point when it has minimum solubility and maximum adsorption. For small molecules, studies show the use of ionic interaction between the drug and matrix materials can be a very effective way to increase the drug loading[18, 44].

DRUG RELEASE

To develop a successful nanoparticulate system, both drug release and polymer biodegradation are important consideration factors. In general, drug release rate depends on:

- Solubility of drug,
- Desorption of the surface-bound/ adsorbed drug,
- Drug diffusion through the nanoparticle matrix,

- Nanoparticle matrix erosion/degradation and
- Combination of erosion/diffusion process.

Thus solubility, diffusion and biodegradation of the matrix materials govern the release process. In the case of nanospheres, where the drug is uniformly distributed, the release occurs by diffusion or erosion of the matrix under sink conditions. If the diffusion of the drug is faster than matrix erosion, the mechanism of release is largely controlled by a diffusion process [44]. The rapid initial release or 'burst' is mainly attributed to weakly bound or adsorbed drug to the large surface of nanoparticles. It is evident that the method of incorporation has an effect on release profile. If the drug is loaded by incorporation method, the system has a relatively small burst effect and better sustained release characteristics. If the nanoparticle is coated by polymer, the release is then controlled by diffusion of the drug from the core across the polymeric membrane. The membrane coating acts as a barrier to release, therefore, the solubility and diffusivity of drug in polymer membrane becomes determining factor in drug release. Furthermore release rate can also be affected by ionic interaction between the drug and addition of auxiliary ingredients. When the drug is involved in interaction with auxiliary ingredients to form a less water soluble complex, then the drug release can be very slow with almost no burst release effect whereas if the addition of auxiliary ingredients e.g., addition of ethylene oxide-propylene oxide block copolymer (PEO-PPO) to chitosan, reduces the interaction of the model drug bovine serum albumin (BSA) with the matrix material (chitosan) due to competitive electrostatic interaction of PEO-PPO with chitosan, then an increase in drug release could be observed.

Various methods which can be used to study the in vitro release of the drug are:

I. Side-by-side diffusion cells with artificial or biological membranes,
II. Dialysis bag diffusion technique,
III. Reverse dialysis bag technique,
IV. Agitation followed by ultracentrifugation/centrifugation,
V. Ultra-filtration or centrifugal ultra-filtration techniques.

Usually the release study is carried out by controlled agitation followed by centrifugation. Due to the time-consuming nature and technical difficulties encountered in the separation of nanoparticles from release media, the dialysis technique is generally preferred[19].

INHALATION TECHNIQUES AND DEVICES

1. Pressurized metered-dose inhaler:

The most common device for inhaled drug delivery is the pMDI. The pMDI consists of a pressurized canister and a chamber outfitted with a mouthpiece and protective cover. The canister contains a medication, a surfactant and/or a solvent, and a liquid propellant such as chlorofluorocarbons (CFCs). CFCs must be removed from pMDIs according to The Montreal Protocol on Substances That Deplete the Ozone Layer. Environmentally friendly propellants such as hydrofluoroalkanes (HFAs) are rapidly replacing CFCs in pMDIs.

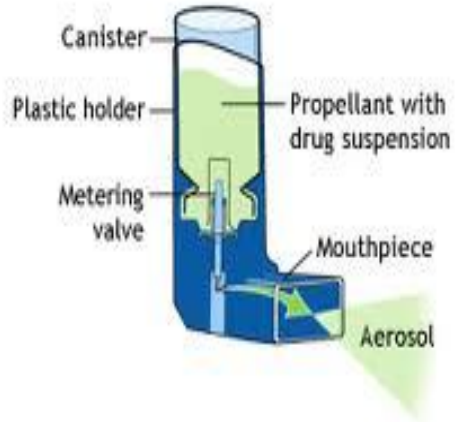

Fig. 7: pressurized metered-dose inhaler

The inhaler itself is designed to deliver exact doses of medication. When the canister is pressed, a one-way metering valve is opened by the trigger mechanism in the activator body. The medication is aerosolized near the

opening of the mouthpiece, at which point the patient must inhale it. The aerosol consists of droplets in varying diameters. When the medication is inhaled, large droplets are deposited in the mouth, pharynx, and larynx, whereas smaller droplets make their way toward the lower airways [43]. Manufacturers of HFA-driven devices have improved the design characteristics and drug formulations to address some of the drawbacks of the older pMDIs [14]. They have decreased the velocity and increased fine particle dose in an attempt to improve the therapeutic ratio of the drug. The new designs deliver a more consistent dose throughout the life of the canister, thus eliminating the tail off effect (reduction of drug output as the device nears empty). Chlorofluorocarbon-driven devices deliver reduced doses when exposed to cold. Hydrofluoroalkane driven canisters deliver consistent doses even when exposed to temperatures as low as –20°C. The new generation of pMDIs produces a warmer spray, which should alleviate the cold freon effect (interruption of inspiration) experienced by some patients in the past, reliable and effective method of delivering inhaled medication, and, when used properly, their efficacy is at least equal to that of other inhalation devices. Their greatest disadvantage is complicated to use effectively. Several studies have revealed that a large number of patients are unable to master the technique[20].

2. Spacing Devices:

Spacing devices were designed to overcome the difficulties experienced when using pMDI. Spacing devices are available in varying forms and sizes. The most efficient spacing devices have a holding chamber and a one-way valve that opens during inspiration and closes during expiration, preventing drug loss caused by poor coordination between actuation of the pMDI and inspiration. Spacing devices also improve the deposition of medication in the lower airways. Essentially, they slow down and suspend small droplets of aerosolized medication for approximately 1 to 2 seconds. This allows time for some of the propellant surrounding the particles of medication to evaporate; hence, the inhaled aerosol is made up of a greater proportion of particles small enough to reach the lower airways. The larger particles that would not reach the lungs remain within the spacing device, thus significantly reducing the deposition of medication in the oropharynx and thereby reducing adverse effects. The use of a spacing device is recommended for patients who take high doses of inhaled corticosteroids to prevent oropharyngeal candidiasis. Patients who are unable to hold their breath for 4 seconds should use a spacing device with a one way valve, allowing the patient to obtain a suitable dose of medication in three to four tidal breaths. Patients who cannot make a tight seal around the mouthpiece—for example, someone suffering from facial paralysis—should use a spacer with a mask attachment. However, many patients find them embarrassing to use in public or cumbersome to carry.

Spacing devices are therefore indicated as follows:

- To overcome difficulties of patients who are unable to use pMDIs correctly (i.e., because of coordination problems, physical or mental handicaps, etc)
- To reduce the risk of adverse effects with inhaled respiratory medications (especially when using high doses of inhaled corticosteroids)
- To decrease or eliminate coughing or arrested inspiration experienced by some patients when using CFC-driven devices.
- To administer inhaled medication during severe exacerbations as recommended by the American Thoracic Society.

Two commonly used examples of spacing devices that have one-way valves are the Aero chamber Plus and the Venta Haler. Aero Chamber plus VHC is a 145-mL rigid cylinder made of polyester (Trudell Medical, London, ON). It has a rubber adapter that makes it compatible with most pMDIs and is available with a mouthpiece or a mask. The Aero Chamber plus VHC with mouthpiece is also outfitted with an audible flow signal (Flow Signal whistle) that will sound if the patient inhales too quickly. The Venta Haler is an elliptical-shaped device made of rigid, transparent plastic with a capacity of 750ml. The Venta Haler was designed to fit GlaxoSmithKline products and therefore does not fit all pMDIs[20, 21].

Fig. 8: Spacing Devices

3. Multidose dry powder inhalers:

Alternative methods of aerosol delivery that are effective and easy to use are increasingly in demand. Some patients also have difficulty using pMDIs correctly because of problems synchronizing the activation of the pMDI and inspiration or because of reaction to the propellants used in pMDIs. Breath activated inhalers were developed in response to these problems. Dry powder inhalers (DPIs) are portable inspiratory flow-driven devices that deliver dry powder formulations of inhaled drugs to the lungs[22, 42].

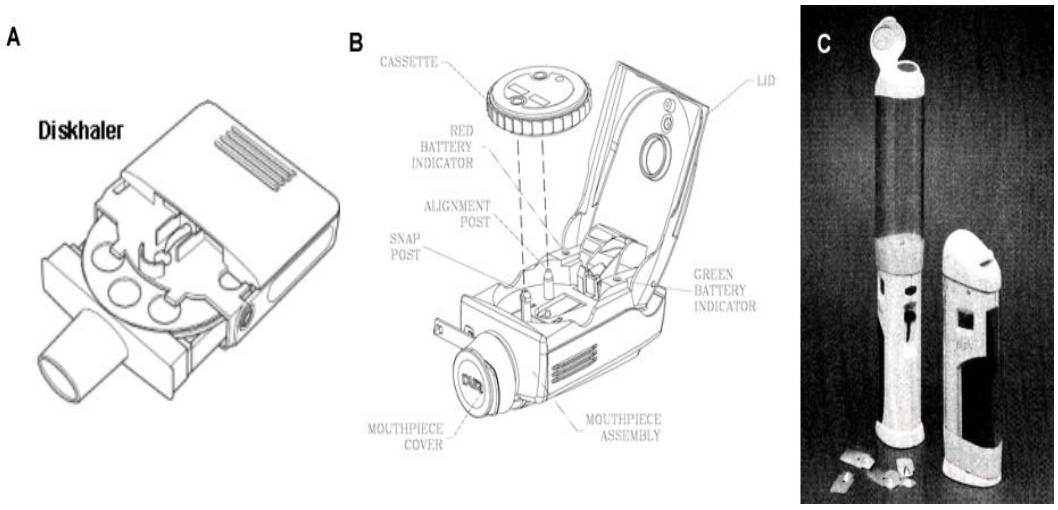

Fig. 9: Multi dose powder inhalers

a) Turbuhaler:

The Turbuhaler is a multi dose breath-actuated metered-dose inhaler that is comprised of components and a metal spring. The medication in the Turbuhaler is in the form of a fine, additive free dry powder (eg, budesonide, terbutaline) with the exception of formoterol and Symbicort (formoterol and budesonide combination), which also contains lactose powder. If the mechanism is activated a second time, either intentionally or inadvertently, the patient cannot inhale a double dose, as only a single dose is aligned at any time with the inhalation channel. The Turbuhaler uses the force of inspiration to lift particles that are deposited onto a dosing disc within the container into the respiratory system. When a patient inhales through the Turbuhaler, the fine powder medication moves through the inhalation channel toward a disaggregation zone, which consists of two spiral channels designed to create turbulent air flow in the mouthpiece (hence the name Turbuhaler). This action further breaks each particle of fine powder into smaller, more therapeutically effective units (particles in diameter < 6 µm). Studies indicate that the minimum inspiratory flow rate needed to attain a therapeutic dose using the Turbuhaler is 30 L/min. Because of the low flows required, the Turbuhaler (Bricanyl) has been quite successfully used in the treatment of acute severe asthma in the emergency department[22, 23].

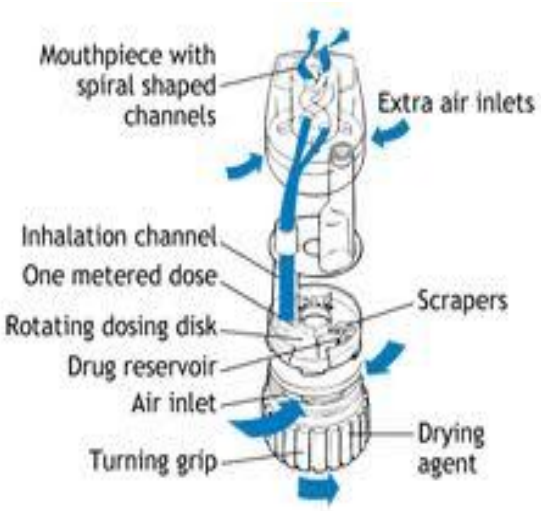

Fig. 10: Turbuhaler

b) Diskhaler:

The Diskhaler delivers dry powder formulations of inhaled drugs such as salbutamol, salmeterol, and beclomethasone in a lactose-based drug carrier. It consists of a case containing a rotating wheel onto which a metallic-filmed medication disk containing four or eight blisters of medication is loaded. A blister is mechanically punctured by lifting the cover. The medication may then be inhaled through the mouth. Subsequent doses are available by rotating the cartridge. Each dose on the blister pack is individually numbered and appears in a small window on the device, allowing the patient to monitor the number of doses taken and how many remain. Every medication disk is hermetically sealed and must remain so to protect the powder medication from humidity that will cause it to clump[24].

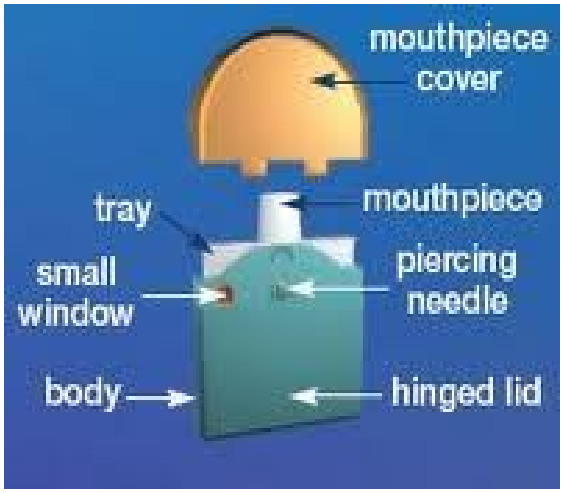

Fig. 11: Diskhaler

c) Diskus:

The Diskus is a new multi dose DPI that contains 60 doses of medication in a lactose-based carrier. The Diskus device currently carries all major classes of inhaled therapy (inhaled corticosteroids, short- and long-acting β_2 agonists, and combination therapy). The outside of the Diskus comprises five main parts: an attached cover that slides open, a thumb grip that uncovers the dose-release lever, a mouthpiece, and a dose counter. There are four wheels inside the Diskus [41]. One wheel contains 60 doses of powder medication individually wrapped in blisters on a foil strip. The individual wrapping protects the powder from humidity and other environmental conditions. Sliding the dose-release lever peels the foil off the top of each dose as it is advanced to the mouthpiece. The peeled foil is then wound on to another wheel. The dose is then inhaled, and the empty blister advances to a fourth wheel. Once the Diskus cover is closed, the lever is automatically returned to the starting position. The Diskus provides a relatively consistent doseover a wider range of flow rates than the other DPIs. Inspiratory flow between 30 and 90 L/min ensures delivery of 90% of the dose [23].

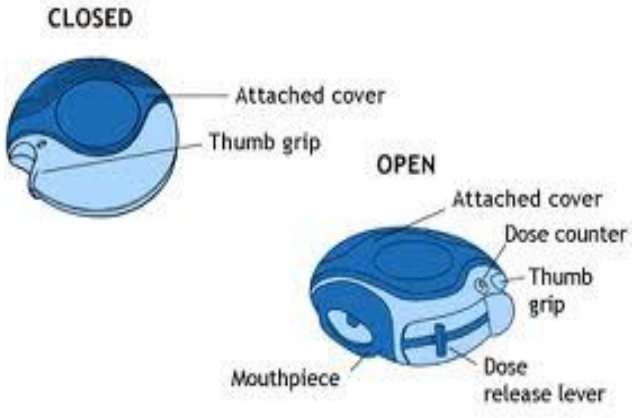

Fig. 12: Diskus

4. Wet nebulization:

Inhaled medication was delivered most commonly via wet nebulization, until recent years when more efficient portable devices became available. Nebulizers break down measured doses of medication in liquid form into a mist of small droplets, called an aerosol, which can then be inhaled through a mask or a mouthpiece. Wet nebulization requires the following:

- An energy source such as a compressor
- Compressed air or oxygen
- A mask or a mouthpiece
- A nebulizer

Compressed air or oxygen is more frequently used in hospitals that have large sources of these gases under pressure. Most patients will use a small portable compressor that is safe and effective for home use [40]. The compressor is powered by electricity and functions by drawing the surrounding air through an external air inlet, forcing it through a small tube to a nebulizer. In the nebulizer, the driving gas is forced through a very small opening called a Venturi, creating a low pressure zone. As a

result of this fall in pressure, the liquid is sucked up from the reservoir through a capillary system, creating droplets. Only the smallest droplets leave the nebulizer, whereas the majority impact on the baffles and walls of the nebulizer and drip back into the reservoir. This process is repeated continuously for several minutes. During nebulization, the solution used to dilute the medication evaporates resulting in an increasing concentration of the medication. A small amount of solution will remain on the baffles and walls of the nebulizer (dead volume), even when nebulization continues until no more spray is produced. Because the drug concentration increases during nebulization, up to 50% of the drug may be left in the reservoir. Several factors will affect drug deposition in the lungs, including the output of the nebulizer and the patient's breathing pattern. Drug output is affected by residual volume and gas flow. The latest nebulizers allow for an initial volume of 2 to 2.5 mL and leave a dead volume of about 0.5 mL Gas flow affects the size of particles released from the nebulizer and the nebulization time. Studies have shown that optimal flow for most currently used nebulizers is between 6 and 10 L/min. Generally, it takes approximately 5 to 10 minutes to administer 2.5 mL of solution at this flow rate. Breathing should be slow and regular, with an occasional deep breath. Additionally, when inhaling nebulized medication, the patient must learn to breathe through the mouth to reduce drug deposits in the nose and pharynx. Contrary to popular belief, wet nebulization is not the most effective aerosol delivery system. In fact, only 1 to 5% of the output from most compressed air nebulizers is delivered to the lower branches of the bronchial tree. A large amount of the medication is lost to the air during the exhalation phase because aerosol output is constant during both inspiration and expiration. According to the Nebulizer Project Group of the British Thoracic Society Standards of Care Committee and the Quebec Pharmacology Advisory Board nebulizers are indicated for the following circumstances:

- For those patients who are unable to use other types of inhalation devices, for example, those who suffer from physical or cognitive deficits.
- In hospital settings for severe dyspnoea and when high doses of medication or oxygen must be administered once able to cooperate, individuals should be given another type of inhalation device and their technique should be reassessed[25].

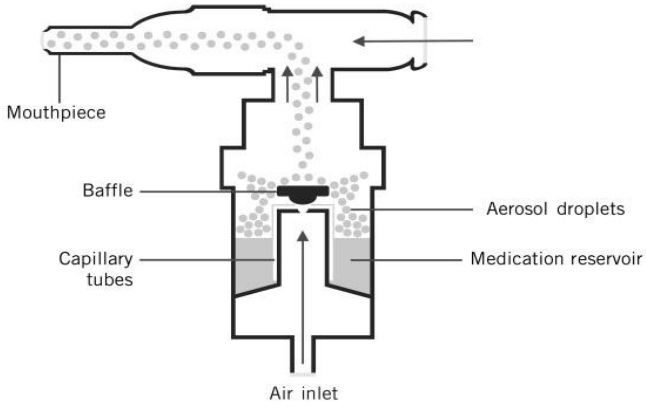

Fig. 13: Nebulizer

EVALUATION OF NANOPARTICLES

1. Methods for macroscopic studies:

a) Animals and organ preparation: For our studies with radio-labeled nanoparticles, the deeply anesthetized (isoflurane 5%) rats were killed by exsanguinations via the prepared abdominal aorta. Like this, about 70% of the total blood volume, estimated from the body weight, was collected. All organs and tissues, the remaining carcass as well as the total excretions were thereafter sampled for radio-analysis:

- Organs: lungs, liver, spleen, kidneys, reproductive organs, brain, heart, gastro-

intestinal tract, blood, skin
- Tissues: samples of muscle and of bone (femur)
- Remainder: carcass beyond the listed tissues and organs
- Excretions: urine and feces, collected separately

To avoid any cross contamination, the organs were collected in toto and all body fluids were immediately removed, when vessels or excretory ducts had to be cut. Organs and tissue samples as well as the entire excretion were collected such that the entire organism was sampled and weighed in wet state.

The application of clean dissection techniques is essential to avoid cross contamination, in particular in inhalation studies, where fur contamination occurs upon whole body or nose-only exposures. Systematic changes of dissection tools and equipment are highly recommended. In addition, whole body vascular perfusion to empty the blood vessels within the organs is recommended to estimate particle retention in the parenchyma[26].

2. Methods for microscopic studies:

Analysis of nanoparticles at the individual particle level by electron microscopy requires (i) adequate organ preservation, (ii) representative tissue sampling, and (iii) unambiguous identification of the nanoparticles in ultrathin tissue sections.

a) Lung fixation:

i. Airway instillation:

The fixative is introduced into the airways of a collapsed lung, in deeply anaesthetized animals or in cadaver lungs. Thereby, the air spaces evenly expand and the interalveolar septa remain unfolded. Note: Airway instillation of (aqueous) fixatives does not preserve the lung lining layer and luminal cells, i.e. macrophages are dislocated from their native positions [27-30].

ii. **Vascular perfusion:**

Fixatives are delivered to the lungs via the blood vessels, while the airways and alveoli remain in their natural air-filled state. This method is less effective in larger airways and especially of large animals, due to larger distance between vasculature and airway surface. Note: Fixation solely with glutaraldehyde, paraformaldehyde, or mixtures thereof, does not adequately preserve the lung lining layer and its associated luminal cells. Osmium tetroxide and uranyl acetate are necessary for cross-linking and stabilizing this laye[27-30].

iii. **Other methods:**

Vapor fixation of lungs at a given pressure is a fast and technically easy method to preserve whole lungs. However, shrinkage and organ distortion occur and ultrastructural preservation of cells is inferior. Non-polar fixatives, i.e. 1% osmium tetroxide dissolved in inert fluorocarbon (FC, Fluorinert™ Liquid, 3 M, and Belgium) are suitable to preserve large airways, either by immersion of excised specimens or by airway instillation. A variety of other fixation techniques have been developed in

view to preserve the inner surface of airways and alveoli, but most of them cannot be used to fix whole lungs or even lung lobes[27-30].

NANOPARTICLE COMPANY DIRECTORY

Company	Products
CytImmune(U.S.A)	Gold nanoparticles for targeted delivery of drugs to tumors
Invitrogen(U.S.A.)	Qdots for medical imaging
Evident(U.S.A.)	Quantum Dots
American Elements (U.S.A.)	Nanoparticles and Quantum Dots
Applied Nanotech(Austin)	Palladium nanoparticle-based hydrogen sensor.
Antaria(Australia)	Zinc oxide nanoparticles used in coatings to reduce UV exposure
Nanoledge(Canada)	Epoxy resins strengthened with nanoparticles
NanoHorizons(U.S.A.)	Fabric treatments containing silver nanoparticles
Nanocs	Gold and silver nanoparticles
Nanosolar(Germany)	Solar cells manufactured in a low temperature process using semiconductor nanoparticles
Sirem(Canada)	Iron nanoparticles to treat groundwater pollutants
NanoEner Technologies(Florida)	Electrodes composed of nanoparticles on a substrate for use in batteries. Partner company Enerdel is developing Li Ion battery packs for use in electric and hybrid vehicles
BASF(Germany)	Fabric enhanced with nanoparticles
Schoeller Technologies(Switzerlan)	Fabric enhanced with nanoparticles (NanoSphere®)
Nanosphere(U.S.A.)	Diagnostic testing using gold nanoparticles to detect low levels of proteins indicating particular diseases
Nanotherapeutics(Florid)	Nanoparticles for improving the performance of drug delivery by oral or nasal methods
Oxonica (U.K.)	Diagnostic testing using gold nanoparticles (biomarkers)
T2 Biosystems(U.S.A.)	Diagnostic testing using magnetic nanoparticles
Z-Medica	Medical gauze containing aluminosilicate nanoparticles which help blood clot faster in open wounds.
ApNano Materials(Israel)	Lubricants enhanced with nanoparticles
Nanomaterials Company(Philadelphia)	Nanopowders and nanostructured materials
Energenics(Singapore)	Diesel additive containing cerium oxide nanoparticle catalyst to reduce fuel consumption
Nanophase(U.S.A.)	Metal oxide nanoparticles
Meliorum Technologies(U.S.A.)	Metal, oxide and silicon nanoparticles
Nanoshel(U.S.A.)	Metal and oxide nanoparticles, carbon nanotubes
Nanopartz(U.S.A.)	Gold, platinum and palladium nanoparticles
Particular(Germany)	Nanoparticles produced by laser ablation
TECNAN	Large-scale production of simple, mixed and doped oxide nanoparticles
3M	Expoxy resins reinforced with silica nanoparticles

CONCLUSION

The review show that, by using nanoparticle, we can able to convert poorly soluble, poorly absorbed and labile biologically active substance into promising deliverable drugs. The core of this system can enclose a variety of drugs, enzymes, genes and is characterized by a long circulation time due to the hydrophilic shell which prevents recognition by the reticular-endothelial system. To optimize this drug delivery system, greater understanding of the different mechanisms of biological interactions, and particle engineering, is still required. Further advances are needed in order to turn the concept of nanoparticle technology into a realistic practical application as the next generation of drug delivery system.

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