ABSTRACT:
Nanosphere systems have great potentials, being 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 nanoparticles technology into a realistic practical application as the next generation of drug delivery system.

Keywords:- Nanospheres, Oral Drug Delivery, Targeted Drug Delivery, Biodegradable, Nanoparticles

INTRODUCTION:
Oral administration of medicines utilizes the highly absorptive capacity of the gut to deliver drugs to the systemic circulation. This is the most widely used and accepted route of drug delivery to the adult population. Key to increasing the range of formulations for oral delivery in terms of improving drug solubility, drug stability and bioavailability using nanocarriers is a primary strategy. The fundamental rationale for application of these technologies for oral drug delivery is the inherent ability of the carrier to modulate pharmacokinetics of the incorporated drug molecules. This is frequently achieved by polymeric protection of the pharmacophore from the destructive elements within the gastrointestinal tract (Roger et al, 2010).
Nanocarriers or nanoparticles can be divided into two main families: nanospheres, which have a homogeneous structure in the whole particle, and nanocapsules, which exhibit a typical core-shell structure. Nanospheres are the spherical particles which have the size between 10-200 nm in diameter and that exhibit some new enhanced size dependent properties in comparison of larger spheres of the same material. Basically the drug is dissolved, entrapped, encapsulated or attached to the matrix of polymer. In the matrix system of polymer the drug is physically and uniformly dispersed (Mahapatro and Singh, 2011).
Biodegradable nanospheres such as modified starch nanospheres, gelatine nanospheres, polypropylene dextran nanospheres and polylactic acid nanospheres can be amorphous or crystalline in nature and these carriers are designed for site-specific drug release in the small intestine to achieve maximal bioavailability in the systemic circulation. Thus drug absorption, distribution and elimination during gut transit are not only determined by the drug itself but also by the tuneable physicochemical properties of the nanospheres. This is achieved by variation in carrier chemical composition, size, interface forces, morphology, surface decoration, associated charge and hydrophile–lipophile balance. Application of this technology is potentially highly advantageous as it not only affords the potential for the improved delivery of gut labile molecules and macromolecules such as peptides as antigens for vaccination, but also affords the opportunity to deliver poorly water soluble and poorly absorbed drugs in target cells.
to formulate a diverse range of novel systems that can give new patent life to known effective pharmacophores (Lee, 2005).

**Absorption of nanocarries through oral route**

Oral delivery is the most commonly used and readily accepted form of drug administration. Many small molecule drugs are successfully administered via the oral route, due to the high absorptive capacity of the GI tract. However, many drugs are not suitable for oral administration due to poor solubility, stability, and/or bioavailability. Encapsulating these drugs in nanoparticles can overcome these limitations, as well as allowing the potential for targeted, sustained delivery in the GI tract (Li and Huang, 2008). Significant barriers in the GI tract exist for nanoparticle formulations. Nanoparticles must withstand the acidic environment of the stomach, as well as the degradative enzymes in the intestines. Also, nanoparticles in the GI tract must penetrate the mucus barrier being secreted by the epithelium. The unique rheological and adhesive properties of mucus protect the epithelium from both mechanical forces and foreign pathogens and particles. Rapid mucus secretion and clearance rates efficiently remove foreign materials, limiting the residence time of orally administered nanoparticles (Ruddy *et al.*, 2009). Many promising studies have been completed with various drugs. However, there is a vast array of *in vitro* systems and animal models that have been used, which has produced discordant results regarding the optimum characteristics for efficient nanoparticle delivery in the GI tract. Additionally, there is significant evidence indicating that efficient oral drug delivery in the GI tract is limited by nanoparticles that adhere to the mucus barrier. Mucus penetrating particles can potentially improve oral drug delivery by penetrating the quickly cleared, loosely adherent mucus layer and be retained longer in the firmly adherent layer. Increased GI tract residence time and increased distribution over the epithelium could lead to more effective treatments (Ponchel and, Irache, 2012)

**Major Goals of designing nanoparticles**

Although the initial properties of nanomaterials studied were for its physical, mechanical, electrical, magnetic, chemical and biological applications, recently, attention has been geared towards its pharmaceutical application, especially in the area of drug delivery. This is because of the challenges with use of large size materials in drug delivery, some of which include poor bioavailability, *in vivo* stability, solubility, intestinal absorption, sustained and targeted delivery to site of action, therapeutic effectiveness, generalized side effects, and plasma fluctuations of drugs. Of recent, several researches in nanodrug delivery have been designed to overcome these challenges through the development and fabrication of nanostructures (Gad, 2008). 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. In addition, the research in nanotechnology provides materials with reduced toxicity, greater safety and biocompatibility, and faster development of new safe medicines (Mohanraj and Chen, 2006).

**The potential uses of nanoparticles as drug delivery device**

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 administration through different routes such as parenteral, oral, nasal, intra-ocular etc (Singh *et al.*, 2010).
- They control and sustain release of the drug during the transportation and at the site of localization so as to achieve increase in drug therapeutic efficacy and reduction in side effects (Parveen *et al.*, 2012)
- Nanostructures have the ability to protect drugs from the degradation in the gastrointestinal tract. It also enables the delivery of drugs that are poorly water soluble. The technology increases oral bioavailability of drugs due to their specialized uptake mechanisms such as absorptive endocytosis and can provide means of bypassing the liver, thereby preventing the first pass metabolism (Rieux *et al.*, 2006).
- Nanostructures are able to penetrate tissues and are easily taken up by cells, allowing for efficient delivery of drugs to target sites of action. i.e. they can pass through the smallest capillary vessels because of their ultra-tiny volume and avoid rapid clearance by phagocytes so that their duration in blood stream is greatly prolonged; they can penetrate cells and tissue gap to arrive at target organs such as liver, spleen, lung, spinal cord and lymph (Parveen *et al.*, 2012)

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. In addition, small particles size and large surface area readily result in limited drug loading and burst release. These practical problems have to be overcome before nanoparticles can be used...
Biodegradable polymers for nanospheres

Over the past few decades, there has been considerable interest in developing biodegradable polymer based nanospheres as effective drug delivery devices. Various polymers have been used in drug delivery research as they can effectively deliver the drug to a target site and thus increase the therapeutic benefit, while minimizing side effects. The controlled release of pharmacologically active agents to the specific site of action at the therapeutically optimal rate and dose regimen has been a major goal in designing such devices. Biodegradable nanoparticles have been used for site-specific delivery of drugs, vaccines and various other biomolecules. A few of the most extensively used biodegradable polymer matrices for preparation of nanoparticles are:

**Poly-D-L-lactide-co-glycolide** (Figure 1): widely utilised to manufacture nano- and microparticles due to its excellent biocompatibility, variable mechanical and biodegradability properties. It undergoes hydrolysis in the body to produce biodegradable metabolite monomers such as lactic acid and glycolic acid. Since lactic acid and glycolic acids are normally found in the body and participate in a number of physiological and biochemical pathways, there is very minimal systemic toxicity associated with the use of Poly-D-L-lactide-co-glycolide (PLGA) for the drug delivery or biomaterial applications. PLGA NPs have been mostly prepared by the emulsification-diffusion, the solvent evaporation and the nanoprecipitation methods. PLGA nanoparticles have been used to develop protein and peptide based nanomedicines, nano-vaccines, and genes containing nanoparticles for in-vivo delivery systems (Feczkó et al., 2011).

**Polylactic acid** (Figure 2): is a biocompatible and biodegradable polymer which is broken down to monomeric units of lactic acid in the body. Lactic acid is a natural intermediate/by product of anaerobic respiration, which is converted into glucose by the liver during the Cori cycle. The use of PLA nanoparticles is therefore safe and devoid of any major toxicity. PLA nanoparticles have been prepared mostly by nanoprecipitation, solvent displacement and solvent evaporation (Chawla and Amiji, 2002).

**Poly-ε-caprolactone (PCL):** is biodegradable and biocompatible synthetic aliphatic polyester that is widely used in drug delivery applications. High permeability to many drugs and a lack of toxicity has made PCL and its derivatives well suited for colloidial drug delivery. It is a highly hydrophobic crystalline polymer that degrades by hydrolysis of its ester linkages under the normal physiological conditions in the human body and has minimal or no toxicity. Therefore, PCL (Figure 3) has grabbed the attention of researchers as a candidate of choice for use in drug delivery and long-term implantable devices. PCL nanoparticles have been prepared mostly by nanoprecipitation, solvent displacement and solvent evaporation. (Shi et al., 2011).

**Chitosan** (Figure 4): is a modified natural carbohydrate polymer prepared by the partial N-deacetylation of the crustacean-derived natural biopolymer chitin. Chitosan nanoparticles are widely studied drug delivery systems. The use of chitosan nanoparticles offers many advantages, providing targeted delivery of drugs, improving the bioavailability and stability of the therapeutic agents against chemical/ enzymatic degradation. There are at least four methods reported for the preparation of chitosan nanoparticles. The four methods are ionotropic gelation, microemulsion, emulsification solvent diffusion and polyelectrolyte complex formation (Shi et al., 2011).
Gelatin: Gelatin (Figure 5) is a naturally occurring polymer with relatively low antigenicity and is extensively used in food and medical products. In addition, its biodegradability, biocompatibility, non-toxicity, ease of chemical modification and cross-linking make gelatin-based nanoparticles an efficient carrier in delivery and controlled release of the drugs. It is known that the mechanical properties such as swelling behavior and thermal properties of gelatin NPs depend significantly on the degree of crosslinking between cationic and anionic groups. These properties of gelatin can be manipulated to prepare desired type of NPs from gelatin. Gelatin nanoparticles can be prepared by the desolvation/coacervation or emulsion methods (Ofokansi, et al., 2010).

Figure 5: Chemical structure of Gelatin.

Albumin: Albumin is an attractive macromolecular carrier that has been shown to be biodegradable, nontoxic, metabolized in vivo to produce innocuous degradation products, non-immunogenic, easy to purify and soluble in water allowing ease of delivery by injection and thus an ideal candidate for nanoparticle preparation (Elzoghby et al., 2012).

Methods of 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:

- Size of nanoparticles required;
- Inherent properties of the drug, e.g., aqueous solubility and stability;
- Surface characteristics such as charge and permeability;
- Degree of biodegradability, biocompatibility and toxicity;
- Drug release profile desired; and
- Antigenicity of the final product.

Nanoparticles have been prepared most frequently by two methods: Dispersion of preformed polymers and Polymerization of monomers. Dispersion of pre-formed polymers is a common technique used to prepare biodegradable nanoparticles from poly (lactic acid); poly(D,L-glycolide); poly(D, L-lactide-co-glycolide) and poly(cyanoacrylate) (Singh et al., 2010). Several methods developed and successfully utilized to prepare Polymeric nanoparticles by dispersing preformed polymers. These include solvent evaporation, salting-out, nanoprecipitation, dialysis, Emulsification–diffusion, miniemulsion cross-linking and supercritical fluid technology (Rapid expansion of supercritical solution and Rapid expansion of supercritical solution into liquid solvent) (Rao and Geckeler, 2011).

Membrane dialysis technique
Dialysis offers a simple and effective method for the preparation of small, narrow-distributed nanoparticles. Polymer is dissolved in an organic solvent and placed inside a dialysis tube with proper molecular weight cutoff. Dialysis is performed against a non-solvent (Figure 6). The displacement of the solvent inside the membrane is followed by the progressive aggregation of polymer due to a loss of solubility and the formation of homogeneous suspensions of nanoparticles. The mechanism of nanoparticle formation by dialysis method is not fully understood at present. It is thought that it may be based on a mechanism similar to that of nanoprecipitation (Rao and Geckeler, 2011).

Figure 6: Schematic representation of osmosis based method for preparation of polymer nanoparticles.

Nanoprecipitation
The nanoprecipitation method is also called as solvent displacement method. The basic principle of this technique is based on the interfacial deposition of a polymer after displacement of a semipolar solvent, miscible with water, from a lipophilic solution. Rapid diffusion of the solvent into non-solvent phase results in the decrease of interfacial tension between the two phases, which increases the surface area and leads to the formation of small droplets of organic solvent (Rao and Geckeler, 2011). Polymers and drugs are dissolved in a polar, water-miscible solvent such as acetone, acetonitrile, ethanol, or methanol. The solution is then poured in a controlled manner (i.e. drop-
by-drop addition) into an aqueous solution with surfactant. Nanoparticles are formed instantaneously by rapid solvent diffusion. Finally, the solvent is removed under reduced pressure and evaporation (Mahapatro and Singh, 2011).

Preparation of nanoparticles is based on size controlled synthesis of nanoparticles by a simple nano-precipitation method. Nanoparticles are obtained by addition of solution into excess absolute ethanol under continuous stirring using a magnetic stirrer at a constant stirring rate. The resulting mixture is then centrifuged and the supernatant is removed to obtain the regenerated nanoparticles, which are rinsed three times with absolute ethanol to remove NaOH and urea. This approach has resulted in the particle size ranging between 300 nm and 400 nm. The presence of surfactants during the precipitation process can produce the reduced mean particle size around 150nm and this may be due to the fact that surfactants have limited the growth of nanoparticles (Chin et al., 2011).

The fluorescent nanoparticles are also prepared by the same nano-precipitation process. Here, fluorescein-labeled acetate is dissolved in water miscible organic solvent (acetone). Distilled water is then added drop-wise to the polymer solution (figure 7). The resulting nanoparticle suspensions are stirred at room temperature until acetone is completely vaporized from the aqueous suspension (Li et al., 2011).

**Figure 7. Schematic representation of the nanoprecipitation technique.**

**Emulsification–diffusion technique or solvent diffusion method**

This is a modified version of solvent evaporation method. In this method, the water-miscible solvent such as acetone or methanol along with a small amount of the water immiscible organic solvent such as dichloromethane or chloroform 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 hydrophobic drug, a multiple w/o/w emulsion needs to be formed with the drug dissolved in the internal aqueous phase (Soppimath et al., 2001).

This method has several advantages such as high yields, high batch to batch reproducibility and easy scaling up. This biphasic system is emulsified with a high speed homogenizer. Then, high purified water is added up to desired volume to force the complete diffusion of the organic solvent to the aqueous phase (Figure 8). Finally, the organic solvent is evaporated under vacuum at 35 °C (Santander-Ortega et al., 2010).

**Water-in-oil (w/o) miniemulsion cross-linking technique**

The (w/o) emulsion cross-linking technique involves the dispersion of the aqueous phase (containing polymer and cross-linkers) in the oil phase with the presence of emulsifiers. This emulsion is quite stable and generates particles through cross-linking reaction. These emulsion droplets help maintain the shape and size of the particles within the dispersed phase. From this technique nanoparticles can easily be obtained if we can produce miniemulsion containing nano-scale droplets. Although instruments such as rotor–stator emulsifiers, sonicators and high-pressure homogenizers can be used to provide mechanical energy to produce miniemulsions, high-pressure homogenizer is much more efficient than others to prepare nanoparticles. Polymeric nanoparticles have been prepared with this miniemulsion technique and shown to be effective for controlled release of drugs such as doxorubicin. The study also demonstrated that the nanoparticles possess good thermal stability, small particle size, low biological toxicity, and slowly released the anticancer drug (Mi et al., 2007).

**Solvent evaporation method**

In this method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate. The drug (hydrophobic drug) is dissolved or dispersed into the preformed polymer solution. The...
mixture of polymer and drug solution is then emulsified in an aqueous solution containing an emulsifying agent to form oil in water (o/w) emulsion. Most commonly used surfactant/emulsifying agents for this purpose are gelatin and polyvinyl alcohol. After formation of a stable emulsion the organic solvent is evaporated by increasing the temperature or reducing pressure along with continuous stirring of the solution (Figure 9). Process parameters such as stabilizer and polymer concentration and stirring speed have a great influence on the particle size of the nanoparticles formed (Mahapatro and Singh, 2011).

**Supercritical fluid technology**

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 ($T_c = 31.1 ^\circ C, P_c = 73.8$ 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 supercritical anti-solvent (SAS) method employs a liquid solvent, eg methanol, which is completely miscible with the supercritical fluid (SC CO$_2$), to dissolve the solute to be micronized (Figure 10). The solution (solute-solvent) is charged with the supercritical fluid in the precipitation vessel containing solute of interest in an organic solvent (e.g. methanol). At the process conditions, because the solute is insoluble in the supercritical fluid, the extract of the liquid solvent by supercritical fluid (anti-solvent) leads to the instantaneous precipitation of the solute, resulting in the formation of nanoparticles i.e. at high pressures, enough anti-solvent will enter into the liquid phase so that the solvent power will be lowered and the solute precipitates. After precipitation, when the final operating pressure is reached, the anti-solvent flows through the vessel so as to strip the residual solvent. When the solvent content has been reduced to the desired level, the vessel is depressurized and the solid product is collected (Rao and Geckeler, 2011).

**Salting out/emulsification–diffusion method**

In this method, the polymer is dissolved in the organic phase, which should be water-miscible, like acetone or tetrahydrofuran (Figure 10). The organic phase is emulsified in an aqueous phase, under strong mechanical shear stress. The aqueous phase contains the emulsifier and a high concentration of salts which are not soluble in the organic phase. Typically, the salts used are 60% w/w of magnesium chloride hexahydrate or magnesium acetate tetrahydrate in 1:3 polymer to salt ratio. Contrary to the emulsion diffusion method, there is no diffusion of the solvent due to the presence of salts. The fast addition of pure water to the o/w emulsion under mild stirring reduces the ionic strength and leads to the migration of the water-soluble organic solvent to the aqueous phase inducing nanosphere formation. The final step is purification of nanoparticles by cross flow filtration or centrifugation to remove the salting out agent (Singh et al., 2010).

**Fig.11:** Experimental set-up for preparation of polymer nanoparticles by rapid expansion of supercritical fluid solution

In the rapid expansion of supercritical solution (RESS) method differs from the SAS process in that the solute of interest is dissolved in a supercritical fluid (such as supercritical methanol) and then the solution is expanded through a small nozzle into a region lower pressure (figure 12). Thus the solvent power of supercritical fluids dramatically decreases and the solute eventually precipitates (Mohanraj and Chen, 2006).
Characteristics of Nanoparticles on Drug Delivery

While nanoparticle characterization is quite similar across disciplines, there are a number of clinically relevant parameters which must be considered for drug delivery applications. Such properties are indicated in the following pages which include the particle size, surface characteristic, drug loading and drug release.

Particle size

Particle size and size distribution are the most important characteristics of nanoparticle systems. They determine the in vivo distribution, biological fate, toxicity and the targeting ability of nanoparticle systems. In addition, they can also influence the drug loading, drug release and stability of nanoparticles. Many studies have demonstrated that nanoparticles of sub-micron size have a number of advantages over microparticles as a drug delivery system. Generally nanoparticles have relatively higher intracellular uptake compared to microparticles and available to a wider range of biological targets due to their small size and relative mobility. It has been found that 100 nm nanoparticles had a 2.5 fold greater uptake than 1 μm microparticles, and 6 fold greater uptake than 10 μm microparticles in a Caco-2 cell line. In a subsequent study, the nanoparticles penetrated throughout the submucosal layers in a rat in situ intestinal loop model, while microparticles were predominantly localized in the epithelial lining. It was also reported that 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. In some cell lines, only submicron nanoparticles can be taken up efficiently but not the larger size microparticles (Singh and Lillard, 2009).

Drug release is affected by particle size. Smaller particles have larger surface area, therefore, most of the drug associated would be at or near the particle surface, leading to fast drug release. Whereas, larger particles have large cores which allow more drug to be encapsulated and slowly diffuse out. Smaller particles also have greater risk of aggregation of particles during storage and transportation of nanoparticle dispersion. It is always a challenge to formulate nanoparticles with the smallest size possible but maximum stability (Mohanjar and Chen, 2006).

In nanoparticle study, the particle size determination is important for further characterization of their colloidal property. Currently, the fastest and most routine methods of determining particle size are photon-correlation spectroscopy, atomic force microscopy, laser diffraction size analyzer 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 electron microscopy (SEM) or transmission electron microscopy (TEM) (Singh and Lillard, 2009).

Surface properties of nanoparticles

Surface coating

The association of a drug to conventional carriers leads to modification of the drug biodistribution profile, as it is mainly delivered to the mononuclear phagocyte system (MPS) such as liver, spleen, lungs and bone marrow. Nanoparticles can easily be recognized by the host immune system when intravenously administered and cleared by phagocytes from the circulation. Apart from the size of nanoparticles, their surface hydrophobicity determines the amount of adsorbed blood components, mainly proteins (opsonins). This in turn influences the in vivo fate of nanoparticles. Binding of these opsonins onto the surface of nanoparticles called opsonization acts as a bridge between nanoparticles and phagocytes. Indeed, once in the blood stream, surface non-modified nanoparticles (conventional nanoparticles) are rapidly opsonized and massively cleared by the macrophages of MPS rich organs (Singh and Lillard, 2009).

Hence, to increase the likelihood of the success in drug targeting by nanoparticles, it is necessary to minimize the opsonization and to prolong the circulation of nanoparticles in vivo. This can be achieved by Surface coating of nanoparticles with hydrophilic polymers/surfactants; Formulation of nanoparticles with biodegradable copolymers with hydrophilic segments such as polyethylene glycol (PEG), polyethylene oxide, polyoxamers, poloxamine and polysorbate 80(Tween 80). Studies show that PEG conformation at the nanoparticle surface is of utmost importance for the opsonin repelling function of the PEG layer (Mo-
Electrical property
The particles in a colloidal suspension or emulsion usually carry an electrical charge. The zeta potential of a system is a measure of charge stability and controls all particle-particle interactions within a suspension. Understanding zeta potential is of critical importance in controlling dispersion and determining the stability of a nanoparticle suspension, i.e. to what degree aggregation will occur over time. The zeta potential of a nanoparticle is commonly used to characterize the surface charge property of nanoparticles. It reflects the electrical potential of particles and is influenced by the composition of the particle and the medium in which it is dispersed. Nanoparticles with a zeta potential above (+/-) 30 mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles i.e. a higher level of zeta potential results in greater electro-static repulsion between the particles, minimizing aggregation/flocculation. Samples with zeta potentials of between -30mV and +30mV typically tend to aggregate, although the precise stability threshold will vary according to particle type. Determining the stability of a sample to minimize aggregation for drug delivery and pharmaceutical applications (high zeta potential) is of great importance in nanoparticle research. 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 (Singh and Lillard, 2009).

The charge in a colloidal suspension or emulsion is more often negative than positive and it may arise in a number of ways. Sometimes the surface of the particles contains chemical groups that can ionize to produce a charged surface. Sometimes the surface itself preferentially adsorbs ions of one sign of charge in preference to charges of the opposite sign. In other cases there may be deliberately added chemical compounds that preferentially adsorb on the particle surface to generate the charge (Santander-Ortega et al., 2009; Suyao et al., 2006).

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: incorporation and adsorption. The encapsulation of the drug in the polymer, dispersion of the drug in the polymer, adsorption of the drug onto the surface of the nanoparticles and chemical binding of the drug to the polymer can be accomplished using incorporation/adsorption techniques. The amount of drugs bound to nanoparticles and the type of interaction between drugs and nanoparticles depend on the chemical structure of the drug, chemical structure of the polymer and the conditions of drug loading (Mohanjar and Chen, 2006).

Drug release
To develop a successful nanoparticulate system drug release pattern, drug release mechanisms and polymer biodegradation are important factors that need due consideration. The drug release from nanoparticles will influence the effectiveness of the proposed application and successful sustained drug delivery. In general, the drug release rate depends on solubility of the drug, desorption of the surface bound/adsorbed drug, drug diffusion through the polymer matrix, nanoparticle matrix erosion/degradation and combination of the erosion diffusion process. Hence, for manipulation of the drug release, a good understanding of the mechanisms of drug release is needed which would involve knowledge of the solubility, diffusion and biodegradation of the matrix. One way to modify the drug release profile is by adopting appropriate polymer matrices (Mohanjar and Chen, 2006). Drug release kinetics also depends upon size of the nanoparticles and the loading efficiency of the drug. The drug loading efficiency will determine the initial burst and the sustained release rate of nanoencapsulated drug molecule. Larger particles have a smaller initial burst release than smaller particles. 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 a drug is faster than the matrix erosion, the release mechanism is predominately through a diffusion process. The rapid initial release or burst of drug seen in release profiles is mainly attributed to weakly bound or adsorbed drug on to the surface (Singh and Lillard, 2009).

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. Drugs are loaded into nanoparticles mainly by incorporating at the time of nanoparticles production. In drug release study on nanoparticles, surface crosslinked nanoparticles have slowed down the release of the drug from the nanoparticles. Slow release of drug in buffer of high
pH has proved that the nanoparticle can be used as a good carrier of drugs. In another study folate conjugated nanoparticles have shown to sustain the release of drugs such as Doxorubicin (Suyao, et al., 2006).

Critical parameters of nanoparticulate formulation evaluation

The critical parameters of a nanoparticulate formulation to set and monitor quality standards have to be based on simplicity (for routine analysis), reliability, and correlation to the in vivo performance. These can include particle size, zeta potential, pH of the suspension, (absence of) visible aggregation, redispersibility (contact angle measurement), assay of the incorporated drug, maximum allowable limit of solvents, residual stabilizer, and degradation products (oligomers/monomers) for ensuring quality assurance (Paola et al., 2012).

Dissolution tests can be developed for nanoparticulate formulations of only the drug or polymer entrapped drug with or without surfactant. Similarly, if the NPs are formulated into a solid-dosage form–like tablet, then a disintegration test has to be developed that will ensure total recovery of constitutive particles in the original nanosize range and with the same physicochemical properties. As the mode of absorption of the drug can be from either a (faster and locally generated) solution or direct uptake through the PPs, the drug release from the NPs within the expected time of residence of the particles in the GIT has to be accounted for in both qualitative and quantitative terms. This is especially important in the light of the fact that NPs can give an initial quicker release for the drug at or near the surface where polymer degradation and dissolution are not controlling the drug release. The drug release before the particles are absorbed (and when uptake is the only mechanism of drug absorption) is not going to contribute to the overall bioavailability of the drug, and thus the drug release has to be seen in the background of the mechanism of drug absorption (Zohri, M. 2009).

Degradation Studies

Degradation in NPs is indicative of their stability and the possible time period and kinetics of release of incorporated drug. The dose of the drug to be incorporated can be calculated by correlating the in vivo detectable levels of NPs with the degradation kinetics over a period of time. Thus, the effective delivery period of the drug from the NPs becomes dependent on the combined effect of polymer degradation and natural scavenging mechanisms of the body. The design of these in vitro studies should be based on the actual physiological environment to which the particles are going to be exposed. Particle size plays a significant role in determining the rate of degradation. As the particle size is reduced, more surface area is available for entry of water into NPs resulting in faster degradation and release of therapeutic agent. Polymer degradation was demonstrated to be biphasic in PLGA NPs, with an initial rapid degradation during the first 20–30 days followed by a much slower phase. It was suggested that the surface associated PVA rather than the particle size plays a dominant role in controlling the degradation of NPs (Nagavarma et al., 2012). 

Storage

Depending on its chemistry and morphology, a polymer will absorb some water on storage in a humid atmosphere. Absorbed moisture can initiate degradation and a change in physicochemical properties, which can in turn affect the performance in vivo. Storage conditions may thus be critical to the shelf life of a polymeric NP formulation. The incorporation of the drug may also affect the storage stability of a polymer matrix. The relative strength of water polymer bonds and the degree of crystallization of polymer matrix are other important factors. To maintain the absolute physicochemical integrity of degradable polymeric drug delivery devices, storage in an inert atmosphere is recommended (Jain et al., 2010).

Commercialization of nanoparticulate systems has not been taken up because of the problems in maintaining the stability of suspensions for an acceptable shelf life. The colloidal suspension, in general, does not tend to separate just after preparation because submicron particles sediment slowly and aggregation effect is counteracted by mixing tendencies of diffusion and convection. However, after several months of storage, aggregation can occur. Additionally, microbiological growth, hydrolysis of the polymer, drug leakage, and/or other component degradation in aqueous environment is possible.

Freeze-drying is a good method to dry nanospheres to increase the stability of these colloidal systems. However, because of their vesicular nature, nanocapsules are not easily lyophilized, as they tend to collapse releasing the oil core.

Applications of nanoparticulate delivery systems

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 (Mäder et al., 2012).

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. 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 (Sivasankar and Kumar, 2010).

Absorption enhancement using non-specific interactions

In general, the gastrointestinal absorption of macromolecules and particulate materials involves either paracellular route or endocytic pathway. The paracellular route of absorption of nanoparticles utilizes less than 1% of mucosal surface area. Using polymers such as Chitosan, starch or poly (acrylate) can increase the paracellular permeability of macromolecules. Endocytic 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 M cells. This shows that a combination of size, surface charge and hydrophobicity play a major role in affinity. This is demonstrated with poly (styrene) nanoparticles and when it is carboxylated (Mora-Huertas et al 2010)

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. 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 endolysosomal compartment to the cytoplasmic compartment. It is 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 strat-
egy could be applied to facilitate bone healing by using PLGA nanoparticles containing therapeutic genes such as bone morphogenetic protein.

**Challenges of nano drug delivery**

Although nanotechnology in drug delivery has been successful, as evidenced by some nano drug products in the market, not all approaches have met with the same success. New nanomaterials being developed come with challenges which have to be surmounted. However some of the challenges encountered have been and are still being tackled by modification of the physicochemical characteristics of the nanomaterials to improve on properties such as long circulation in the blood, increased functional surface area, protection of incorporated drug from degradation, crossing of biological barriers and site-specific targeting. Another challenge of research and development (R&D) of nanomaterials for drug delivery is large scale production. There is always a need to scale up laboratory or pilot technologies for eventual commercialization. A number of nano drug delivery technologies may not be scalable due to the method and process of production and high cost of materials employed. The challenges of scaling up include low concentration of nanomaterials, agglomeration and the chemistry process – it is easier to modify improved performance than at large scale. Maintaining the size and composition of nanomaterials at large scale is also a challenge (Ochekpe, et al. 2009).

Despite the number of patents for nano drug delivery technologies, commercialization is still at its early stage. This is partially due to the fact that most of the research studies in nano drug delivery are carried out by researchers in academia. Therefore, for these technologies to get to the market there has to be increased partnership with the pharmaceutical companies. Unfortunately, a number of the major pharmaceutical industries are yet to consider nanotechnology as one of their priorities due to lack of regulatory guidelines and challenges of scaling up. However, it is envisaged that with the expiration of more patents and market loss, more pharmaceutical industries will take up the production of nano drug products in order to compete favorably. Advances in nano drug delivery technology also provide new challenges for regulatory control. There is an increasing need to have regulations that would account for physicochemical and pharmacokinetic properties of nano drug products, which are different from conventional drug products (Ochekpe, et al. 2009).

The United States’ Food and Drug Administration (FDA) and the European Medicines Evaluation Agency (EMEA) have taken the initiative to identify some possible scientific and regulatory challenges. Furthermore, the International Organization for Standardization has set up a technical committee (TC 229) for the field of nanotechnologies to develop standards pertaining to terminology and nomenclature; measurement and characterization; and health, safety and environment amongst other standards. These standards are still under development.

**Safety issues**

With increased R&D work on nano drug delivery, emerges a concern about the safety of the nanotechnologies in humans. Some of the nanomaterials are biodegradable while some are not; furthermore, the side effects of the by-products present a huge concern. Materials which may be safe at macro scale may not be at nanoscale since there may be change in physicochemical characteristics at nanoscale. These nanomaterials may not clear completely from the body and their accumulation may have several possible effects (Shkumar et al. 2013).

Safety and possible impact nanomaterials should not be considered for the patient population alone but also for the entire manufacturing and disposal processes. Conventional safety measures in a pharmaceutical factory may not be appropriate for the development and fabrication of nanomaterials. Also extra measures are to be taken to protect the environment from increased envisaged negative impacts of nanomaterials. Although reduced cost to the patients is envisaged to be one of the advantages of nanotechnology since fewer materials are expected to go into production as compared to bulk production; it is doubtful if this will be so, as successful commercialization will be expensive. There is also the general public reluctance to embrace nanotechnology based on the unavailability of documented safety guidelines. However, despite these challenges, nano drug delivery is a development that cannot be ignored and so the challenges will be tackled with time (Mónica et al. 2013).

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