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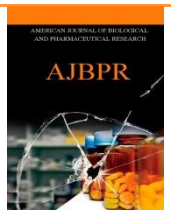

HUMAN SERUM ALBUMIN CARRIER FOR NEVIRAPINE NANOPARTICLES – A REVIEW

Umasankar K* and Jayachandra Reddy P

Department of Pharmaceutics,
Krishna Teja Pharmacy College, Chadalawada Nagar, Tirupati - 517506, Andhra Pradesh, India.

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ABSTRACT

Human serum Albumin (HSA) is a bio-macromolecule and has a great attention in both fundamental and applied medication due to its biodegradability, non-toxicity non-immunogenicity and regulatory function. For example, HSA could stabilize the ingredients in vaccines and modify the surface of medical devices. In addition the antioxidant property of HSA to construct possible innovated structure for therapeutic carriers. In fact HSA were used in frabicating albunex and ABI 007 for clinical purpose. Nanoparticles are prepared by following methods such as a) Amphiphilic macromolecules crosslinking b) Polymerization based methods c)Polymer precipitation methods d)Ionic gelation method. Considering the above factors, it is understood that delivery of nevirapine nanoparticles can greatly improve its solubility and bioavailability. HSA is a naturally occurring non immunogenic polymer and is considered advantageous for development of nevirapine nanoparticles. Desolvation method to prepare the nanoparticles. Therefore in the present study nevirapine nanoparticles was developed using HSA as a polymer by desolvation method and the nanoparticles was evaluated for physicochemical, in-vitro and in-vivo release characteristics.

INTRODUCTION

Viral disease is one of the most prevalent diseases in the world. The viral diseases are commonly occurring worldwide AIDS, Dengue, Encephalitis, Hepatitis, Yellofever. Among all the viral diseases AIDS is most dangerous and incurable disease. HIV virus comes from the Congo in 1959 and 1960 [1]. A recent study states that a strain of HIV probably moved from Africa to Haiti and then entered united states around 1969. India is one of the largest and most populated countries in the world with over one billion in habitants. Of this number it is estimated that around 2.27 million people are currently living with HIV, which indicates that there are more people with HIV in india than in any other country in the world [2].

Currently HIV is treated with anti-HIV drugs includes lamivudine, stavudine and zidovudine and nevirapine etc. Nevirapine is particularly indicated in the therapy of HIV as non-nucleoside reverse transcriptase inhibitor, and combination of lamivudine, stavudine and nevirapine is recommended for effective control of HIV. They are available as tablet dosage forms commercially. However nevirapine being poorly soluble may pose dissolution limited absorption problem resulting in poor bioavailability of the drug and therefore difficulty in controlling HIV infection. Different pharmaceutical approaches are followed to increase the dissolution of NVP. Solid dispersion of NVP with polyvinylpyrolidine (PVP k30) enhance the solubility of Nevirapine [3].

Corresponding Author

Umasankar K
Email:- umasankar73@gmail.com

Nanoparticles have become most active areas of research in the field of drug delivery due to their ability to deliver drugs to the right place, at appropriate times, and in right dosage. Nanoparticles can be defined as solid micron, colloidal particles ranging from 1nm to 1000nm in diameter, generally but not necessarily made of natural or synthetic polymers, in which drugs can be adsorbed, entrapped, encapsulated or covalently attached and are produced by mechanical or chemical means.

A pure anhydrous form of nevirapine in microspheres was prepared by sublimation and condensation of the drug and was reported 30% more solubule than the pure anhydrous drug and 140% more soluble than the semihydrate form. These microspheres are suitable for parentral dosage form inhalation therapy and injection. Liposomes of nevirapine were developed using egg phospholipid to cholesterol ratio at 9:1 which shows a prolonged release of NVP upto 1320 min at pH. Nevirapine nanosuspensions for iv was developed for targeting viral resvoirs in body was assessed the in-vitro protein absorption was carried out using 2-D PAGE. Bare nanosuspensions and surface modified nanosuspension with serum albumin, polysaccharide and PEG were compared. Solid lipid nanoparticles and nano structured liquid carriers containing nevirapine were reported. These formulations are coated with formulations human serum albumin. An accelerated release of NVP was reported from nanocarriers. When incubated with DODAB-stabilised SLNSs, the viability of HBMECs reduced [4].

The in-vitro and in-vivo performance of NPs depends on the type of polymers used in the development of NPs. Biodegradable polymers are most preferred for NPs because these are non toxic easly metabolized and eliminated from the body. Among the biodegradable polymers, Albumin NPs have gained high binding capacity with various drugs and well tolerated without any serious side effects.

HIV is the causative agent for AIDS. It is a sexually transmitted disease. Infection is aided by Langerhans cells in mucosal epithelial. The CD4+ T-lymphocytes have surface receptors to which HIV can attach. The infection extends to lymphoid tissues which contain follicular dendrites that can become infected and provide a reservoir for infection of CD4+ T-lymphocytes. When HIV infects a cell, it must use its reverse transcriptase enzyme to transcribe its RNA to host cell proviral DNA. It is this proviral DNA that directs the cell to produce additional HIV virions which are released.

Epidemiolgy of HIV

The genome of HIV contains only three major genes: **env, gag, and pol**. These genes direct the formation of the basic components of HIV. The **env gene** directs production of an envelope precursor protein **gp160** which undergoes proteolytic cleavage to the outer envelope glycoprotein **gp120,** which is responsible for tropism to CD4+ receptors, and transmembrane glycoprotein **gp41**, which catalyzes fusion of HIV to the target cell's membrane. The **gag gene** directs formation of the proteins of the matrix **p17**, the "core" capsid **p24**, and the nucleocapsid **p7**. The **pol gene** directs synthesis of important enzymes including reverse transcriptase **p51** and **p66**, integrase **p32**, and protease **p11** [5]**.**

In addition to the CD4 receptor, a coreceptor known as a chemokine is needed for HIV infection. Coreceptors include CXCR4 and CCR5. Their presence on cells can aid binding of the HIV envelope glycoprotein gp120, promoting infection. Initial binding of HIV to the CD4 receptor is mediated by conformational changes in the gp120 subunit, but not sufficient of fusion. The chemokine receptors produce a conformational change in the gp41 subunit which allows fusion of HIV. The differences in chemokine coreceptors explains how different strains of HIV may infect cells selectively. There are strains of HIV known as T-tropic strains which selectively interact with the CXCR4 to infect lymphocytes. The M-tropic strains of HIV interact with the CCR5 to infect macrophagesains.

Mechanism and Transmission of HIV Infection

HIV primarily infects CD4 cell-surface receptor molecules, using them to gain entry (Figure 1). Many cell types share common epitopes with this protein, though CD4 lymphocytes play a crucial role. In macrophages and in some other cells lacking CD4 receptors, such as fibroblasts, an Fc receptor site or complement receptor site may be used instead for entry of HIV. Cells of the mononuclear phagocyte system, principally blood monocytes and tissue macrophages, T lymphocytes, B lymphocytes, natural killer lymphocytes, dendritic cells, hematopoietic stem cells, endothelial cells, micro glial cells in brain, and gastrointestinal epithelial cells are the primary targets of HIV infection [6]. The virus is crossing the mucosa of the genital tract to infect CD4+ T-lymphocytes. A Langerhans cell in the epithelium is shown in red in this diagram.

HIV is transmitted through direct contact of a mucous membrane or the bloodstream with a bodily fluid containing HIV, such as blood, semen, vaginal fluid, preseminal fluid, and breast milk. This transmission can involve anal, vaginal or oral sex, blood transfusion, contaminated hypodermic needles, exchange between mother and baby during pregnancy, childbirth, breastfeeding or other exposure to one of the above bodily fluids.

Role of Nevirapine in HIV Infection

Nevirapine falls in NNRTI class of antiretrovirals. Both nucleoside and non-nucleoside RTIs inhibit the same target, the reverse transcriptase enzyme, an essential viral

enzyme which transcribes viral RNA into DNA. Unlike nucleoside RTIs, which bind at the enzyme's active site, NNRTIs bind allosterically at a distinct site away from the active site termed the NNRTI pocket.
Nevirapine is not effective against HIV-2, as the pocket of the HIV-2 reverse transcriptase has a different structure, which confers intrinsic resistance to the NNRTI class.

Nevirapine is a prescription medicine approved by the U.S. FDA for the treatment of HIV infection in adults and children. Nevirapine is always used in combination with other anti-HIV medicines. It is a type of anti-HIV medicine called a NNRTI. NNRTIs work by binding to and blocking HIV reverse transcriptase, an HIV enzyme. This prevents HIV from replicating and lowers the amount of HIV in the blood [7].

Nevirapine comes in the following forms and strengths:

- 200-mg immediate-release tablets (brand name: Viramune).
- 100-mg extended-release tablets (brand name: Viramune XR).
- 400-mg extended-release tablets (brand name: Viramune XR).
- 50-mg/5 mL oral suspension (brand name: Viramune).

A single dose of nevirapine given to both mother and child reduced the rate of HIV transmission by almost 50% compared with a very short course of zidovudine (AZT) prophylaxis. Using Nevirapine to Prevent Mother-to-Child HIV Transmission During Breastfeeding is scheduled for completion in March 2011.

A major concern with this approach is that NNRTI resistance mutations are commonly observed in both mothers and infants after single-dose nevirapine, and may compromise the response to future NNRTI-containing regimens. A short course of maternal zidovudine/lamivudine is recommended by the U.S. Public Health Service Task Force to reduce this risk.

Nanotechnology

Nanotechnology, the term derived from the Greek word Nano, meaning dwarf, applies the principles of engineering, electronics, physical and material science, and manufacturing at a molecular or submicron level. The materials at nanoscale could be a device or a system or these could be supramolecular structures, complexes or composites. An early promoter of nanotechnology, Albert Franks, defined it as 'that area of science and technology where dimensions and tolerances are in the range of 0.1nm to 100nm'. Nano technology is expected to make significant advances in biomedical applications, including in the areas of gene therapy, drug delivery, imaging, and novel drug discovery techniques.

Nanotechnology is hailed as a new generation of technology with the potential to revolutionise many facets of the world we live in. This includes virtually all aspects of daily life, including health and health care, the manufacturing and use of materials and equipment, the environment and protection thereof. It is said to be increase manufacturing production at significantly reduced costs. Products of nanotechnology will be smaller, cheaper, lighter yet more functional and require less energy and fewer raw materials to manufacture.

Nanotechnology can be defined as having the following features

- It involves research and development at the 1 nm–100 nm range
- It creates and uses structures that have novel properties because of their small Size
- It builds on the ability to control or manipulate at the atomic and molecular scale

At the nano-scale the interactions between atoms display 'exotic' properties that are absent at larger scale because at this level atoms leave the realm of classical physical properties behind and enter the realm of quantum mechanics. Nanotechnology includes a bewildering array of activities including: molecular manufacturing, supramolecular and self assembly/organization; biomimicry; nanoparticles (e.g. Bucky balls and carbon nano tubes), nanospheres, nano cups and nanorods; nanobots (nanorobots); colloids, micelles, vesicles and nano-emulsions; clathrate complexes and intercalation compounds.

Nanotechnology in drug delivery

The development of delivery systems for small molecules, proteins and DNA has been impacted to an enormous degree over the past decade by nanotechnology, and has led to the development of entirely new and somewhat unpredicted fields. For the pharmaceutical industry, novel drug delivery technologies represent a strategic tool for expanding drug markets. The technology can address issues associated with current pharmaceuticals such as extending product life, or can add to their performance and acceptability, either by increasing efficacy or improving safety and patient compliance. This technology is permitting the delivery of drugs that are highly water- insoluble or unstable in the biological environment. Advantages of Nano sizing of drugs has the potential to: Increase surface area, enhance solubility, increase rate of dissolution, increase oral bioavailability, more rapid onset of therapeutic action, decrease the dose needed, decrease fed/fasted variability and decrease patient to patient variability.

In recent trend, Nano drug delivery may occur through gold nanospheres and rods, nanowires, nanotriangles, nanostars, nanocubes, and nanorice. Nanoplatforms include organic nanostructures, polymeric nanoparticles, lipid systems-liposomes, self assemblies-micelles, dendrimers, and carbon nanostructure-nanotubes.

Inorganic nanostructures include metal nanoparticles and nanoshells, silicon nanostructure, nanocrystals, and quantum dots. Hybrid nanostructures, combining two to three of those previous listed can also be produced. Studies were described in which polymeric nanoparticles used for tumor-targeted deliver, Gelatin-based nanoparticles used for gene delivery and nanoemulsions for oral and intravenous delivery. Gadolinium-loaded nanoemulsion has been used in animals for brain imaging, and used for imaging within the eye to observe the results of various drug delivery modalities.

The benefits of Nanotechnology are

- The lifespan of the blockbuster drugs can be resurrected by reformulating the drugs through novel drug delivery system.
- The effective patent protection can be enhanced.
- Drug delivery formulation involves low cost research compared to that for the discovery of new molecules.
- Minimizing use of expensive drugs would reduce the cost of the product.

Nanoparticles

Nanoparticles are solid colloidal particles with diameters ranging from 1-1000 nm. They consist of macromolecular materials and can be used therapeutically as adjuvant in vaccines or drug carriers, in which the active ingredient is dissolved, entrapped, encapsulated, adsorbed or chemically attached. Polymers used to form nanoparticles can be both synthetic and natural polymers. There are two types of nanoparticles depending on the preparation process nanospheres and nanocapsules. Nanospheres have a monolithic-type structure (matrix) in which drugs are dispersed or adsorbed on to their surfaces. Nanocapsules exhibit a membrane-wall structure and drugs are entrapped in the core or adsorbed on to their exterior. In recent years, biodegradable polymeric nanoparticles, coated with hydrophilic polymer such as poly (ethylene glycol), 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.

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 [8].

The Advantages of using nanoparticles as a drug delivery system include the following

- Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.
- They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.
- Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity.
- Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.
- The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc.

In spite of these advantages, nanoparticles do have limitations. For example, their small size and large surface area can lead to particle particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms, limited drug loading and burst release. These practical problems have to be overcome before nanoparticles can be used clinically or made commercially available.

Related works on nanoparticles with anti HIV drugs

In the present study, an attempt was made to develop nanoparticulate delivery system for highly water soluble drug lamivudine. Chitosan nanoparticles of drug lamivudine were prepared by ionic gelation technique. The method was able to produce discrete, free flowing and uniform sized particles. All the formulations showed high process yield and drug loading capacity. Among the different batches, Formulation F1 (drug polymer ratio 1:1) was selected as the ideal formulation, after considering their better drug loading capacity, and *in vitro* drug release. Based on the observations, it can be concluded that the formulated nanoparticulate delivery system of highly water soluble drug lamivudine using widely accepted and physiologically safe polymer was capable of exhibiting sustained release properties for a period of 24 h. They are thus may be reduce frequency of dosing, thereby minimizing the occurrence of side effects, improve bioavailability and increase the effectiveness of the drug.

Albumin nanoparticles of anti viral drug azidothymidine were prepared and evaluated for brain specific delivery after intravenous administration. Long circulatory polyethyleneglycolated (PEGylated) albumin nanoparticles of azidothymidine were prepared by ultra-emulsification method using chemical cross linking by glutaraldehyde. Surface of PEGylated nanoparticles was modified by anchoring teansferrin as a ligand for brain targeting. Fluorescence studies revealed the enhanced uptake of transferring-anchored nanoparticles in brain

tissue when compared with unmodified nanoparticles. A significant enhancement of brain localization of azidothymidine was observed for transferring anchored PEGylated albumin nanoparticles**.**

In the study of nevirapine nanosuspensions were prepared by high-presssure homogenization characterized. A crystalline NS of nevirapine for intravenous injection was developed assessed regarding its targeting potential to viral reservoirs in body .To determine the intereactions of the nanocrystals with proteins,in vitro protein absorption studies in plasma were carried out using 2-D PAGE. The in vitro protein rejecting and accepting proteins were studied as a function of stabilizer of the nanocrystals Bare NS and surface modified NS (eg serum albumin, polysaccharide and PEG) were compared regarding their protein absorption patterns [9].

Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) coated with human serum albumin (HSA) were fabricated for formulating Nevirapine (NVP). Here NLCs contained low melting point oleic acid (OA) in the internal liquid phase. The results revealed that the two nanoparticles were uniformly distributed with the average diameter ranging from 145 to 180nm. The surface HSA neutralized the positive charge of dimethyldioctadecyl ammonium bromide (DODAB) on SLNs and NLCs and reduced their zeta potential. In a fixed ratio of solid lipids, SLNs entrapped more NVP than NLC. The incorporation of OA also reduced the thermal resistance of NLCs and accelerated the release of NVP from the nanocarriers. When with DODAB-stabilized SLNs, the viability of human brain microvascular endothelial cells reduced. However the surface HSA increased the viability of HBMECs about 10% when the cancentration of SLNs was higher than 0.8mg/ml. HSA grafted SLNs and NLCs can be effective formulations in the delivery of NVP for viral therapy [10].

These methodologies are conveniently classified as follow

1) Amphiphilic macromolecules cross-linking
a) Heat cross-linking
b) Chemical cross-linking
2) Polymerization based methods
a) Polymerization based methods
b) Emulsion (micellar) polymerization
c) Dispersion polymerization
d) Interfacial condensation polymerization
e) Interfacial complexation
3) Polymer precipitation methods
a) Solvent extraction/evaporation
b) Solvent displacement (nanoprecipitation)
c) Salting out
4) Ionic gelation method

Nanoparticles can be prepared from proteins, polysaccharides and synthetic polymers. The selection of matrix materials is dependent fallowing factors including[28]
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) Antigencity of the final product.

Most frequenty Nanoparticles have been prepared by three methods.
1) Dispersion of preformed polymers.
2) Polymerization of monomers; and
3) Coacervation

Other methods such as superficial fluid technology and particle replication in non-wetting templates have described in the literature for production of nanoparticles. Dispersion of preformed polymers is a common technique used to prepare biodegradable nanoparticles from poly (lactic acid) (PLA); poly (D, L-glycoside), PLG; poly (D, L-lactide-co-glycolide) (PCA) 21. This technique can be used in various ways as described below.

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 emulgent to form 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 reducing the pressure or by continuous stirring. Particle size was found to influence 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.

Spontaneous emulsification or solvent diffusion method

In this method, the water miscible solvent and 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 aqueous phase.

Polymerization method

In this method, monomers are polymerized to form nanoparticles in an aqueous solution. Drug is incorporated either by 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. Nanocapsules formation and their particle size depend on the concentration of the surfactants and stabilizers used [11].

Cocervation or ionic gelation method

This method involves a mixture of two aqueous phases, one is the polymer chitosan, a di-block co-polymer ethylene oxide or propylene oxide (PEOPPO) and the other is a polyanions 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.

Production of nanoparticles using superficial fluid technology

A superficial 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 is the most widely used supercritical fluid because of its mild critical conditions (Tc = 31.1^0C, Pc = 73.8 bars), non-toxicity, non-inflammability, and low price. The most common techniques involving supercritical fluids are supercritical anti-solvent (SAS) and rapid expansion of critical solution. The process of SAS employs a liquid solvent, e.g. methanol, which is miscible with the supercritical fluid to dissolve the solute to be micronized; 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.

Polyelectrolyte complex (PEC)

Mechanism of PEC formation involves charge in neutralization between cationic polymer and DNA leading to a fall in hydrophilicity as the polyelectrolyte component self assembly. Several cationic polymers (i.e. gelatin, polyethylenimine) also have this property. The nanoparticles spontaneously formed after addition of DNA solution into Chitosan dissolved in acetic acid solution, under mechanical stirring at or under room temperature. The complexes size varied from 50 to 700 nm [12].

Microemulsion method

Chitosan NP prepared by microemulsion technique was first developed by Maitra, 1999). In this method, a surfactant was dissolved in N-hexane. Then, chitosan in acetic solution and glutaraldehyde were added to surfactant/hexane mixture under continuous stirring. Nanoparticles were formed. The system was stirred overnight to complete the cross-linking process, which the free amine groups of chitosan conjugate with glutaraldehyde. The organic solvent is then removed by evaporation under low pressure. The cross-linked chitosan NP and excess surfactant were obtained. The excess surfactant was then removed by precipitate with CaCl2 and then the precipitant was removed by centrifugation. The final nanoparticles suspension was dialyzed before Lyophilization. This technique offers a narrow size distribution of less than 100 nm and the particle size can be controlled by varying the amount of glutaraldehyde that alters the degree of cross-linking.

Freeze drying of nanoparticles

Protective excipients, such as carbohydrates, are widely used in freeze-drying to ensure redispersibility and to avoid aggregation or size changes of nanoparticles[50]. Glucose and lactose were evaluated as cryo- and lyo protectants for the L- PLA nanoparticles because these nanoparticles could not survive during the drying process without protectants. Even the smallest tested amount of glucose (weight ratio glucose: nanoparticles 1:4) was found to protect the nanoparticles, although the appearance of the dried material was translucent and sticky, and its redispersibility was poor. When lactose was used as a protectant, it enhanced the appearance of the cake (the dried material) as a white powder, eligible for a freeze-dried formulation. Redispersion of the nanoparticle was possible, but as a form of visible aggregates. Further freeze-thawing experiments revealed that already the freezing step (with lactose) destroyed the particles. Next, the two carbohydrates were used together to combine the cryoprotective functionality of glucose and the lyoprotective functionality of lactose. The best result, prolonged Tyndall effect (opalescence in the dispersion) after redispersion of the dried formulation and good quality nanoparticles were obtained, when the amount of lactose was double the amount of glucose. The weight ratios of glucose and lactose to the nanoparticles were 1:2 and 1:1, respectively. Additionally, when an extra stabilizer, Tween 80, was used during the nanoparticle preparation or during the redispersion, the freeze-dried cake could be redispersed more easily with increased stability (prolonged Tyndall effect).

The good cryoprotective results with glucose probably arise from its ability to bind water molecules to the amorphous phase which it forms during the freezing step. Part of the water in the frozen glucose remained non-frozen (even 32% w/w). That water acted as a plasticizer and as a spacing matrix reducing the pressure of ice crystals against the nanoparticles and preventing harmful aggregation caused by freeze concentration, respectively. At the same time, insufficient cryoprotective function of lactose derived most likely from its lower water binding activity. However, as a combination with glucose, lactose reduced the amount of water to a level where the interaction of glucose with water was reduced and, thus, the formation of ice crystal was slightly promoted. This enabled sufficient evaporation of water during the drying and formation of a proper cake. Tween 80 improved the freeze-drying result as it acted as a steric stabilizer and increased the hydrophilicity of the nanoparticles. A hydrophilic surface enhances the redispersion properties of the freeze-dried nanoparticles.

Following advantages are cited for the freeze drying of nanopartilces[29]:

- Prevention from degradation and/or solubilization of the polymer.
- Prevention from drug leakage, drug desorption and/or drug degradation.
- Easy to handle and store and helps in long-term prevention/conservation of nanoparticles.

Criteria for ideal polymeric carriers for nanoparticles & nanoparticle delivery systems

Polymeric carriers

- Easy to synthesize and characterize
- Inexpensive
- Biocompatible
- Biodegradable
- Non-immunogenic
- Non-toxic
- Water soluble

Nanoparticle delivery systems

- Simple and inexpensive to manufacture and scale-up
- No heat, high shear forces or organic solvents involved in their preparation process.
- Reproducible and stable
- Applicable to a broad category of drugs; small molecules, proteins and polynucleotides
- Ability to lyophilize
- Stable after administration
- Non-toxic

Table 1. Polymers Used for the Preparation of Nanoparticles

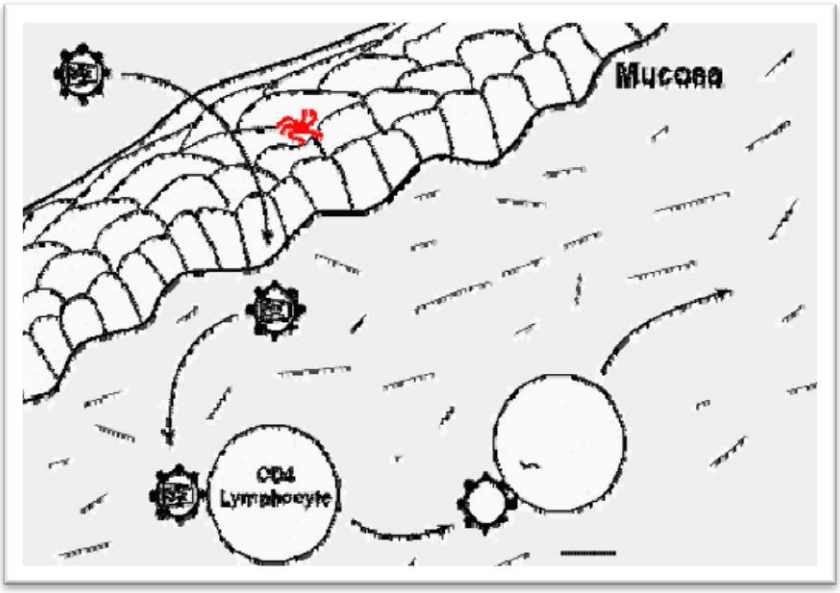
Polymer use	Technique	Candidate drug
Hydrophilic	Heat denaturation and cross-linking in w/o emulsion	Hydrophilic
Albumin, gelatin	Desolvation and cross-linking in aqueous medium	Hydrophobic and protein affinity
Alginate, chitosan	Cross-lining in aqueous medium polymer	Hydrophilic
Dextarn	precipitation in an organic solvent	Hydrophilic
Hydrophobic	Emulsion polymerization	Hydrophilic
Poly(alkylcyanoacrylates)	Interfacial o/w polymerization	Hydrophobic
Polyesters	Solvent extraction-evaporation	Hydrophilic& Hydrophobic
Poly (lactic acid), poly (lactide-co-glycolide), poly (e-cprolactone)	Solvent displacement Salting out	Soluble in polar solvent Soluble in polar solvent

Table 2. Characteristics of nanoparticles on drug delivery Different parameters and characterizaton methods of nanoparticles

Parameter	Characterization method
Particle size and distribution	Photo correlation spectroscopy (PCS) Laser defractometry Transmission electron microscopy(TEM) Scanning electron microscopy(SEM) Mercury porositometry
Charge determination	Laser Doppler anemometry Zeta potentiometer
Surface hydrophobicity	Water Doppler Anemometry Rose Bengal (dye) binding Hydrophobic interaction chromatography X-ray photoelectron spectroscopy
Chemical analysis of surface	Static secondary ion mass spectroscopy
Carrier-drug interaction	Differential scanning calorimetry (DSC)

Nanoparticles dispersion stability	Critical flocculation temperature (CFT)
Release profile	*In vitro* release characteristics under physiologic and sink conditions
Drug stability	Bioassay of drug extracted from nanoparticles chemical analysis of drug

Fig 1. Mechanism of HIV



Particle Size

Particle size and size distribution are the most important characteristics of nanoparticle systems. They determine the *in vivo* distribution, biological fate and targeting ability of nanoparticle systems, drug loading, drug release and stability of nanoparticles.

Nanoparticles of sub-micron size have a number of advantages over microparticles. Generally nanoparticles have relatively higher intracellular uptake compared to microparticles and available to wider range of biological targets due to their small size and relative mobility.100nm nanoparticles had a 2.5 fold greater uptake than 1µm microparticles and 6 fold greater uptakes than 10µm microparticles in a CACO-2 cell line. Nanoparticles can cross the blood-brain barrier following the opening of tight junctions by hyper osmotic mannitol, which may provide sustained delivery of therapeutic agents for difficult-to-treat diseases like brain tumors. Tween 80 coated nanoparticles have been showed to cross the blood-brain barrier. In some cell lines, only submicron nanoparticles can be taken up effectively but not the larger size microparticles. The recent literature shows ophthalmic nanosuspension that proves to be boon for drugs that exhibit poor soluble in lachrymal fluid.

Drug release is affected by particle size. Smaller particles have larger surface area, leading to fast drug release. Whereas, larger particles have large cores which allow more drug to be encapsulated and slowly diffuse out.

Method of determining particle size is by photon-correlation spectroscopy or dynamic light scattering. Photon-correlation spectroscopy requires the viscosity of the medium to be known and determines the diameter of the particle by Brownian motion and light scattering properties. The results obtained by photon-correlation spectroscopy are usually verified by scanning or transmission electron microscopy (SEM or TEM).

Surface Properties of Nanoparticles

A part from the size of nanoparticles, their surface hydrophobicity determines the amount of adsorbed blood components, mainly proteins. This is turn influences the *in vivo* fate of nanoparticles. Binding of these opsonins on nanoparticles called opsonization acts as a bridge between nanoparticles and phagocytes. The association of a drug to conventional carriers leads to modification of the drug biodistribution profile, as it is mainly delivered to the mononuclear phagocytes system such as liver, spleen, lungs and bone marrow. Generally, IgG, compliment C_3 components are used for recognition of foreign substances specially macromolecules.

Hence, it is necessary to minimize the opsonized and to prolong the circulation of nanoparticles *in vivo*. This can be achieved by surface coating of nanoparticles with biodegradable copolymers with hydrophilic segments such as polyethylene glycol (PEG) Polyethylene oxide, polyoxamer, poloxamine and polysorbate 80 (Tween 80).

The zeta potential of a nanoparticle is commonly used to characterize the surface charge property of nanoparticles. Nanoparticles with a zeta potential above (+/-) 30 mV shows stable suspension, as the surface charge prevents aggregation of the particles. The zeta potential can also be used to determine whether a charged active material is encapsulated within the centre of the *nanocapsule* or adsorbed onto the surface.

Drug-polymer interactions

Drug loading can be performed during the preparation of nanoparticles or by adsorbing/absorbing in preformed particles. Within the particle-forming polymer, drug can be present as a solid solution (individual drug molecules) or as a solid dispersion (amorphous/crystalline drug). It can be adsorbed on the particle surface or bound chemically within the nanoparticles. The preparation process can also modify the crystal structure of the drug. The polymer is usually amorphous or semi-crystalline. DSC, (powder) x-ray diffractometry and FTIR are commonly used techniques to reveal the physicochemical state and possible interactions of the drug and the polymer in pharmaceutical micro- and nanoparticles. Polymer MW is determined e.g. by SEC.

DSC detects phase transitions such as glass transition, (exothermic) crystallization and (endothermic) melting: the nanoparticle sample is heated and changes in heat flow, compared to reference, are registered. Crystallinity/amorphicity properties are obtained from XRPD analysis when diffraction pattern of the x-ray from the sample is determined as a function of scattering angle.. In FTIR, a vibrational spectrum, characteristics for a given crystal structure, is obtained.

Absence of the drug melting peak and diffraction peaks of the crystal structure of the drug in DSC thermo gram and XRPD pattern, respectively, are usually signs of amorphous or molecularly dispersed drug within the polymer .It can also indicate that the amount of drug is lower than the detection limit of the instrument. Drug polymer interactions (e.g. plasticizing effect of drug on polymer) or polymorph change of the drug can be detected as peak shifts in DSC thermogram, band shifts in FTIR spectra or as new reflections in XRPD pattern. Correspondingly, smoothened XRPD pattern, increased cold crystallization exotherms (DSC) or some band shifts to higher wave numbers (FTIR) indicate increased amorphicity of the polymer.

Drug loading

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 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). For small molecules, use of ionic interaction between the drug and matrix materials can be a very effective way to increase the drug loading [13].

***In-vitro* 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: (1) solubility of drug; (2) desorption of the surface bound/adsorbed drug; (3) drug diffusion through the nanoparticle matrix; (4) nanoparticle matrix erosion/degradation; and (5) combination of erosion/diffusion process.

CONCLUSION

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 controlled by a diffusion process. 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 nanoparticle is coated by polymer, the release is controlled by diffusion. 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 auxillary ingredients. Addition of ethylene oxide-propylene oxide block copolymer (PEO-PPO) to chitosan, reduces the interaction of the model drug bovine serum albumin (BSA) with chitosan due to competitive electrostatic interaction of PEO-PPO with chitosan, then an increase in drug release could be observed.

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