



Biodegradable Polymer Based Nanoparticles: A Novel Approach

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ABSTRACT

Nanoparticles are defined as solid colloidal particles that include both nanospheres and nanocapsules. Nanoparticulated systems show promise as active vectors due to their capacity to release drugs. Their subcellular size allows relatively higher intracellular uptake than other particulate systems. They can improve the stability of active substances having size range 10-100 nm. The use of nanoparticles as carriers for the delivery of therapeutic materials to target tissues has become popular in recent years and has demonstrated great potentials for the treatments of a wide range of diseases polymeric nanoparticles may actually provide better results for drug delivery compared with lipid-based nanoparticles because they may increase the stability of the drugs or proteins being transported. Polymeric nanoparticles may also contain beneficial control release mechanism. Nanoparticles made from natural polymers that are biodegradable have the ability to target specific organs and tissues in the body. They have the ability to carry DNA for gene therapy, and they have the ability to deliver larger molecules such as proteins, peptides. There are various methods for preparation of nanoparticles such as Homogenizer, Ultra Sonicator, Spray Milling, Supercritical Fluid Technology, Electro spray, Ultracentrifugation, and Nanofiltration. A few common polymer materials used for drug delivery studies are polybutyl cyanoacrylate (PBCA), poly (isohexyl cyanoacrylate) (PIHCA), polylactic acid (PLA), or polylactide-co-glycolide (PLGA). Human serum albumin (HSA) and chitosan are also materials of interest. PBCA undergoes degradation through enzymatic cleavage of its ester bond on the alkyl side chain to produce water-soluble by products.

Keywords: Nanoparticles, PBCA, Nanospheres, Nanocapsules, Homogenizer, Ultracentrifugation.

INTRODUCTION

The concept of nanoparticles and drug targeting was inspired by a visit of one of the giants in science – Paul Ehrlich – to Karl Maria von Weber's opera "Der Freischütz" (Greiling, 1954). In this opera, so-called "Freikugeln", made by calling the spirit of the devil, play a central role. These Freikugeln always hit their goal, even if the rifleman did not aim properly or if the goal was out of reach. Paul Ehrlich, who had been working for a long time on the staining of bacteria and tissues, after attending this opera thought that this concept of targeted delivery could greatly improve drug therapy. He called the delivery system that would be used in this type of therapy "Zauber kugeln" – English "Magic Bullets". Being a medical doctor with a great interest in bacteriology and immunology, he had something like antibodies in mind, but with this idea the concept of nanoparticles and of drug targeting was born.¹

Nanoparticles can be defined as particulate dispersions or solid particles with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. In recent years, the number of patents and products in this field is increasing significantly. Several terminologies have been used to describe nanoparticulate drug delivery systems. In most cases, either polymers or lipids are used as carriers for the drug, and the delivery systems have particle size distribution from few nanometers to few hundred nanometers.²

Poor bioavailability, inadequate stability and shelf life, immunogenicity, short plasma half-life, and poor penetration across biological membranes are issues of polymeric protein carriers that have been demonstrated *in vivo*. To develop ideal therapeutic polymeric nanoparticles, several key features including enhanced drug solubility, controlled release of molecules, prevention of initial bursting, avoidance of undesirable side effects, improved drug distribution, and targeting of diseased tissue must be considered for practical applications³.

Polymeric nanoparticles formulated from biodegradable polymers are being widely explored as carriers for controlled delivery of different agents including proteins, peptides, plasmid DNA (pDNA), and low molecular weight compounds. Self-assembling polymer or block/graft copolymers that can form nanostructures have been extensively investigated in the field of biotechnology and pharmaceuticals. In general, hydrophobic interactions, electrostatic forces, hydrogen bonds, van der Waal forces, or combinations of these interactions are available as the driving forces for the formation of the polymer complexes. Numerous investigators have shown that the biological distribution of drugs. Biodegradable polymers provide sustained release of the encapsulated antigen and degrade in the body to nontoxic, low molecular weight products that are easily eliminated. The biodegradation rate and the release kinetics of loaded drugs can be controlled by the composition ratio and the molecular weight of the polymer and block/graft



copolymers. Furthermore, by modulating the polymer characteristics, one can control the release of a therapeutic agent from the nanoparticles to achieve a desired therapeutic level in a target tissue for the required duration for optimal therapeutic efficacy.⁴

Advantages

1. Particle size and surface characteristics of nanoparticles can be easily manipulated to achieve both passive and active drug targeting after parenteral administration.
2. They control and sustain release of the drug during the transportation and at the site of localization, altering organ distribution of the drug and subsequent clearance of the drug so as to achieve increase in drug therapeutic efficacy and reduction in side effects.
3. Controlled release and particle degradation characteristics can be readily modulated by the choice of matrix constituents. Drug loading is relatively high and drugs can be incorporated into the systems without any chemical reaction; this is an important factor for preserving the drug activity.
4. Site-specific targeting can be achieved by attaching targeting ligands to surface of particles or use of magnetic guidance.
5. The system can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc.⁵

Disadvantages

1. Solvent and high-temperature incompatibility for low-cost polydimethylsiloxane microchannels. Higher costs and complexities in the fabrication of glass and silicon microdevices.
2. Current methods are not applicable to all classes of nanoparticles. Not all properties can be characterized, such as drug encapsulation and release, and signal-to-noise ratio.
3. Higher costs and complexities in the fabrication and operation compared with well plates. Might not be reusable and if reusable, it would be difficult to keep sterile.
4. Lack of methods to translate data from small-scale organisms to other species. Pharmacokinetics or biodistribution cannot be determined.
5. Difficult to build systems at low-cost that are comparable to a batch reactor able to prepare grams or kilograms of nanoparticles.⁶

Types of Nanoparticles

Preparation Methods of Nanoparticles

Biodegradable nanoparticles can be prepared from a variety of materials such as proteins, polysaccharides and synthetic biodegradable polymers. The selection of the

base polymer is based on various designs and end application criteria.

It depends on many factors such as 1) size of the desired nanoparticles, 2) properties of the drug (aqueous solubility, stability, etc.) to be encapsulated in the polymer, 3) surface characteristics and functionality, 4) degree of biodegradability and biocompatibility, and 5) drug release profile of the final product.

Depending upon selection of desired criteria for the preparation of the nanoparticles, the methods can be classified as following 1) dispersion of preformed polymers, 2) polymerization of monomers and 3) ionic gelation method for hydrophilic polymers.¹³

Dispersion of preformed polymers this is the most commonly used technique to prepare biodegradable nanoparticles from poly-lactic acid (PLA); poly-D- L-glycolide (PLG); poly-D- L-lactide-co-glycolide (PLGA) and poly-cyanoacrylate (PCA). This technique can be used in several ways as described below.

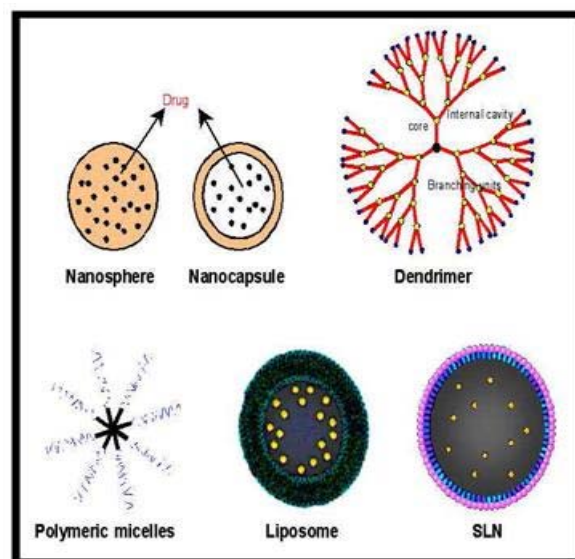


Figure 1: Types of nanoparticles¹⁷

Solvent Evaporation Method

In this method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate, which is also used as the solvent for dissolving the hydrophobic drug.

The mixture of polymer and drug solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form oil in water (o/w) emulsion.

After the formation of stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring.

Particle size was found to be influenced by the type and concentrations of stabilizer, homogenizer speed and polymer concentration. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed. As shown in fig.2.¹⁵

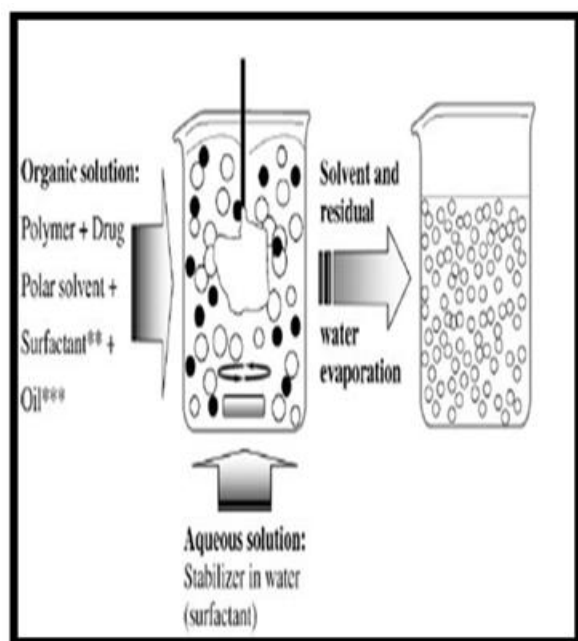


Figure2: Solvent Evaporation Method

Polymerization method

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

Coacervation or ionic gelation method

Much research has been focused on the preparation of nanoparticles using biodegradable hydrophilic polymers such as chitosan, gelatin and sodium alginate. Calvo and co-workers developed a method for preparing hydrophilic chitosan nanoparticles by ionic gelation. The method involves a mixture of two aqueous phases, of which one is the polymer chitosan, a di-block co-polymer ethylene oxide or propylene oxide (PEO-PPO) and the other is a polyanion sodium tripolyphosphate. In this method, positively charged amino group of chitosan interacts with negative charged tripolyphosphate to form coacervates with a size in the range of nanometer. Coacervates are formed as a result of electrostatic interaction between two aqueous phases, whereas, ionic gelation involves the material undergoing transition from liquid to gel due to ionic interaction conditions at room temperature.⁷ As shown in fig.3.¹⁵

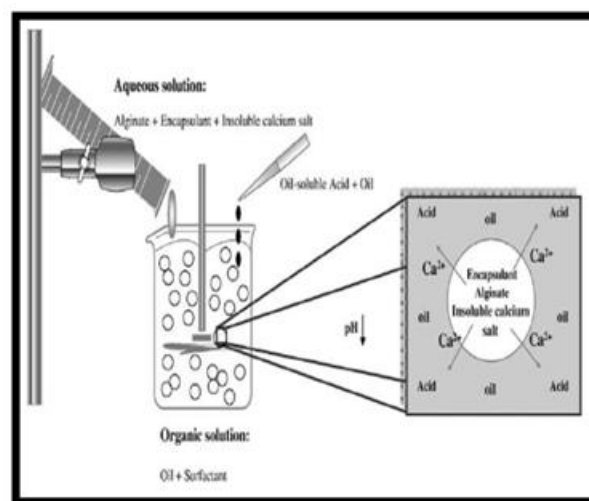


Figure 3: Coacervation or ionic gelation method

Spontaneous Emulsification Or Solvent Diffusion Method

This is a modified version of solvent evaporation method. In this method, the water miscible solvent along with a small amount of the water immiscible organic solvent is used as an oil phase. Due to the spontaneous diffusion of solvents an interfacial turbulence is created between the two phases leading to the formation of small particles. As the concentration of water miscible solvent increases, a decrease in the size of particle can be achieved. Both solvent evaporation and solvent diffusion methods can be used for hydrophobic or hydrophilic drugs. In the case of hydrophilic drug, a multiple w/o/w emulsion needs to be formed with the drug dissolved in the internal aqueous phase.⁸

Double Emulsion and Evaporation Method

The emulsion and evaporation method suffer from the limitation of poor entrapment of hydrophilic drugs. Therefore to encapsulate hydrophilic drug the double emulsion technique is employed, which involves the addition of aqueous drug solutions to organic polymer solution under vigorous stirring to form w/o emulsions. This w/o emulsion is added into second aqueous phase with continuous stirring to form the w/o/w emulsion. The emulsion then subjected to solvent removal by evaporation and nano particles can be isolated by centrifugation at high speed. The formed nanoparticles must be thoroughly washed before lyophilisation. In this method the amount of hydrophilic drug to be incorporated, the concentration of stabilizer used, the polymer concentration, the volume of aqueous phase are the variables that affect the characterization of nanoparticles.

Salting Out Method

Salting out based on the separation of a water-miscible solvent from aqueous solution via a salting-out effect. Salting-out is based on the separation of a water miscible solvent from aqueous solution via a salting-out effect. Polymer and drug are initially dissolved in a solvent which

is subsequently emulsified into an aqueous gel containing the salting out agent (electrolytes, such as magnesium chloride and calcium chloride, 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 solvent into the aqueous phase, thus inducing the formation of nanospheres. Several manufacturing parameters can be varied including stirring rate, internal/external phase ratio, concentration of polymers in the organic phase, type of electrolyte concentration and type of stabilizer in the aqueous phase. This technique used in the preparation of PLA, Poly (methacrylic) acids, and Ethyl cellulose nanospheres leads to high efficiency and is easily scaled up. Salting out does not require an increase of temperature and therefore may be useful when heats sensitive substances have to be processed. The greatest disadvantages are exclusive application to lipophilic drug and the extensive nanoparticles washing steps. As shown in the fig.4.¹⁵

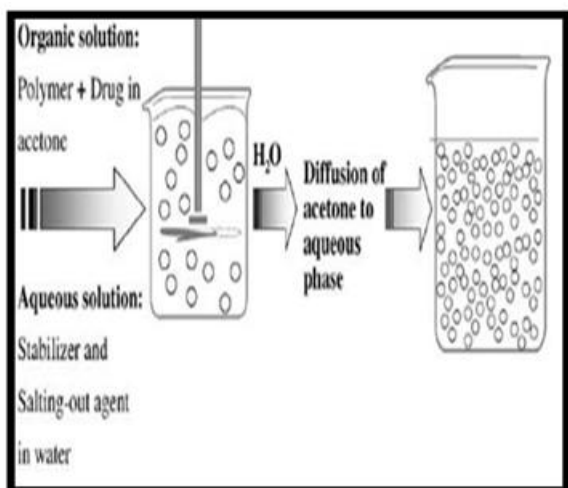


Figure4: Salting Out Method

Solvent Displacement / Precipitation method

Solvent displacement involves the precipitation of a preformed polymer from an organic solution and the diffusion of the organic solvent in the aqueous medium in the presence or absence of surfactant. Polymers, drug, and or lipophilic surfactant are dissolved in a semipolar water miscible solvent such as acetone or ethanol. The solution is then poured or injected into an aqueous solution containing stabilizer under magnetic stirring. Nanoparticles are formed instantaneously by the rapid solvent diffusion. The solvent is then removed from the suspensions under reduced pressure. The rates of addition of the organic phase into the aqueous phase affect the particles size. It was observed that a decrease in both particles size and drug entrapment occurs as the rate of mixing of the two phase increases. Nano precipitation method is well suited for most of the poorly soluble drugs. Nanosphere size, drug release and yield

were shown to be effectively controlled by adjusting preparation parameters. Adjusting polymer concentration in the organic phase was found to be useful in the production of smaller sized nanospheres through restricted to a limited range of the polymer to drug ratio.⁹

Microemulsion/colloidal method

NP prepared by appropriate amount of water, oil, surfactant and an alcohol- or amine-based co-surfactant produced clear and homogeneous solutions that Hirai called microemulsions. Microemulsion is a technique for the synthesis of nanoparticles in which two immiscible fluids such as water in oil (W/O) or oil in water (O/W) or water in supercritical carbon dioxide (W/Sc. CO₂) become a thermodynamically stable dispersion with the aid of a surfactant. A typical emulsion is a single phase of three components, water, oil and a surfactant. Normally oil and water are immiscible but with the addition of a surfactant, the oil and water become miscible because the surfactant is able to bridge the interfacial tension between the two fluids. Microemulsion consists of surfactant aggregates that are in the ranges of 1 nm to 100 nm. The location of water, oil and surfactant phases affects the geometry of aggregate. The micro-emulsion is said to be oil in water (O/W) if water is the bulk fluid and oil is in less quantity, with small amounts of surfactant. Similarly, the system is said to be water in oil (W/O), if oil is the bulk fluid and water is present in less quantity. The product of oil in water and surfactant (O/W) is called micelles, which is an aggregate formed to reduce free energy. Hydrophobic surfactants in nanoscale oil and micelles point toward the center of aggregate, whereas the hydrophobic head groups toward water, the bulk solvent. The water in oil microemulsion carries oil or organic solvent as bulk. The system is thermodynamically stable and called reverse micelles.¹⁰

Iontropic gelation method

Chitosan NP prepared by ionotropic gelation technique was first reported by Calvo and has been widely examined and developed by Janes. The mechanism of chitosan NP formation is based on electrostatic interaction between amine group of chitosan and negatively charge group of polyanion such as tripolyphosphate. This technique offers a simple and mild preparation method in the aqueous environment. First, chitosan can be dissolved in acetic acid in the absence or presence of stabilizing agent, such as poloxamer, which can be added in the chitosan solution before or after the addition of polyanion. Polyanion or anionic polymers was then added and nanoparticles were spontaneously formed under mechanical stirring at room temperature. The size and surface charge of particles can be modified by varying the ratio of chitosan and stabilizer.¹¹

Desolvation

Desolvation is a thermodynamically driven self-assembly process for polymeric materials, which prepares nanoparticles of a controlled size. The obtained nano-

capsules are vesicular, where the drug is essentially encapsulated in the central core and surrounded by a polymeric sheath. Polymeric nanoparticles form particles of different sizes depending on preparation conditions such as protein content, pH, ionic strength, cross-linking agent concentration, agitation speed, and amount of desolvating agent.³

Supercritical fluid technology

Conventional methods such as solvent extraction-evaporation, solvent diffusion and organic phase separation methods require the use of organic solvents which are hazardous to the environment as well as to physiological systems. Therefore, the supercritical fluid technology has been investigated as an alternative to prepare biodegradable micro- and nanoparticles because supercritical fluids are environmentally safe. A supercritical fluid can be generally defined as a solvent at a temperature above its critical temperature, at which the fluid remains a single phase regardless of pressure. Supercritical CO₂ (SC CO₂) is the most widely used supercritical fluid because of its mild critical conditions (T_c = 31.1 °C, P_c = 73.8 bars), nontoxicity, non-flammability, and low price. The most common processing techniques involving supercritical fluids are supercritical anti-solvent (SAS) and rapid expansion of critical solution (RESS). The process of SAS employs a liquid solvent, eg methanol, which is completely miscible with the supercritical fluid (SC CO₂), to dissolve the solute to be micronized; at the process conditions, because the solute is insoluble in the supercritical fluid, the extract of the liquid solvent by supercritical fluid leads to the instantaneous precipitation of the solute, resulting the formation of nanoparticles. The use of a modified SAS method for formation of hydrophilic drug dexamethasone phosphate drug nanoparticles for microencapsulation purpose. RESS differs from the SAS process in that its solute is dissolved in a supercritical fluid (such as supercritical methanol) and then the solution is rapidly expanded through a small nozzle into a region lower pressure, Thus the solvent power of supercritical fluids dramatically decreases and the solute eventually precipitates. This technique is clean because the precipitate is basically solvent free. RESS and its modified process have been used for the product of polymeric nanoparticles. Supercritical fluid technology technique, although environmentally friendly and suitable for mass production, requires specially designed equipment and is more expensive.⁷

Spray drying

Some of the challenges faced by this technique include the production of small-sized nanoparticles and the need for innovative methods to increase the drug-entrapment efficiency. However, when compared with other methods, it provides a relatively rapid and convenient production technique that is easy to scale up, involves mild processing conditions, and has relatively less dependence on the solubility characteristics of the drug

and the polymer. In this method, a solution or dispersion (w/o) of a drug in an organic solvent containing the polymer is sprayed from the sonicating nozzle of a spray dryer and subsequently dried to yield nanoparticles.¹⁶

Characterization of Nanoparticles

Nanoparticles are generally characterized by their size, morphology and surface charge, using such advanced microscopic techniques as scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM). The average particle diameter, their size distribution and charge affect the physical stability and the *in vivo* distribution of the nanoparticles. Electron microscopy techniques are very useful in ascertaining the overall shape of polymeric nanoparticles, which may determine their toxicity. The surface charge of the nanoparticles affects the physical stability and redispersibility of the polymer dispersion as well as their *in vivo* performance.⁹

Determination of particle size

The particle size and size distribution of the acyclovir loaded PLGA (50:50) nanoparticles were characterized by laser light scattering using Particle size Analyzer (Malvern Mastersizer Hydro-2000 SM, UK). The obscuration level was set between 7 to 11 %, distilled water was used as medium.

Determination of Encapsulation Efficiency

Estimation of Free Drug

The free drug (per 100mg of formulation) was estimated by taking said quantity of formulation in dialysis bag (cellophane membrane , molecular weight cut off 10000-12000 Da, Hi-Media, India) which was tied and placed into 100ml water (pH =7) kept at 37°C on magnetic stirrer. At predetermined time intervals, 5ml of the samples were withdrawn by means of a syringe. The volume withdrawn at each interval was replaced with same quantity of fresh water (pH=7) maintained at 37 °C. The samples were analyzed for free drug by measuring the absorbance at 252nm using UV-visible spectrophotometer (Shimadzu UV-1700) after suitable dilution. Above described process of withdrawing sample and analysis was continued till a constant absorbance was obtained.

Estimation of encapsulated drug

Encapsulated drug (per 100mg of formulation) was estimated by taking residue formulation remained in dialysis membrane after estimation of free drug content, as described above. Quantity left behind in dialysis membrane was added to acetone (10ml) to dissolve PLGA and filtered. Residue remaining on filter paper was dissolved in 100ml of water (pH=7) kept at 37 °C and after removing supernatant, sample was analyzed for drug content by measuring the absorbance at 252nm using UV-visible spectrophotometer (Shimadzu UV- 1700) after suitable dilution. The percentage of drug entrapped and



the percentage of free drug are calculated by following Eq.

Formulae to calculate % free drug and % drug entrapment

$$\% \text{ free drug} = \frac{\text{Amount of free drug present in 100mg of formulation}}{\text{Total amount of drug present in 100mg of formulation}} \times 100$$

$$\% \text{ Drug entrapment} = \frac{\text{Amount of encapsulated drug present in 100mg of formulation}}{\text{Total amount of drug present in 100mg of formulation}} \times 100$$

Statistical Analysis

The results from factorial design were evaluated using PCP Disso 2000 V3 software. Step-wise backward linear regression analysis was used to develop polynomial equations for dependent variables particle size (Y1) and % drug entrapment (Y2) which bear the form of equation-1:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 + \dots + 1$$

Where Y is dependent variable, b_0 arithmetic mean response of nine batches, and b_1 estimated coefficient for factor X_1 the main effects (X_1 and X_2) represent average result of changing one factor at a time from its low to high value. The interaction term (X_1X_2) shows how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate non-linearity. The validity of the developed polynomial equations was verified by preparing check point formulation (C).¹²

Dynamic light scattering (DLS)

Currently, the fastest and most popular method of determining particle size is photon-correlation spectroscopy (PCS) or dynamic light scattering (DLS). DLS is widely used to determine the size of Brownian nanoparticles in colloidal suspensions in the nano and submicron ranges. Shining monochromatic light (laser) onto a solution of spherical particles in Brownian motion causes a Doppler shift when the light hits the moving particle, changing the wavelength of the incoming light. This change is related to the size of the particle. It is possible to extract the size distribution and give a description of the particle's motion in the medium, measuring the diffusion coefficient of the particle and using the autocorrelation function. The photon correlation spectroscopy (PCS) represent the most frequently used technique for accurate estimation of the particle size and size distribution based on DLS.

Scanning Electron microscopy

Scanning electron microscopy (SEM) is giving examination with direct visualization. The techniques based on electron microscopy offer several advantages in morphological and sizing analysis; however, they provide limited information about the size distribution and true population average. For SEM characterization, nanoparticles solution should be first converted into a dry powder, which is then mounted on a sample holder followed by coating with a conductive metal, such as gold, using a sputter coater. The sample is then scanned with a focused fine beam of electrons. The surface characteristics of the sample are obtained from the

secondary electrons emitted from the sample surface. The nanoparticles must be able to withstand vacuum, and the electron beam can damage the polymer. The mean size obtained by SEM is comparable with results obtained by dynamic light scattering. Moreover, these techniques are time consuming, costly and frequently need complementary information about sizing distribution.⁹

Transmission electron microscope

TEM operates on different principle than SEM, yet it often brings same type of data. The sample preparation for TEM is complex and time consuming because of its requirement to 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, uranyl acetate, etc, 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.

Atomic force microscopy

Atomic force microscopy (AFM) offers ultra-high resolution in particle size measurement and is based on a physical scanning of samples at sub-micron level using a probe tip of atomic scale. Instrument provides a topographical map of sample based on forces between the tip and the sample surface. Samples are usually 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 prime advantage of AFM is its ability to image non-conducting samples without any 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 mathematical treatment. Moreover, particle size obtained by AFM technique provides real picture which helps understand the effect of various biological conditions.

Surface Charge

The nature and intensity of the surface charge of nanoparticles is very important as it determines their interaction with the biological environment as well as their electrostatic interaction with bioactive compounds. The colloidal stability is analyzed through zeta potential of nanoparticles. This potential is an indirect measure of the surface charge. It corresponds to potential difference between the outer Helmholtz plane and the surface of shear. The measurement of the zeta potential allows for predictions about the storage stability of colloidal dispersion. High zeta potential values, either positive or



negative, should be achieved in order to ensure stability and avoid aggregation of the particles. The extent of surface hydrophobicity can then be predicted from the values of zeta potential. The zeta potential can also provide information regarding the nature of material encapsulated within the nanocapsules or coated onto the surface.⁹

Fourier Transform Infrared Spectroscopy (FTIR)

Advancements in computing techniques have enabled FTIR to become a popular tool to characterize various types of materials including polymers. FTIR is used for both qualitative and quantitative purposes. Molecular reaction mechanisms of biomolecules have been studied using time resolved FTIR. In Pharmaceutical research, FTIR is used to identify and analyze structure of drugs, excipients, polymorphism and dissolution. Drug polymer interaction studies can be performed using this technique in dosage forms containing nanoparticles. The FT-IR spectra of pure drug and nanoparticles loaded with drug were recorded to check drug polymer interaction and stability of drug.¹⁶

Drug loading

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

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

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

Drug release

To develop a successful nanoparticulate system, both drug release and polymer biodegradation are important consideration factors. In general, drug release rate depends on: (1) solubility of drug; (2) desorption of the surface bound/adsorbed drug; (3) drug diffusion through the nanoparticle matrix; (4) nanoparticle matrix erosion/degradation; and (5) combination of erosion/diffusion process. Thus solubility, diffusion and biodegradation of the matrix materials govern the release

process. In the case of nanospheres, where the drug is uniformly distributed, the release occurs by diffusion or erosion of the matrix under sink conditions. If the diffusion of the drug is faster than matrix erosion, the mechanism of release is largely controlled by a diffusion process. The rapid initial release or 'burst' is mainly attributed to weakly bound or adsorbed drug to the large surface of nanoparticles. It is evident that the method of incorporation has an effect on release profile. If the drug is loaded by incorporation method, the system has a relatively small burst effect and better sustained release characteristics. If the nanoparticle is coated by polymer, the release is then controlled by diffusion of the drug from the core across the polymeric membrane. The membrane coating acts as a barrier to release, therefore, the solubility and diffusivity of drug in polymer membrane becomes determining factor in drug release. Furthermore release rate can also be affected by ionic interaction between the drug and addition of auxillary ingredients. When the drug is involved in interaction with auxillary ingredients to form a less water soluble complex, then the drug release can be very slow with almost no burst release effect; whereas if the addition of auxillary ingredients e.g., addition of ethylene oxide-propylene oxide block copolymer (PEO-PPO) to chitosan, reduces the interaction of the model drug bovine serum albumin (BSA) with the matrix material (chitosan) due to competitive electrostatic interaction of PEO-PPO with chitosan, then an increase in drug release could be observed. Various methods which can be used to study the *in vitro* release of the drug are: (1) side-by-side diffusion cells with artificial or biological membranes; (2) dialysis bag diffusion technique; (3) reverse dialysis bag technique; (4) agitation followed by ultracentrifugation/centrifugation; (5) Ultra-filtration or centrifugal ultra-filtration techniques. Usually the release study is carried out by controlled agitation followed by centrifugation. Due to the time-consuming nature and technical difficulties encountered in the separation of nanoparticles from release media, the dialysis technique is generally preferred.⁷

Specific Applications of Biodegradable NPS

Tumor targeting

The rationale of using nanoparticles for tumor targeting is based on

- 1) Nanoparticles will be able to deliver a concentrate dose of drug in the vicinity of the tumor targets via the enhanced permeability and retention effect or active targeting by ligand on the surface of nanoparticles.
- 2) Nanoparticles will reduce the drug exposure of health tissues by limiting drug distribution to target organ.

The polymeric composition of nanoparticles such as type, hydrophobicity and biodegradation profile of the polymer along with the associated drug's molecular weight, its localization in the nanospheres and mode of incorporation technique, adsorption or incorporation,



have a great influence on the drug distribution pattern *in vivo*.⁵

Nanoparticles for Oral delivery

In recent years, significant research has been done using nanoparticles as oral drug delivery vehicles. Oral delivery of drugs using nanoparticles has been shown to be far superior to the delivery of free drugs in terms of bioavailability, residence time, and biodistribution. 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. The drugs may also be susceptible to gastrointestinal degradation by digestive enzymes. The advantage of using polymeric nanoparticles is to 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.

Another study showed that an antifungal drug encapsulated in particles of less than 300 nm in diameter was detected in the lungs, liver, and spleen of mice seven days post oral administration. The oral-free formulations on the other hand were cleared within 3 hours post administration. For this application, the major interest lies in lymphatic uptake of the nanoparticles by the Peyer's patches in the GALT (gut associated lymphoid tissue). There have been many reports as to the optimum size for Peyer's patch uptake ranging from less than 1 μm to 5 μm . However, it has also been shown that microparticles remain in the Peyer's Patches while nanoparticles are disseminated systemically. Nanoparticles can be engineered not only for oral absorption, but can also be used to deliver a drug directly to the source for gastrointestinal uptake, thereby protecting the drug from low pH and enzymes in the stomach. The pH-sensitive nanoparticles made from a poly (methylacrylic acid and methacrylate) copolymer can increase the oral bioavailability of drugs like cyclosporine-A by releasing their load at a specific pH within the gastrointestinal tract. The pH sensitivity allows this to happen as close as possible to the drug's absorption window through the Peyer's patches.

Nanoparticles for vaccine/gene delivery

Polynucleotide vaccines/DNA vaccines/plasmid 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/DNA 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, its localization to the nucleus of these cells, and ensuring that the integrity of the polynucleotides 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. Reported that following their intracellular uptake and endolysosomal escape, nanoparticles could release DNA at a sustained rate resulting in continuous 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.⁷

Nanoparticles for drug delivery into the brain

The blood-brain barrier (BBB) is the most important factor limiting the development of new drugs for the central nervous system. The BBB is characterized by relatively impermeable endothelial cells with tight junctions, enzymatic activity and active efflux transport systems. It effectively prevents the passage of water-soluble molecules from the blood circulation into the CNS, and can also reduce the brain concentration of lipid-soluble molecules by the function of enzymes or efflux pumps. Consequently, the BBB only permits selective transport of molecules that are essential for brain function. Strategies for nanoparticle targeting to the brain rely on the presence of and nanoparticle interaction with specific receptor-mediated transport systems in the BBB. For example polysorbate 80/LDL, transferrin receptor binding antibody (such as OX26), lactoferrin, cell penetrating peptides and melanotransferrin have been shown capable of delivery of a self non transportable drug into the brain via the chimeric construct that can undergo receptor-mediated transcytosis. It has been reported poly (butylcyanoacrylate) nanoparticles was able to deliver hexapeptide dalargin, doxorubicin and other agents into the brain which is significant because of the great difficulty for drugs to cross the BBB. Despite some reported success with polysorbate 80 coated NPs, this system does have many shortcomings including desorption of polysorbate coating, rapid NP degradation and toxicity caused by presence of high concentration of polysorbate 80. OX26 MAbs (anti-transferrin receptor MAbs), the most studied BBB targeting antibody, have been used to enhance the BBB penetration of liposomes.⁵



Long circulating nanoparticles

To be successful as a drug delivery system, nanoparticles must be able to target tumors which are localized outside MPS-rich organs. In the past decade, a great deal of work has been devoted to developing so-called “stealth” particles or PEGylated nanoparticles, which are invisible to macrophages or phagocytes. A major breakthrough in the field came when the use of hydrophilic polymers (such as polyethylene glycol, poloxamines, poloxamers, and polysaccharides) to efficiently coat conventional nanoparticle surface produced an opposing effect to the uptake by the MPS. These coatings provide a dynamic “cloud” of hydrophilic and neutral chains at the particle surface which repel plasma proteins. As a result, those coated nanoparticles become invisible to MPS, therefore, remained in the circulation for a longer period of time. Hydrophilic polymers can be introduced at the surface in two ways, either by adsorption of surfactants or by use of block or branched copolymers for production of nanoparticles.⁷

Absorption enhancement using non-specific interactions

In general, the gastrointestinal absorption of macromolecules and particulate materials involves either paracellular route or endocytotic pathway. The paracellular route of absorption of nanoparticles utilises less than 1% of mucosal surface area. Using polymers such as chitosan, starch or poly (acrylate) can increase the paracellular permeability of macromolecules. Endocytotic pathway for absorption of nanoparticles is either by receptor-mediated endocytosis, that is, active targeting, or adsorptive endocytosis which does not need any ligands. This process is initiated by an unspecific physical adsorption of material to the cell surface by electrostatic forces such as hydrogen bonding or hydrophobic interactions. Adsorptive endocytosis depends primarily on the size and surface properties of the material. If the surface charge of the nanoparticles is positive or uncharged, it will provide an affinity to adsorptive enterocytes though hydrophobic, whereas if it is negatively charged and hydrophilic, it shows greater affinity to adsorptive enterocytes and M cells. This shows that a combination of size, surface charge and hydrophilicity play a major role in affinity. This is demonstrated with poly (styrene) nanoparticles and when it is carboxylated.²

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.^{57,58} 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.^{59,60}

For this intrinsic process, mucoprotein is required, which is prepared by the mucus membrane in the stomach and binds specifically to cobalamin.

The mucoprotein completely reaches the ileum where resorption is mediated by specific receptors.⁵

Other Nanoparticles Applications

Healthcare/medical

1. Bone growth promoters
2. Biocompatible coatings for implants
3. Sunscreens (e.g. using ZnO and TiO₂) / cosmetics
4. Biolabeling and detection (e.g. using Au)
5. Carriers for drugs with low water solubility
6. Fungicides (e.g. using ZnO)
7. MRI contrast agents (e.g. using superparamagnetic iron oxide)
8. New dental composites
9. Biological binding agents (e.g. for high phosphate levels)
10. Antiviral, antibacterial (e.g. Ag), anti-spore non-chemical creams and
11. powders (using surface tension energy on the nanoscale to destroy biological particles)¹⁴

Future of Opportunities and Challenges

Nanoparticles have already been applied as drug delivery systems with great success. Nanoparticles provide massive advantages regarding drug targeting, delivery and with their potential for combine diagnosis and therapy and one of the major tools in Nanomedicine. These are many technical, challenges in developing the following techniques:- Virus-like systems for intracellular systems, Architecting of biomimetic polymers, control of sensitive drugs, functions (of active drug targeting, bioresponsive triggered systems, systems interacting with me body smart delivery), nanochips for nanoparticle release, carriers for advanced polymers for the delivery of therapeutic peptide / proteins. Drug delivery techniques were established to deliver or control the amount & rate. Most major and established internal research programmes on drug delivery that are formulations and dispersion containing components down to nano sizes.⁹



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