Nanoparticles and their Therapeutic Applications in Pharmacy

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ABSTRACT

Nanotechnology is providing solutions several pharmaceutical drug delivery issues. With the emergence of nanotechnology, researchers become more interested in studying the unique properties of nanoscale materials. Nanoparticles are attractive tool in pharmaceutical and biomedical fields. These particulate systems have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug/proteins. Nanoparticles have been used in-vivo to protect the drug/proteins molecules in the systemic circulation, targeting of the drug to the chosen sites and to deliver the drug at a controlled and sustained rate to the site of action. Various types polymers have been used in the formulation of nanoparticles for drugs, proteins, and hormone delivery are discussed in this article. It also describes various methods of preparation, advantages, disadvantages and their applications in biomedical fields. It provides an overview of characterization of nanoparticles, storage, and commercially available nanoformulations.

KEYWORDS: Nanosuspension; Preparations; Storage; Characterization; Nano-applications.

Introduction

‘Nanoscience’ can be defined as study of phenomenon and manipulation of materials at atomic and molecular scales. The word ‘nano’ is derived from Latin word, which means Dwarf. Nano size refers to one thousand millionth of a particular unit thus nanometer is one thousand millionth of a metre (i.e., 1 nm = 10^{-9} m). ‘Nanotechnology’ is related to design characterization, production and applications of structures, devices and systems by controlling shape and size at nanometer scale. ‘Pharmaceutical nanotechnology’ embraces applications of nanoscience to pharmacy as nanomaterials, and as devices like drug delivery, diagnostic, imaging and biosensor. ‘Nanomedicine’ is defined as submicron size (< 1 µm) modules, used for treatment, diagnosis, monitoring and control of biological system.

Nanoparticles are solid particles or dispersion with a size ranges from 10-1000 nm. In this drug is entrapped or adhere or dissolved in on the polymer matrix. Depend upon the method of preparation nanospheres or nanocapsules were obtained. In nanocapsules drug is confirmed or embedded in the cavity of a polymer membrane, where as in nanospheres drug is physically or uniformly dispersed. Apart from the structure, nanocapsules differ from nanospheres in their size and degree of polymerization. Nanocapsules are generally larger than nanospheres and degree of polymerization is higher in nanocapsules than nanospheres. Nanospheres are easily lyophilized than the nanocapsules because nanocapsules are easily collapsed by freeze drying (see Fig 1).

The submicron size of nanoparticles exhibit distinct advantages over microparticles, including relatively higher intracellular uptake (M cells) compared with microparticles. In terms of intestinal uptake, apart from their particle size, nanoparticle nature and charge properties seem to influence the uptake by intestinal epithelia. Uptake of nanoparticles obtained from hydrophobic polymers seems to be higher uptake effect than that of particles with more hydrophilic surfaces thus more hydrophilic particles may be rapidly eliminated (Jung et al., 2000).

Nanoparticles prepared by using hydrophobic polymers such as polystyrene, polycaprolactone, acrylic polymers shows uncharged or positively charged which provides an affinity to follicle epithelia as well as absorptive enterocytes, whereas negatively charged nanoparticles shows low affinity to any type of intestinal tissues. In opposite or contrast nanoparticles with hydrophilic, negatively charged show an increase in

Fig. 1. Difference between nanosphere and nanocapsule.

systemic circulation, targeting of the drug to the chosen sites and to deliver the drug at a controlled and sustained rate to the site of action. Various types polymers have been used in the formulation of nanoparticles for drugs, proteins, and hormone delivery are discussed in this article. It also describes various methods of preparation, advantages, disadvantages and their applications in biomedical fields. It provides an overview of characterization of nanoparticles, storage, and commercially available nanoformulations.
bioadhesive properties and easily absorbed by M-cells and enterocytes, combination of both positively and negatively charges and increased hydrophilicity of matrix material seem to effect gastrointestinal uptake in positive sense. Figure 2 shows uptake and transport of nanoparticles (Catarina et al., 2006).

![Fig. 2. Intracellular trafficking of nanoparticles.](image)

Following their uptake, nanoparticles are transported through early endosomes to the sorting endosomes. A fraction of nanoparticles recycles back to the cell exterior while another fraction is transported to secondary endosomes/lysosomes from where nanoparticles escape into the cytoplasm. Nanoparticles that escape into the cytoplasm could act as intracellular reservoirs for sustained release of the encapsulated therapeutic agent. Nanoparticle surface charge is plays a vital role in targeting drug delivery system. In the bloodstream, generally conventional nanoparticles (no surface modification) and negatively charged particles can be rapidly opsonized and massively cleared by the fixed macrophages. We are very familiar with that, the reticuloendothelial system, mainly the liver and spleen, is a major obstacle to active target delivery because of its ability to recognize these systems, remove them from systemic circulation, and, consequently, avoid the effective delivery of the nanoparticles to organs other than those of the reticuloendothelial system (Kumar et al., 2001).

To control the opsonization process by modifying the surface of nanoparticle with hydrophilic polymers or coating or attaching of polyethylene glycol (PEG) to the PLGA or PLA, these are usual for the delivery of proteins, peptides absorption.

The major goals in designing nanoparticles as a delivery system are to increase the saturation solubility of poorly soluble drugs by adopting control particle size with surface properties like charge (either cation or anion) 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 (Mohanraj et al., 2006). Incase of liposomes also have inherent with these properties but it also some problems such as low encapsulation, leakage of water soluble drugs in the blood components and short term stability such a way that nanoparticles have more significant than liposomes. In case of polymeric lipid nanoparticles it is a combination of lipid and polymer carriers and it enhances GIT absorption and Plasma concentration in blood as well as more stable in biological fluid and more stable while storing (Sanyog et al., 2012) (see Fig.3).

![Fig. 3. Polymeric lipid nanoparticles (PLNs).](image)

These polymeric nanoparticles shows specific advantages especially over the liposomes such as increase the stability of drugs/proteins which possess control release properties (Vila et al., 2002 and Mu et al., 2003).

Advantages of nanoparticles over other novel drug delivery system

1. The allowable size of nanoparticles to be administered via intravenously unlike colloidal system which could occlude in blood capillaries and needle.
2. Due to its small size than microspheres and liposomes, they can easily pass through the sinusoidal spaces in the bone marrow and spleen as compared to other systems with long circulation time.
3. Due to their larger surface area, nanoparticles have higher loading capacity.
5. Nanoparticles are safe and effective in site specific and targeted drug delivery systems.
6. To enhances the targeting moieties by adhering monoclonal antibodies with nanoparticles for specificity.
7. It improves the solubility of poorly water soluble drugs.
8. It improves bioavailability by reducing the fluctuations in the therapeutic ranges.
9. It reduces the toxicity of liver.
10. They offer a significant improvement over traditional oral and intravenous methods of administration in terms of efficiency and effectiveness.
Disadvantages of nanoparticles
1. Due to its high surface area and energy they tend to high aggregation in biological systems.
2. Quickly scavenged by RES resulting in low biological half-life.
3. Residual amount of organic solvent (nano-suspension) causes toxicity.
4. Highly immunogenicity or foreignness.
5. Long and expensive to cost.

Nanoparticle Preparation
Different techniques like dispersion of performed polymer, polymerization and ionic gelation techniques

Methods for preparation of nanoparticles from dispersion of preformed polymer
These include nanoprecipitation, solvent evaporation, solvent diffusion/emulsification, salting out and super critical fluids. Polymers used in nanoparticle preparation of drug formulations are listed in Table 1.

Nanoprecipitation
It is also called as solvent displacement method (Kipp et al., 2003; Zili et al., 2005; Trotta et al., 2001 and Zhang et al., 2006). The drug is dissolved in an organic solvent and this solution is mixed with a miscible antisolvent. Rapid addition of a drug solution to an antisolvent leads to sudden supersaturation of the mixed solution, and generation of fine crystalline or amorphous solids. Precipitation of an amorphous material may be favored at high supersaturation when the solubility of the amorphous state is exceeded. This method is basically applicable to lipophilic drugs because of the miscibility of the solvent with the aqueous phase, and it is not an efficient means to encapsulate water-soluble drugs. This method has been applied to various polymeric materials such as PLGA, PLA, PCL and poly (methyl vinyl ether-comaleic anhydride) (PVM/MA) (Nagavarma et al., 2012).

Solvent evaporation
Solvent evaporation method is the first method for the preparation of nanoparticles (Soppimath et al., 2001). It involves two steps, first step is emulsification of the polymer solution into an aqueous phase and the second step polymer solvent is evaporated, inducing polymer precipitation as nanospheres. Using different solvents such as chloroform and ethyl acetate in which polymer is dispersed and subjected to aqueous solution containing surfactants where the polymer precipitates in the form of nanospheres in which the drug is finely dispersed in the polymer matrix network. The solvent is subsequently evaporated by increasing the temperature under pressure or by continuous stirring. The size of nanoparticles can be controlled by adjusting the stir rate, type and amount of dispersing agent, viscosity of organic and aqueous phases, and temperature (Tice et al., 1985).

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Drug and particle size</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(methylmethacrylate) copolymers</td>
<td>Doxorubicin 300 nm</td>
<td>Rolland et al., 1986</td>
</tr>
<tr>
<td>Poly(methylycyanoacrylate)</td>
<td>Vinblastine 200-300 nm</td>
<td>Couvreur et al., 1980</td>
</tr>
<tr>
<td>Polyethylcyanoacrylate</td>
<td>Insulin 500 nm</td>
<td>Radwan et al., 2002</td>
</tr>
<tr>
<td>Polybutylycyanoacrylate</td>
<td>Progestrone 250 nm</td>
<td>Li et al., 1986</td>
</tr>
<tr>
<td>Poly(methylcyanoacrylate)</td>
<td>Triamcinolone 500 nm</td>
<td>Krause et al., 1986</td>
</tr>
<tr>
<td>Poly(isobutylcyanoacrylate)</td>
<td>Indomethacin 220-240 nm</td>
<td>Grgsoy et al., 1989; Ammoury et al., 1991</td>
</tr>
<tr>
<td>Polycaprolactone</td>
<td>Gatifloxacin 300-600 nm</td>
<td>Patil et al., 2013</td>
</tr>
<tr>
<td></td>
<td>Cyclosporine A 100-200 nm</td>
<td>Molpeceres et al., 1996</td>
</tr>
<tr>
<td>PLGA</td>
<td>Tomaxifen 165 nm</td>
<td>Jain et al., 2011</td>
</tr>
<tr>
<td></td>
<td>Doxocubicin 160 nm</td>
<td>Jain et al., 2011</td>
</tr>
<tr>
<td></td>
<td>Amphotericin B 253 nm</td>
<td>Sanyog et al., 2012</td>
</tr>
<tr>
<td>Poly(lacticacid/poly(glycolic acid) copolymer</td>
<td>Indomethacin 168 nm</td>
<td>Barichello et al., 1999; Ne’mati et al., 1996</td>
</tr>
<tr>
<td></td>
<td>Cyclosporine 170 nm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Valproic acid 166 nm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ketoprofen 187 nm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vancomycin 187 nm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insulin 105-170 nm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Doxorubicin 274 nm</td>
<td></td>
</tr>
<tr>
<td>Poly lactic acid</td>
<td>Doxorubicin 270 nm</td>
<td>Ne’mati et al., 1996; Fessi et al., 1989</td>
</tr>
<tr>
<td></td>
<td>Taxol 260 nm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dexamethasone 300 nm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vitamin K 270 nm</td>
<td></td>
</tr>
<tr>
<td>Chitosan</td>
<td>5-flurouracil</td>
<td>Sivabalan et al., 2011</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>Doxorubicin 200-1500 nm</td>
<td>Widder et al., 1979</td>
</tr>
<tr>
<td>Gelatin</td>
<td>mitomycin C 280 nm</td>
<td>Yoshioka et al., 1981</td>
</tr>
</tbody>
</table>
Emulsification/solvent diffusion

Emulsification/solvent diffusion (ESD) was proposed in the literature based on the use of organic solvents, and then it was adapted to the following salting-out procedure. The polymer is dissolved in a partially water-soluble solvent such as acetone and saturated with water to ensure the initial thermodynamic equilibrium of both liquids. In fact, to produce the precipitation of the polymer and the consequent formation of nanoparticles, it is necessary to promote the diffusion of the solvent of the dispersed phase by dilution with an excess of water when the organic solvent is partly miscible with water or with another organic solvent in the opposite case. Subsequently, the polymer-water saturated solvent phase is emulsified in an aqueous solution containing stabilizer (Tween 80, PVA, Poloxamer etc.), leading to solvent diffusion to the external phase and the formation of nanoparticles or nanocapsules, according to the oil-to-polymer ratio. Finally, the solvent is eliminated by evaporation by magnetic stirring and then filtrate for collecting nanoparticles. This method has several advantages, such as high encapsulation efficiency, high batch-to-batch reproducibility, ease of scale-up, and narrow size distribution. Disadvantages are the high volumes of water to be eliminated from the suspension and the leakage of water-soluble drug into the saturated-aqueous external phase during emulsification, reducing encapsulation efficiency. Several nanoparticles were prepared with different polymers such as PLGA for Doxorubicin (Yoo et al., 1999), plasmid DNA loaded PLA nanoparticles (Perez et al., 2001).

Salting out

Salting out is based on the separation of a water-miscible solvent from aqueous solution via a salting-out effect. Polymer and drug are initially dissolved in a solvent such as acetone, which is subsequently emulsified into an aqueous gel containing the salting-out agent (electrolytes, such as magnesium chloride, calcium chloride, and magnesium acetate, or non-electrolytes such as sucrose) and a colloidal stabilizer such as polyvinylpyrrolidone or hydroxyethylcellulose. This oil/water emulsion is diluted with a sufficient volume of water or aqueous solution to enhance the diffusion of acetone into the aqueous phase, thus inducing the formation of nanospheres. The selection of the salting-out agent is important, because it can play an important role in the encapsulation efficiency of the drug. Both the solvent and the salting-out agent are then eliminated by cross-flow filtration (Quintanar et al., 1998).

Supercritical fluid technology: It is a technology and it is suitable for removing the impurities remaining in the drug loaded particles or polymer matrix which are toxic in nature. The supercritical fluid technology becomes more attractive and acceptable method due to purity of obtaining particles without any trace of organic solvents. The nanoparticles can be prepared by using:

Rapid Expansion of Supercritical Solution (RESS): The polymer is solubilized in a supercritical fluid and the solution is expelled through nozzle and polymer is precipitated with enhanced solubility and free of solvent or solvent mixture. High molecular weight polymers (> 10,000) are slightly or insoluble in supercritical fluids, low molecular weights are applicable.

Supercritical Anti-Solvent (SAS): The polymer solution in an organic solvent is incorporated along with the supercritical fluid taken in a precipitated vessel. The anti-solvent is added at high pressure, so that the solvent power is reduced and the solvent get precipitated. 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 volume of solvent reaches the desired level the vessel is depressurized and the solid product is collected.

Gas Anti-Solvent Technique (GAS): This is modification of supercritical anti-solvent. In this, solution of polymer in suitable solvent is injected in supercritical fluid, such that the solvent is extracted by the supercritical fluid, causing the supercritical fluid-insoluble polymer to precipitate as fine particles. This method has been successfully used for the preparation of nano as well as micro particles.

Nanoparticles obtained by polymerization of a monomer

It includes following methods such as emulsion polymerization, interfacial polymerization and interfacial polycondensation.

Emulsion polymerization

This method is easy and fastest method of preparing nanoparticles and is classified into two categories based on the continuous organic and aqueous phase.

In case of continuous organic phase methodology, dispersion of monomer into an emulsion or inverse microemulsion, or into a material in which the monomer is not soluble (nonsolvent). This method is very less applicable because of organic solvents. In the aqueous continuous phase the monomer is dissolved in continuous phase which is usually water and is free of surfactant and emulsifiers. The polymerization of monomer can be started or initiated by different mechanisms. First, initiation monomer molecule dissolved in the continuous phase collides with initiator molecules that might be formation of Ions or free radicals or gamma irradiation of monomer molecules or UV light radiation might be also used then chain growth starts when initiated monomer or radicle collide with other monomer molecules according to that of anionic polymerization mechanism (Vauthier et al., 2003). Phase separation and formation of solid particles can take place before or after termination of the polymerization reaction (Kreuter et al., 1982). PMMA nanoparticles are suitable adjuvants for vaccines and are produced by a radical emulsion polymerization mechanism generally without emulsifier (Kreuter et al., 1976). In the preparation of poly-methylmethacrylate (PMMA) nanoparticles single methylmethacrylate (MMA) was used. The preparation of PMMA nanospheres is simple and drugs can be successfully entrapped, but two drawbacks have to be
kept in mind. First, polymerization requires a chemical or physical initiation, and second, PMMA nanospheres are not biodegradable.

**Interfacial polymerization**

It is very rapid polymerization occurring during seconds initiated by ions present in the medium. Cyanoacrylate monomer and drug were dissolved in a mixture of an oil and absolute ethanol. This mixture was then slowly extruded through a needle into a well-stirred aqueous solution, with or without some ethanol or acetone containing surfactant. Nanocapsules are formed spontaneously by polymerization of cyanoacrylate after contact with initiating ions present in the water. The resulting colloidal suspension can be concentrated by evaporation under vacuum (Khouri et al., 1986). An advantage of interfacial polymerization techniques is high-efficiency drug encapsulation (e.g., insulin with 95%) (Couvreur et al., 2002).

**Interfacial polycondensation**

Polymeric nanoparticles can be also prepared by the interfacial polycondensation of the lipophilic monomer, such as phthaloyldichloride and the hydrophilic monomer, diethylene triamine, in the presence and absence of the surfactant (Montasser et al., 2002). These nanoparticles were smaller than 500 nm. A modified interfacial polycondensation method was also developed. In this case, polyurethane polymer and poly (ether urethane) copolymers were chosen and successfully applied as drug carriers for α-tocopherol. Polyurethane and poly (ether urethane) based nanocapsules were synthesized by interfacial reaction between two monomers (Bouchemail et al., 2004).

**Ionic gelation technique**

Calvo and coworkers was first developed a method by using hydrophilic chitosan polymer (Calvo et al., 1997). The method involves a mixture of two aqueous phases, of which one is the polymer chitosan, a di-block co-polymer ethylene oxide or propylene oxide (PEO-PPO) and the other is a polyanion sodium tripolyphosphate (STPP). 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.

**Characterization of Nanoparticles**

**Particle size:** (Udupa et al., 2013): Particle size, distribution and morphology are the most important parameters of characterization of nanoparticles. Morphology and size are measured by using different electron microscopy such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM). These are the tools for determining the size and distribution.

**Scanning Electron Microscopy (SEM)**

It is one of the versatile instruments to study and visualize nanoparticles. It has highly magnified, detailed three dimensional images is created by SEM, which is not possible by ordinary light microscope. Construction of SEM is simple and easy it consists of electron source, lenses, sample stage and one detector. This works under vacuum so preparation of sample to be prepared carefully and special procedure to be adopted. In case of biological samples need special drying shriveling. The images obtained from SEM with detail information regarding external morphology, texture, crystalline structure and orientation. Scanning mode can generate images in 5 μm to 1 cm width of sample and magnification up to 30,000X and spatial resolution 50-100 nm. The disadvantage of SEMs vacuum sensitive materials cannot be analyzed, not detect the elements with atomic weight less than 11 and coating of samples with gold is major problem.

**Transmission Electron Microscopy (TEM)**

TEM works on the principle as SEM but in TEM a focused monochromatic beam of electrons is transmitted through the sample. The type of data obtained is same. The sample preparation for TEM is complex and time consuming because of its requirement to be sample must be ultra thin for the electron transmittance. The nanoparticles dispersion is deposited onto support grids or films. To make nanoparticles withstand the instrument vacuum and facilitate handling, they are fixed using either a negative staining material, such as phosphotungstic acid or derivatives, and uranyl acetate or by plastic embedding. Alternate method is to expose the sample to liquid nitrogen temperatures after embedding in vitreous ice. The surface characteristics of the sample are obtained when a beam of electrons is transmitted through an ultra-thin sample, interacting with the sample as it passes through (Molpeceres et al., 2000).

**Atomic Force Microscopy (AFM)**

Atomic force microscopy offers ultra-high resolution in particle size measurement and is based on a physical scanning of samples at sub-micron level using a specific probe detector tip of atomic scale (Muhlen et al., 1996). Instrument provides (relating to the arrangement or accurate representation of the physical features of an area) topographical map of sample based on forces between the tip of the detector and the sample surface. Scanning has been done by two process. Such as scanned in contact or noncontact mode depending on their properties. In contact mode, the topographical map is generated by tapping the probe on to the surface across the sample and probe hovers over the conducting surface in non-contact mode. The most advantage of AFM is its ability to image non-conducting samples without any type of specific treatment, thus allowing imaging of delicate biological and polymeric nano and microstructures. AFM provides the most accurate description of size and size distribution and requires no applicable of mathematical treatment. Moreover, particle
size obtained by AFM technique gives real picture which helps understand the effect of various biological conditions (Polakovic et al., 1999).

**Drug loading and entrapment efficiency**

Drug loading can be done by two methods. Incorporation method incorporating at the time of nanoparticles preparation and adsorption method adsorbing the drug after formation of nanoparticles by incubating the carrier with a concentrated drug solution. After loading of drug, entrapment efficiency were calculated by given formula

\[
\text{Entrapment efficiency} = \frac{X_{\text{total drug}} - X_{\text{free drug}}}{X_{\text{total drug}}} \times 100
\]

**Surface modification of nanoparticles and its influences**

Surface modification is important to introduce drugs in the blood stream for “stealth” invisibility of the body’s natural defense systems. The mononuclear phagocytic system (MPS) eliminates them from the blood stream efficiently unless the particles are achieve target site of action. Longer circulation time increases the probability for the nanoparticles to reach their target sites. Small particles (<100 nm) with a hydrophilic surface have the greatest ability to evade the mononuclear phagocytic system (MPS) (Feng et al., 2004). This is because the foreign particles are rapidly cleared by mononuclear phagocyte system (MPS), one of the body’s innate defense mechanism. The process of opsonization is one of the most important biological barriers to nanoparticles based controlled drug delivery. However, opsonin proteins present in the blood serum quickly bind to conventional non-stealth nanoparticles, allowing macrophages of the mononuclear phagocytic system (MPS) to easily recognize and remove these drug delivery devices before they can perform their designed therapeutic function.

Opsonization of injected particles by antibodies in circulation, attachment of opsonized particles to the macrophages and subsequent internalization by phagocytosis are important steps in the clearance of particles by MPS. To address these limitations, several methods have been developed to mask nanoparticles from the mononuclear phagocyte system. Of these methods, the most preferred is the adsorption or grafting of poly-ethylene glycol (PEG) to the surface of nanoparticles. PEG has property of repelling opsonization against the MPS, hydrophilic, non-ionic polymer that has been shown to exhibit excellent biocompatibility. The primary reasons for using PEG, improve long blood circulation, reduces the interaction between the nanoparticles and the enzymes of the digestive fluids and increases uptake of encapsulated drug in the blood stream and lymphatic tissue (Tobio et al., 2003). Hydrophilic polymers such as PEG, poloxamers, polysorbate 80, TPGS, polysorbate 20, polysaccharides like dextran and different type of copolymers can be used to efficiently coat conventional nanoparticles surface leading to variation in the surface properties. These coating of nanoparticles repels plasma binding proteins. Zeta potential is also commonly used to determine the surface charge property of nanoparticles. Zeta potential above (+/-) 30 mV have been shown to be stable in 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 nanoparticles or adsorbed onto the surface (Couvreur et al., 2002).

**Storage of the Nanoparticles**

Generally, a colloidal suspension is stable and does not tend to separate as a result of slow deposition due to the mixing tendencies of diffusion and convection. However, some agglomeration can occur. To prevent a complete precipitation, it is necessary to incorporate some additives. Chemical integrity of drug is also a fundamental aspect of the overall stability evaluation of the nanoparticles. Some parameters are crucial for the stability, such as the duration of contact with the aqueous environment when the drug is water soluble, the surrounding pH when drug degradation is pH dependent, and light exposure when the drug is light sensitive. Stability studies are thus important and can be performed according to the drug and to the polymer properties. There are some methods to increase the stability of the nanoparticles. Lyophilization (freeze-drying) seems to be a highly stabilizing process. It is generally applied to enhance the physicochemical stability of the nanoparticles to achieve a pharmaceutically acceptable product, especially in cases in which the storage conditions are unfavorable. This technique involves the freezing of the suspension and subsequent elimination of its water content by sublimation under reduced pressure. After complete desiccation, nanoparticles are obtained in the form of a dry powder that is easy to handle and store. In most cases the freeze-dried particles are readily dispersible in aqueous solutions. In some systems the ease of redispersion depended on the manufacturing process. Ultra sonication was applied by some authors to ensure complete redispersion of nanoparticles. Freezing is the most aggressive step of the freeze-drying operation for colloidal operations. It is thus important to improve the nanoparticle resistance by addition of a cryoprotectant to avoid alteration of the suspension. Sometimes cryoprotectants like glucose, trehalose, mannitol, and sorbitol were added to ensure redispersibility or to allow of the suspension during the cooling and to avoid crystallization of the liquid suspension. It is also important to be aware of the presence of pharmaceutical excipients, usually used for purposes of isotonicity (e.g., glucose) or stabilization (e.g., dextran and surfactants). Such excipients are indeed cryoprotectants that facilitate the aqueous reconstitution of the freeze-dried product. Sanyog jain et al., 2012 proved 5% W/V trehalose to an optimized nanosuspension proved that particle size remain same, entrapment efficiency and sedimentation rate is unity (Esquisabel et al., 1997).
TABLE 2
Currently available marketed nanoformulations.

<table>
<thead>
<tr>
<th>Brand</th>
<th>Generic name</th>
<th>Indication</th>
<th>Company</th>
<th>Innovator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapamune</td>
<td>Rapamycin, Sirolimus</td>
<td>Immunosuppressant</td>
<td>Elan Nanosystem</td>
<td>Wyeth</td>
</tr>
<tr>
<td>Emend</td>
<td>Aprepitant</td>
<td>Anti-emetic</td>
<td>Elan Nanosystems</td>
<td>Merck &amp; Co.</td>
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<tr>
<td>Tricor</td>
<td>Fenofibrate</td>
<td>Hypercholesterolemia</td>
<td>Abbott Laboratories</td>
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<tr>
<td>Megace ES</td>
<td>Megestrol</td>
<td>Anti-anorexic</td>
<td>Elan Par Pharmaceuticals</td>
<td></td>
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<tr>
<td>Triglide</td>
<td>Fenofibrate</td>
<td>Hypercholesterolemia</td>
<td>IDD-P SkyePharma</td>
<td>Sciele Pharma Inc.</td>
</tr>
<tr>
<td>Paxseed</td>
<td>Paclitaxel</td>
<td>Anti-inflammatory</td>
<td>Angiotech</td>
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TABLE 3
The technologies and patents/patent regarding nanoformulations.

<table>
<thead>
<tr>
<th>Nanocrystal</th>
<th>Company</th>
<th>Patent/patent application examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrosol</td>
<td>Novatis</td>
<td>GB 22 69 536, GB 22 00 048</td>
</tr>
<tr>
<td>Nanomorph™</td>
<td>Soligs/Abbott</td>
<td>D 1963 7517</td>
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<tr>
<td>Nanocrystal™</td>
<td>Elan Nanosystems</td>
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<tr>
<td>Dissocubes®</td>
<td>SkyPharma</td>
<td>US 5,858,410</td>
</tr>
<tr>
<td>Nanopure</td>
<td>PharmaSol</td>
<td>PCT/EP00/063</td>
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<tr>
<td>NANOEDGE™</td>
<td>Baxter</td>
<td>US 6,884,436</td>
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</table>

Applications of Nanoparticle Delivery Systems

Targeting drug delivery by encapsulation

Nanoencapsulation of drugs increases drug efficacy, specificity, tolerability and therapeutic index of corresponding drugs (Table 2) (Vijay et al., 2012). Many patents exist for such products (Table 3) (Chingunpituk et al., 2007). They have many advantages in the protection of premature degradation and interaction with the biological environment, enhancement of absorption into a selected tissue, bioavailability, retention time and improvement of intracellular penetration (Safa et al., 2000; Schroder et al., 1998; Raghuvanshi et al., 2002; Kreutera et al., 1997; Fassas et al., 2003; Jeanchristophe 1996; Alexis et al., 2008). Several disease related drugs or bioactive molecules are successfully encapsulated to improve bioavailability, bioactivity and control delivery. Nanomedicines of the dreadful diseases like cancer, AIDS, diabetes, malaria, prion disease and tuberculosis are in different trial phase for the testing and some of them are commercialized. Nanomedicine formulation depends on the choice of suitable polymeric system having maximum encapsulation (higher encapsulation efficiency), improvement of bioavailability and retention time.

Different drugs with various polymeric (PLA, PLGA, PCL, chitosan, gelatin) nanoparticles show impact upon surface modification, bioavailability and drug release mechanisms. P.S. Kumar et al., formulated insulin-loaded nanoparticles of PLGA is used to maintain the integrity of insulin during formulation and delivery were prepared by solvent evaporation technique, higher encapsulation efficiency of 75% is observed (Kumar et al., 2006). G. Mittal et al., formulated Estradiol encapsulated PLGA nanoparticles have been prepared by emulsion diffusion evaporation method to improving the oral bioavailability and decreasing the dosage frequency, thereby minimizing the dose dependent adverse effects and maximizing the patient’s compliance (Mittal et al., 2007). J. Matsumoto et al., Progesterone-loaded PLA–PEG–PLA nanoparticles have been prepared by solvent evaporation method. The amount of drug release increases as the PEG content and molecular weight of PLA–PEG–PLA copolymers increased (Matsumoto et al., 1999).

Nanoparticles for oral delivery of 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 (Damge et al., 1990). Sanyog Jain et al., enhancing the oral absorption and hypoglycemic activity of insulin via encapsulation in folate-(FA) coupled polyethylene glycolylated (PEG) polylactide-coglycolide (PLGA) nanoparticles (FA-PEG-PLGA NPs) size 260 nm, exhibited a two fold increase in the oral bioavailability (double hypoglycemia) without any hypoglycemic shock (Sanyog et al., 2012). The surface area of human mucosa 200 times that of skin (Brandtzaeg et al., 1997). The GIT provides a variety of physiological and morphological barriers against protein or peptide delivery.

(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 (Lee et al., 1990).
M cells of small intestine prevent uptake of particulate matter from the environment. The most important strategy to overcome the gastrointestinal barrier is to deliver the drug in a colloidal carrier system is nanoparticles, which is capable of enhancing the interaction mechanisms of the drug delivery system and the epithelia cells in the GI tract.

**Nanoparticles for drug delivery into the brain**

The blood-brain barrier (BBB) is the most important limiting factor for the development of new drugs for the central nervous system due to its relatively impermeable endothelial cells with tight junctions, enzymatic activity and active efflux transport systems. BBB is impermeable for the water soluble drugs from blood circulation to CNS and it is selectively permeable for lipophilic and small size molecules.

PEG-coated NPs have vital role as they have been accounted as potentially useful tool to deliver drugs into the brain. PEG coating also causes enlarging of the molecule/particle and slows down kidney ultrafiltration and, thereby allowing better accumulation into the brain and other permeable tissues by the passive enhanced permeation and retention mechanism. It also provides protein shielding which reduces proteolysis within the serum and tissues, and hinders immune surveillance of surface epitopes. PEGylation improves the pharmacokinetic profile of molecules by reducing opsonization, phagocytosis and clearance by the liver and reticuloendothelial system (Bhatt et al., 2013).

**Nanoparticles for ophthalmic delivery**

To overcome the poorly soluble drugs in lachrymal secretions of eye these nanoformulations plays a vital role and could proved. Nanosuspension of nanoparticles offers most advantage of prolong residence time in cul-de-sac, which is most important for the ocular diseases for effective treatment and also maintain tonicity with respect to the eye. The dissolution rate and intrinsic solubility is depend on the lachrymal fluid the intrinsic dissolution rate of the drug will vary because of the constant inflow and outflow of lachrymal fluids. The intrinsic dissolution rate of the drug will vary because of the constant inflow and outflow of lachrymal fluids. Pignatello et al., identified the stability of cloricromene in ophthalmic formulation and enhanced bioavailability at ocular level by utilizing the solvent evaporation method (Pignatello et al., 2006). Adibkia et al., prepared Eudragit RS100 loaded piroxicam nanoparticles using similar method for control the inflammatory symptoms in rabbits with endotoxin-induced uveitis (EIU). The study suggested the non-invasive implementation of the piroxicam Eudragit RS-100 nanosuspensions as a safer controlled ocular delivery of anti-inflammation agents for inhibition of the uveitis symptoms (Adibkia et al., 2007).

**Topical formulations**

Drug nanoparticles can be incorporated into creams and water-free ointments. The nanocrystalline form leads to an increased saturation solubility of the drug in the topical dosage form, thus enhancing the diffusion of the drug into the skin. Micellar nanoparticle is a technology applicable for topical applications. This technology allows high concentrations of drug to penetrate the skin and functionally create a drug depot in the stratum corneum and epidermis. This route of delivery provides similar advantages of patch technology in avoiding both contact with the gastrointestinal tract and hepatic first-pass effects, and is cosmetically more acceptable to many patients (Müller et al., 1999). Solid lipid nanoparticles are used to cure some skin diseases like acne, atopic eczema, psoriasis) because of high permeability through the skin, greater surface area and strong scattering pattern. SLN formulations applied on skin to reduces the systemic adverse effect of drugs. For example, Vitamin A can be incorporated in SLN to reduces side effect of Vitamin A (Shim et al., 2004; Kohli and Alpar et al., 2004; Yamaguchi et al., 2005).

Micellar nanoparticle (MNP) is a multiphase nanoemulsion. MNPs behave like a pseudo-patch or patchless-patch. The active pharmaceutical ingredient easily penetrates in to skin then to blood very easily and quickly. Example Estrasorb (17β-estradiol in Estrasorb) Novavax’s first internally developed FDA approved product and the only emulsion-based formulation in the topical estrogen replacement market. Estrasorb is the world’s first nano-engineered topical dosage form that is approved by the US FDA for hormone replacement therapy (Wright, 1997).

**Nanoparticles for diagnostic applications**

The nanoparticles are used to diagnose cancer and treated. Magnetic Nanoparticles being a sub-family of nanomaterials show remarkable new phenomena such as superparamagnetism, high saturation field, extra anisotropy contributions or shifted loops after field cooling. These phenomena arise from finite size and surface effects that dominate the magnetic behaviour of individual nanoparticles. Small size gives effective surface area, low sedimentation rate, tissular diffusion and reduces dipole-dipole moment. The magnetic property of nanoparticles (MNP) offers an advantage that it provide selective attachment to a functional molecule, confer magnetic properties to the target, and allow manipulation and transportation to a desired location through the control of a magnetic field produced by an electromagnet or permanent magnet.

MNPs can be easily controlled by external magnetic field gradients. This helps to transport the MNPs into human tissue and be directed and concentrated within the target tissue by means of external magnetic field, especially in cancer tumor. Nanotechnology has found many new ways in detecting cancer cells and how far the disease has spread throughout the body. A couple of these new cancer detecting nanoparticles such as gold nanoparticles and magnetic iron oxide nanoparticles encased in a biocompatible material. Magnetic iron oxide nanoparticles encased in a biocompatible material can make detecting cancer cells easier, even if the cancer cells are small and clearer so there is less mistakes in the
detecting process. These particles stick to the tumor cells turning them into little magnets which are then attracted to the tip of a biopsy needle (Charles et al., 1986). Instead of using biopsies, MRI's can be used to distinguish malignant lymph nodes which can help in telling how far cancer has spread.

**Cosmetic applications**

Solid lipid nanoparticles are one type of nanoparticle formulated from physiological lipids. These lipids have some inherent properties such as Occlusive nature and Ultraviolet ray protection which allows their use in cosmetics. The enhanced permeability of the lipid nanoparticles through horny dead layers and allows skin hydration due to occlusive property.

Wising et al., studied the influence of solid lipid nanoparticles on skin hydration and viscoelasticity. These are highly effective carrier for cosmetic creams, which are intended to increases skin hydration (Wising et al., 2003). Solid lipid nanoparticles give physical protection due to their particulate matter. Physical sunscreens act by reflecting and scattering UV rays. This effect of physical sunscreens depends upon the particles refractive index, the size of the particles and thickness of formulation films on skin. The solid lipid nanoparticles are better a scattering light than liquid emulsion droplets (Patel et al., 2006).

**Nanoparticles for gene delivery**

Nanoparticles loaded with plasmid DNA, Vaccines could also serve an effective sustain delivery by escaping from the Endolysosomal compartment to cytoplasmic compartment (Panyam et al., 2002). Hedley et al., reported that following their intracellular uptake and endolysosomal escape, nanoparticles could release DNA at a sustained rate resulting in sustained gene expression. This gene delivery strategy could be applied to facilitate bone healing by using PLGA nanoparticles containing therapeutic genes such as bone morphogenic protein (Hedley et al., 1998).

**Pulmonary delivery**

It means drugs administered for their local or systemic effect through bronchial tree or through lungs. Alveoli are the functional units of lungs which have high surface area, high epithelial permeability and rich vasculature make this route popular for rapid absorption and free from the first pass metabolism. The unique size of nanoparticles not only increases its absorption but also enhances and controls the release and transport of drugs. The preferable drugs for this route of delivery are antiasthmatic (cromolyn sodium), bronchodilators (salbutamol) and steroidal anti-inflammatory agents such as betamethasone (Li et al., 2010).

**References**


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