WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 3, 1456-1471. Review Article ISSN 2277– 7105

NANO FORMUATIONS: PREPARATIONS AND STABILITY CHALLENGES

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Article Received on
19 Jan. 2020,

Revised on 09 Feb. 2020,
Accepted on 29 Feb. 2020,

DOI: 10.20959/wjpr20203-16951

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ABSTRACT

Nano drug delivery system is engineered technologies that use nanoparticle size ranges from 1-100nm to the target drug delivery by controlled release of therapeutic agent. This modern form of a drug delivery system reduce both dosage and dosage frequency. Novel drug delivery system is that it is ahead of conventional dossage forms, it bring forth optimum dose at the right time and right location, well regulated use of exorbitant drugs and excipients, depletion on production cost, advantages to patients, superior therapy. Nanoformulations are formulations of nano medicine. The nano medicine compass nanoparticles for the treatment and diagnosis of disease in more efficacious way. The drug is loaded on the nano carrier which are used for the dispatch of drug to the target tissue meticulous or controlled manner. Disparate nanocarriers used in drug delivery system are liposomes, solid lipid nanoparticles, carbon nanotube, virus based etc. Application of these particle provide expeditious and intense development in the field of medicine, health and technology. Nano formulations in target drug delivery used to treat cardio-vascular disease, diabetics and cancerous tumours. Many challenges observed are protein Corona, toxicity, stability etc. These challenges should be solved to enhance the potential and make more effective in physical, chemical, and therapeutic formulations.

KEYWORDS: Organic and inorganic nano carriers, preparations and challenges.

INTRODUCTION

Nano formulation triggers intense interest over past decades in the field of medicine. The principle for nano medicine pharmaceutical application is to change the drug compounds to nanoparticles. ie ultrafine particle with surrounding layer and having size less than 100nm. The foundation lay on the physiochemical properties, color etc. According to the shape they can be 0D, 1D, 2D, or 3D the nanoparticle contain mainly 3 layers, surface shell and core. These new generation molecule play significant role in medication, manufacturing and material, environment, electronics and energy harvesting.

Organic Nanocarriers

1. Liposomes: Liposomes in drug delivery showed markedly altered pharmacokinetic properties compared to free drug in solution. Liposomes are spherical vesicle composed of phospholipids or steroids having an aqueous core enclosed by lipid bilayer. They have single or multiple bilayer membrane assembly formed of natural or synthetic lipids. Those containing one layer are termed as small unilamellar or large unilamellar based on their size. If more than one bilayer presentthey are called as multilamellar vesicles. Interaction of liposomes with cells can be realized by absorption. Fusion, endocytis and lipid transfer. Drugs in liposomal formulations are anti-cancer drugs, neurotransmitters, antibiotics, anti-inflammatory and antirheumatic.[1,2]

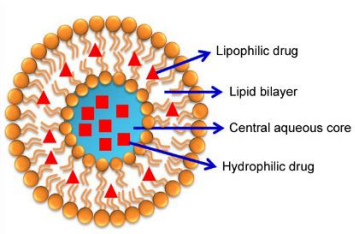

Fig. 1: Diagrammatic representation of liposome struacture.

2. Dendrimers: Dendrimers are frequently branched macromolecules with various arms originating from the central core. Produced from natural or synthetic components, which include sugar nucleotide and aminoacids. Dendrimers are drug delivery system owing to their inimitable characteristics, including distinctive molecular weight, increased number of branching, multi -valency, spherical shape and monodispersed macromolecules. It comprises of branched layer consisting of reiterating units, numerous active terminal groups and

initiator core. Drugs can be loaded to cavities in the dendrimers cores through hydrogen bond, chemical linkages or hydrophobic interaction. Each level of added branches to the core throughout the synthesis process is called as a generation. Dendrimers has been extensively used in the field of biomedicine including gene delivery. The drug is attached to the dendritic boundary with the help of acid labile or disulphide linkers. The drug release is triggered by introducing a masked 4 amino cinnamyl alcohol linker. Dendrimers providean excellent platform for the drug delivery of drugs such as folic acid santimicrobial drugs.[3,4]

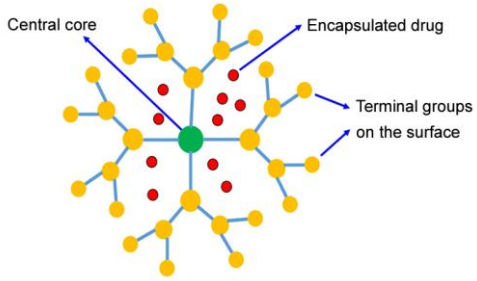

Fig. 2: Structure of dendrimer.

3. Polymeric Nanoparticles: PNPs are solidcolloidal particles made up of biodegradable polymers. PNPs can be classified as nanospheres and nanocapsules. Nanospheres type of PNPs disperse or entrap the drug in the polymer matrix. Nanocapsule type of PNPs the drug is dissolved or dispersed in liquid core of oil or water encapsulated by a solid polymeric membrane. A number of methods developed to prepare PNPs depending upon composition and desired properties of PNPs. The method can be conveniently classified into two categories, namely dispersion of performed polymers and direct polymerization of monomers. The method involving the dispersion of performed polymers include solvent evaporation, salting out, nano precipitation dialysis and supercritical fluid technology. These biodegradable polymers should be stable in blood, non-toxic and non-thrombogenic. Drug released by desorption, diffusion or nanoparticle erosion in target tissue. Drugs used for delivering are carboplastin.[5,6]

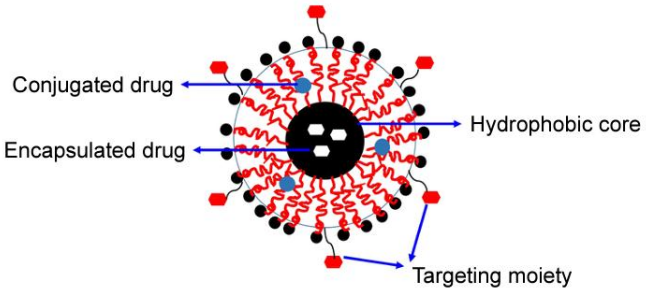

Fig. 3: Structure of PNPs.

4. Solid lipid nano particles: Colloidal drug carrier prepared by dispersing melted solid lipid in water in which the emulsifiers are used to stabilize the dispersion. Methods used for its prepration are high pressure homogenization and microemulsification SLNs have highly lipophilic lipid matrix for drugs to be dispersed or dissolved. It is similar to nano emulsion but the only difference is lipid that are solid at room temperature are used in SLNs where as liquid lipids in nano emulsions. SLNs score better in controlled drug delivery lack of biotoxicity and improved bioavailability of poorly watersoluble drugs. Drug can be either dispersed homogenously in lipid matrix of SLNs, incorporated into shell surrounding lipid core. Or incorporated into the core surrounded by a lipid shell. SLNs are natural platform for anti cancer drugs are docetaxei, doxorubicin, paclitaxel, methotrixate and 5- flurouracil.[7,8]

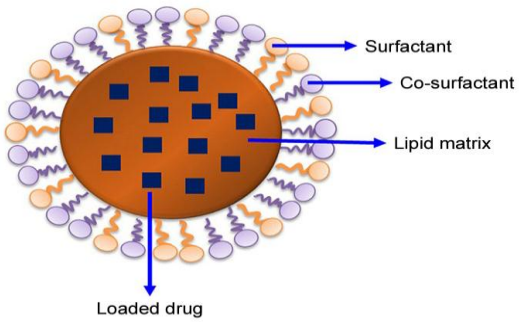

Fig. 4: Structure of SLNs.

5. Virus based nanoparticles: These are virus like nanoparticles, self assembled robust protein cages having uniform nano structure. VNPs are included in drug delivery gene therapy, vaccination, imaging and targeting. it offers attractive features such as morphological uniformity, biocompatibility, easy surface fuctionalisation and availability. VNPs are obtained from the plant virus, mottle virus, cowpea mosaic virus, tobacco mosaic virus, insect viruses etc. for the delivery of drug it can either be physically entrapped or

chemically attached to VNPs. In physical entrapment, simple or natural process of supra molecular self assembly or reassembly of viral protein capsid used to load the drug. In chemical attachment drug loaded through covalent attachment of drug molecules to certain reactive sites on capsid.[9,10]

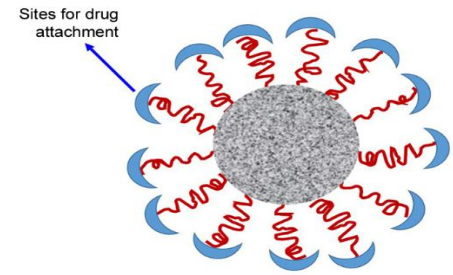

Fig. 5: Structure of VNPs.

6. Polymericmicells: PMs are colloidal nanoparticles formed by the assembly of synthetic amphiphilic di or tri block copolymers in an aqueous medium. These exposed to aqueous environment above particular concentration called critical micells concentration forms the micells. The hydrophilic constitute the shell and hydrophobic constitutes the core the PM core allows entrapment of hydrophobic drugs and control the drug release while the hydrophilic shell stabilize the core and enhance the solubility. The drug can be loaded by physical entrapment or chemical attachment. Methods for preparation are dialysis, solvent evaporation method.

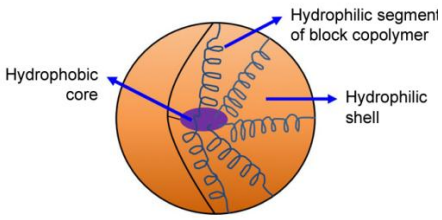

Fig. 6: Polymeric micells.

Inorganic nanoparticles

1. Carbon nanotubes: CNTs are nano hollow tube like assemblies of carbon atom discovered byIiijima. CNTs belonging to the family of fullerence. These are formed by graphene sheets roll up into a tube like structure, they are classified into single walled cabon nanotubes and multi walled carbon nanotubes. CNTs production techniques are discharge, laser ablation and thermal or plasma enhanced chemical vapourdiposition. The features of

CNTs include nano needle shape, hollow monolithic structure, high aspect ratio, ultra light weight, high mechanical strength, high electrical and thermal conductivity. it has needle like shape which allows to cross membrane via endocytosis or needle like penetration. applications of CNTs include targeted delivery of anti cancer drugs.

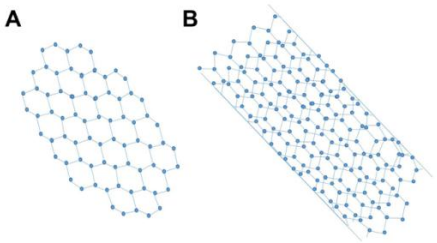

Fig. 8: Structure of SWCNTs.

2. Silica Nano particles: SNPs are biocompatible with a variety of diagnostics and therapeutic applications. These are classified as xerogel and mesoporous. Mesoporous silica able to host large amount of drugs by virtue of its honey comb-like structure with hundreds of spores. It provide ease surface functionalization for controlled and targeted drug delivery. SNPs are applicable in the field of biomedicine. Act as effective carrier for drugs including camptothecin, paclitaxel, methotrexate.[11]

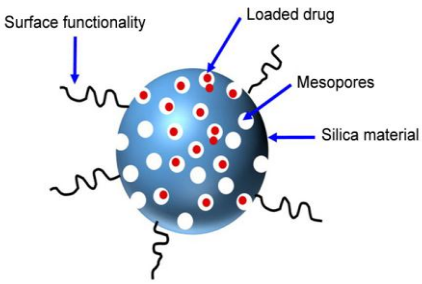

Fig. 9: Structure of MSNs.

METHODS AND DISCUSSIONS

Preparation of Nanoparticles

The properties of PNPs have to be optimized depending on the particular application. In order to achieve the properties of interest, the mode of preparation plays a vital role. Thus, it is highly advantageous to have preparation techniques at hand to obtain PNPs with the desired properties for a particular application. Different techniques like polymerization, preformed polymers or ionic gelation etc are used. The selection of appropriate method for the preparation of nanoparticles depends on the physicochemical character of the polymer and

the drug to be loaded. The primary manufacturing methods of nanoparticles from preformed polymer includes.

1. Emulsion-Solvent Evaporation Method

This is one of the most frequently used methods for the preparation of nanoparticles. Solvent evaporation was the first method developed to prepare PNPs from a. In this method, polymer solutions are prepared in volatile solvents and emulsions are formulated. In the past, dichloromethane and chloroform preformed polymer were widely used, but are now replaced with ethyl acetate which has a better toxicological profile. 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 ultra-sonication may be employed.[12]

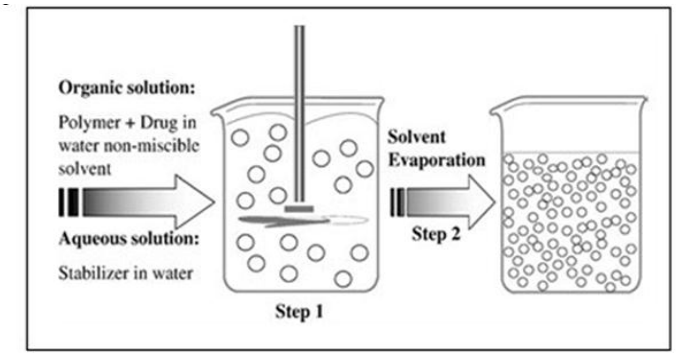

Fig. 10: Schematic representation of solvent evaporation technique.

2. Salting Out Method

Salting out based on the separation of a water-miscible solvent from aqueous solution via a salting-out effect. Salting-out is based on the separation of a water miscible solvent from aqueous solution via a salting-out effect. Salting out does not require an increase of temperature and therefore may be useful when heat sensitive substances have to be processed. The greatest disadvantages are exclusive application to lipophilic drug and the extensive nanoparticles washing steps. Fig.11. Schematic representation of the salting out technique.[13]

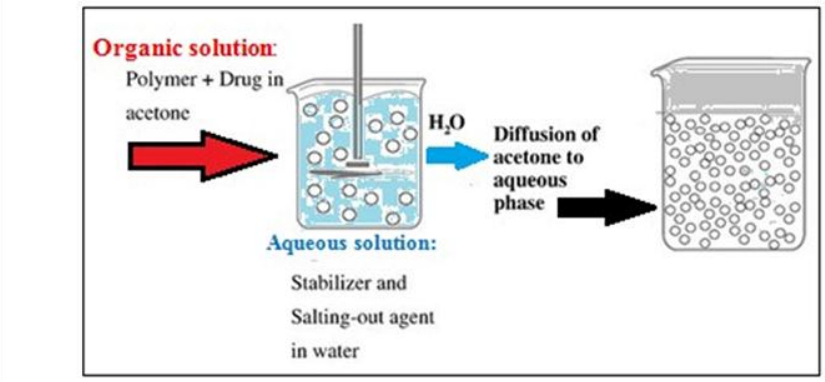

Fig. 11: Schematic representation of salting out technique.

3. Dialysis Emulsions- Diffusion Method

This is another widely used method to prepare nanoparticles. The encapsulating polymer is dissolved in a partially water-miscible solvent (such as propylene carbonate, benzyl alcohol), and saturated with water to ensure the initial thermodynamic equilibrium of both liquids. Subsequently, the polymer-water saturated solvent phase is emulsified in an aqueous solution containing stabilizer, leading to solvent diffusion to the external phase and the formation of nanospheres.

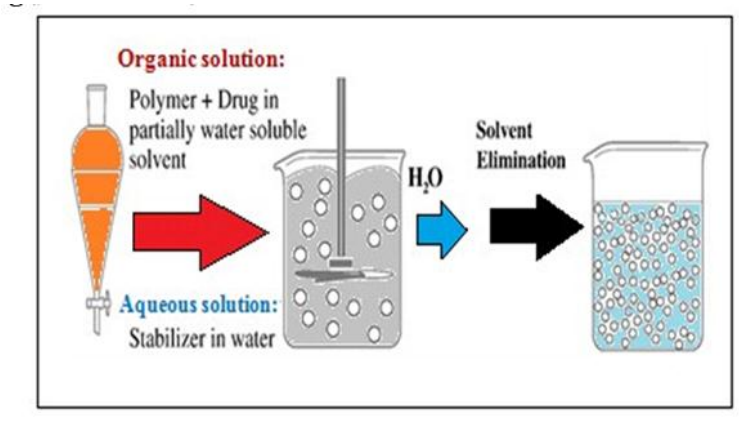

Fig. 12: Schematic representation of emulsification /solvent diffusion technique.

4. Solvent Displacement / Precipitation method

Solvent displacement involves the precipitation of a preformed polymer from an organic solution and the diffusion of the organic solvent in the aqueous medium in the presence or absence of surfactant. Nano particles are formed instantaneously by the rapid solvent diffusion. The solvent is then removed from the suspensions under reduced pressure. The rates of addition of the organic phase into the aqueous phase affect the particles size. It was observed that a decrease in both particles size and drug entrapment occurs as the rate of

mixing of the two phase increases. Nano precipitation method is well suited for most of the poorly soluble drugs.[14]

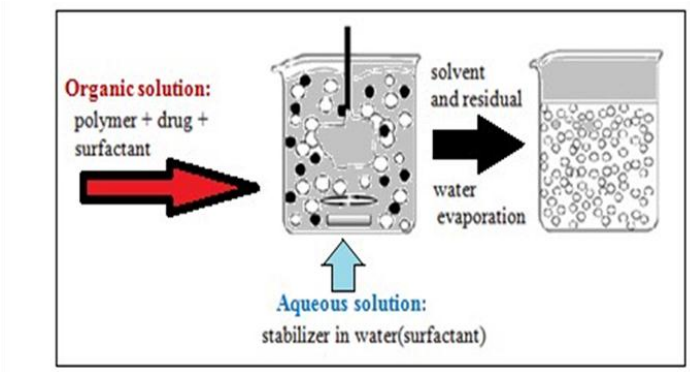

Fig. 13: Schematic representation of Nanoprecipitation technique.

Challenges facing on Nanoformulations

I) Stability

Issues with solidification process of nanosuspensions When stable nanosuspensions are unattainable, the solid dosage form is the ultimate solution. The most common solidification processes are freezedrying and spray drying.

a) Chemical stability

Since drug nanocrystals are usually dispersed in nano suspensions with limited solubility, the possibility of chemical reactions is not as substantial as that insolution. 8°C. No visible degradation product was observed with are covered of mor ethan 99% On the other hand, paclitaxel solution with methanol as co-solvent showed clear degradation only after 48hrs room temperature.

b) General stability issues

Nano suspensions Stability issues associated with nanosuspensions have been widely investigated and can be categorized as physical and chemical stability. The common physical stability issues include sedimentation / creaming, agglomeration, crystal growth and change of crystallinity state.

c) Sedimentation or creaming

Drug particles can either settle down or cream up in the formulation medium depending on their density relative to the medium. The sedimentation rate is described by Stokes' law

which indicates the important role of particle size, medium viscosity and density difference between medium and dispersed phase in determining the sedimentation rate.

d) Crystal growth

Crystal growth in colloidal suspensions is generally known as Ostwald ripening and is responsible for changes in particle size and size distribution. Ostwald ripening is originated from particles solubility dependence on their size. Small particle with higher saturation solubility than larger one according to Ostwald Freundlich equation, creating a drug concentration gradient between the small and large particles.

e) Change of crystalline state

Crystalline state is one of the most important parameters affecting drug stability, solubility, dissolution and efficacy. The main issue with crystalline state change is the transformation between amorphous and crystalline state. The high energy top-down manufacturing techniques tend to create partially amorphous nano suspensions and some bottom-uptechniques can create completely amorphous particles.[15]

6. Overcoming stability of nanoformulation

Among the challenges faced by pharmaceutical industries the major one is poor water solubility of drug in aqueous media. Complexation with cyclodextrins, solid dispersion, micronization, emulsion, liposomes, salt formation, inclusion complex. Any toxic effects of nano materials conjugation to dendrimers are used for poor water soluble system.

II). Toxicity

Toxicity will be specific to the type of base material, size, shape and coatings. However, to determine and understand the toxic effects of nano materials, strategies and interpretation of the data must be done correctly and assumptions taken into consideration. Intoxicity studies of nanoparticles, different research groups used different cell lines, culturing conditions, and incubation times. With our understanding about the nature of nano particles during toxicity test, it is difficult to compare results from different research groups and determine whether the cytotoxicity observed is physiologically relevant.

a) Polymeric Nanoparticles

Polymeric NPs are biocompatible, surface modifiable and are capable of sustained drug release. They show potential for applications in the treatment of various pulmonary

conditions such as asthma, chronic obstructive pulmonary disease (COPD), tuberculosis (TB) and lung cancer as well as extrapulmonary conditions such as diabetes. Biological capping materials reduce cytotoxicity by mimicking the physiological environment, thus 'hiding' from the immune system. However, the possibility of enzymatic degradation due to biophysical resemblance needs further investigation.[16]

b) Carbon Nanotubes (CNTs)

CNTs are frequently used for *in vivo* inhalation models and can be subdivided into SWCNTs and MWCNTs with the former being considered more cytotoxic. This difference in toxicity has been attributed to the larger surface area of SWCNTs compared to the multi-layered alternative. CNTs show biomedical potential in areas such as drug delivery, photodynamic therapy (PDT) and as tissue engineering scaffolds. Exposure of animals to MWCNTs results in contradictory reports with some authors appending toxicities in the range of asbestos poisoning whereas other studies found MWCNTs to be biocompatible and far from cytotoxic. Such discrepancies may be explained by subtle variations in nanotube composition and ways of administration and warrants further investigation.[17]

c) Silica (SiO_2) nanoparticles

Silica NPs are already in wide-spread use in the non-medical field as additives to chemical polishing, cosmetics, varnishes and food stuffs Apart from concentration dependence, silica NPs further exhibited particle size-dependent cellular toxicity with smaller diameters causing more harm than bigger. However, subsequent longer-term studies utilizing the same NPs showed the induction of anti-inflammatory mediators and the reversibility of inflammatory and fibrotic changes to levels close to the control. Fibrogenic mediators (IL-4, IL-10 and IL-13) were upregulated shortly after exposure to silica NPs and contributed to fibrotic changes. These were counteracted by the over expression of matrix-metalloproteinases (MMP), particularly MMP-2 and interferon gamma. Further to the expression of antifibrotic mediators, eventual recovery of lung tissue may be associated with the time-dependent reduction of NP concentration in the alveoli. Some studies suspect diffusion and translocation of NPs away from the lung tissue via the systemic circulation and deposition in extra-pulmonary organs. The above suggests that it is theoretically feasible and within acceptable safety limits to use moderate doses of silica NPs; however high-dose toxicity profiles warrant further investigations.

d) Silver Nanoparticles

The most common route of pulmonary exposure to silver NPs (AgNP) is via the occupational inhalation of airborne particles during manufacturing. Repeated administration of AgNP for 4 weeks showed similar results. In contrast, sub- chronic inhalation for 13 weeks at a maximum concentration (5 times the limit) revealed time-and dose-dependent alveolar inflammatory and granulomatous changes as well as decreased lung function. Such results suggest that while high-dose chronic exposure to AgNP has the potential to cause harm, under current guidelines and limits such excessive particle inhalation.

III) Dermal targets

The skin is the largest organ of the body and functions as the first-line barrier between the external environment and the internal organs of the human body. Consequently, it is exposed to aplethora of non-specific environmental assaults within the air as well as to distinct and potentially toxic substances within creams, sprays or clothing. Topically applied NPs can potentially penetrate the skin and access the systemic circulation and exert adverse effects on a systemic scale.

a) Titanium dioxide (TiO_2) Nanoparticles

TiO_2 NPs have several properties which make them an advantageous ingredient for commercial sun screens and cosmetics. They exhibit UV-light blocking properties and confer better transparency and aesthetics to creams. Near total recovery of sunscreen after 15 tape strippings with no TiO_2 deposition in hair follicles or skin layers. It could be argued that different degrees of permeation and toxicity correlate with surface coatings and functionalizations of TiO2 NPs as well as with the number of follicular pores within the skin facilitating particle uptake.[18]

b) Liver targets

Being the site for first-pass metabolism, the liver is particularly vulnerable to NP toxicity and has consistently been shown to accumulate administered substances, even long after cessation of exposure. Thorough evaluation of NP mediated hepato cellular toxicity thus remains of prevailing importance.

IV). Zeta potential[19]

Zeta potential of colloidal system and nanomedicines as well as their size exert different effect on various properties of nano drug delivery systems. Zeta potential improves drug release profile at specific site and maintain stability in dosage forms.

a). Effect of zeta potential on gene delivery system

Transaction efficiency of gene vector depends on the particle size and zeta potential. The intracellular trafficking of particle and subsequent transfection efficiency depends on particle size and zeta potential of vector/pDNA particles.

b). Effect of zeta potential on macrophage uptake

Nanoparticles having large surface area by volume ratio, nanoparticle tend to agglomerate and adsorb proteins. When they bind to protein they cleared by macrophages before reaching the target cell. To increase the circulation time of nanoparticle in blood either by modifying particle surface and minimize the protein absorption. Protein absorption is attributed to electrostatic interaction of nanoparticles which can be controlled by variation in surface charges. Surface charge variations can be determined by measuring the zeta potential. Nanoparticle having positive zeta potential shows excellent protein absorption. nanoparticles with high surface charge and large particle size are phagocytized more efficiently by murine macrophage. Slight difference in the particle size and surface charge shows implication in the cellular uptake of nanoparticles and different mechanism involved in uptake process.

c) Effect of zeta potential on occular drug delivery

Positive zeta potential is necessary for the ocular drug delivery. It facilitates effective adhesion to the cornea epithelial surface, prolonging the drug release and bioavailability in tissues of eye due to the electrostatic interaction between the positive and negative charge present at the corneal surface. Positive charge can facilitate an effective adhesion to the corneal surface and account for strong interaction with negatively charged mucosa of the conjunctiva.

d) Effect zeta potential on the uptake of Nanoparticles from gut

Uptake of nanoparticles from gut depends on particle size, surface charge and physiochemical properties of the particles. Antigen loaded cationically charged particles are beneficial for the uptake of particles from gut. Positively charged drug loaded particle

expected to interact with negatively charged salicylic acid and fucose residues of mucin in intestine by electrostatic interaction.

e) Effect of zeta potential on respiratory Nano drug delivery

This character attributes to difference in zeta potential and surface hydrophobicity, which in turn altered absorption pattern of positively charged surfactant proteins to the nanoparticles. Cytochrome c is able to overcome repulsive coloumbic forces and adsorb onto similar charged surfaces. So it is evident that a balance between electrostatic and hydrophobic interaction governs adsorption of cytochrome C to nanoparticle. The positive surface charge of sterylamine-PEG-PLA nanoparticle cause increased pulmonary side effects with transient systemic toxicity mainly on WBC.

f) Effect of zeta potential on pharmacokinetics

Particle size and surface charge of nanoparticle maintain biodistribution and pharmacokinetic properties in the body. Altering the size, conformation and charge on the nanoparticles biological behavior. Negatively charged nano emulsions are rapidly cleared from body.

g) Effect on pharmaceutical properties

Controlled drug release- drug release can be controlled by regulating dissolubility and zeta potential. For instance, chitosan showed that it could interact with negatively charged (acidic) drugs are incorporated into films and this might effect physiochemical properties and drug release. Drug loading efficiency, Zeta potential stability of nanoparticles, Problems-positively charged lipids are not approved by FDA for clinical use because zeta potential of nanoparticles is essential factor for their cytotoxicity. Interaction of positive and negative charge membrane, the positive charge posses destabilizing membrane and destructive effect. Factors depends on the cytotoxicity of positively charged nanoparticles are charge density and type of cationic fuctionality, structure and sequence, molecular weight, conformational flexibility.

VI). Protein corona

It is now well recognized that nanoformulations intended for translational medicine applications should be developed and studied taking into consideration the potential impact of the biological milieu in which they are studied and intended to be administered, One prominent event occurring is surface coverage of most, maybe not all NP, by a complex multilayer of proteins, called the “protein corona”. A sudden switch of nanoformulation

physicochemical characteristics may take place within seconds or minutes of contact with the biological matrix, altering their surface properties, potentially generating an immune response, and hence modifying fate, toxicity and targeting.[20]

CONCLUSION

The nanoparticles show a wide range of different parameters possibly impacting on their pharmacokinetics, and therefore their efficacy/toxicity balance. When studying the pharmacokinetics of nanocarriers, one must consider three distinct pharmacokinetic profiles: the systemic one, the tumor microenvironment one and the tumor one. In addition, discriminating free released drug and drug encapsulated or conjugated to its carrier is critical. This singularity plus the wide variety of factors possibly impacting on the ADME process may contribute to the greater pharmacokinetic variability described with liposomes by Schell and colleagues.

In pharmaceutical research and development, the important biopharmaceutical characteristics of drug candidates can be listed as 1) solubility; 2) protein corona; 3) metabolic stability; and 4) systemic pharmacokinetics and pharmacodynamics; these factors would have major impact on the drug ability and develop ability of new pharmaceutical products. Nano drug approaches might resolve the biopharmaceutical problems related to imprecise control of drug release, poor stability, limited pharmacokinetic behaviour, and toxicity of the active ingredient.

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