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Formulation, Characterization and Application on Nanoparticle: A Review

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ABSTRACT

Particles having diameter in range between 10-1000 nm are known as Nanoparticles. They are used as targeted delivery system for delivery of small and large molecules by changing their pharmacodyanmic and pharmacokinetic properties. They are not new to the environment as their existence had been found since long time for e.g. Pollutant in air but they had been studied and formulated for various beneficial purposes such as drug delivery, tissue targeting, cancer treatment, diagnostic agent and for imaging purpose. They had been prepared from different polymer which extent the therapeutic effect as well as reduces side effect. This review discussed about various methods of preparation, their characterization techniques, such as drug loading, release and the applications of Nanoparticles along with some marketed products.

Keywords: application, characterization, drug release, Nanoparticles.

Introduction

The solid colloidal microscopic particles with size ranging between 10-1000 nm are known as nanoparticles. [1]. They can be defined as system which contain active ingredient dissolved, encapsulated or adsorbed in matrix material which are used as target delivery system.

To see the effect of drug in target tissue, to increase stability against degradation through enzymes and for solubilization at intra-vascular route nanoparticles have been used [2]. For the safe administration of nanoparticle through intravenous route they are formulated in the form injection which consist spherical amorphous particle. Formulations are less toxic in nature because in this co-solvent is not used to solubilize the drug. Eukaryotic or prokaryotic cells had been founded to be larger in size then nanoparticle but for comparisons of their size they belong to size of virus or antibody [3]. During the designing of nanoparticle some control has to taken in

care such as their release pattern, their size and surface properties which determine site-specific action at optimal rate with right dose regimen [4].

Advantages of nanoparticles [4, 5, 6, 7]
1) They are suitable for different routes of administration.
2) Carrying capacity of nanoparticles is high.
3) Shelf-stability of drug increases.
4) Ability to sustain and control drug release patterns.
5) Suitable for combination therapy where two or more drug can be co-delivered.
6) Both hydrophobic and hydrophilic drug can be incorporated.
7) System increases the bioavailability of drugs.
8) Imaging studies can be done by utilizing them.
9) It is used for targeted drug delivery of drugs.
10) Development of new medicines which are safer.

Disadvantages of nanoparticles [8, 9, 10]
a) The manufacturing costs of nanoparticle are high which result in overall product cost.
b) Solvents are toxic in nature which is used in the preparation process.
c) Can start immune response and allergic reactions in body.
d) Extensive use of poly (vinyl alcohol) as stabilizer may have toxicity issues.
e) Nanoparticles are difficult to handle in physical form because particle-particle aggregation occurs due their small size and large surface area.

Formulation
Preparation of nanoparticle
In the preparation of nanoparticles different types of matrix material are used such as polysaccharides, synthetic polymer and proteins. Various factors are involved in selection of matrix material to be used in preparations which are [11].

(i) Required nanoparticle size.
(ii) Permeability and surface charge of nanoparticle.
(iii) Level of biodegradability and biocompatibility must be optimum.
(iv) Material must not be toxic.
(v) Solubility profile and stability of drug should not be affected.
(vi) It should show desired drug release profile.
(vii) Must not be immnunogenic.

Following are methods which are used in formulation of nanoparticles [4].

1. Dispersion of preformed polymers.
2. Polymerization method.
3. Coacervation or ionic gelatin method.
4. Supercritical fluid technology [12].

Table-1: Polymer used for the preparation of nanoparticle [2].

Technique	Candidate drug	Polymer used
Heat denaturation and cross linking in w/o emulsion	Hydrophilic	**Hydrophilic** Albumin ,Gelatin
Desolvation and cross linking in water	Hydrophilic and protein affinity	**Hydrophilic** Albumin ,Gelatin
Cross-linking in water	Hydrophilic and protein affinity	**Hydrophilic** Alginates and chitosan
Polymer precipitation in an organic solvent	Hydrophilic	**Hydrophilic** Dextran
Emulsion polymerization	Hydrophilic	**Hydrophobic** Poly(alkylcyanoacrylates)
Interfacial O/W polymerization	Hydrophobic	**Hydrophobic** Poly(alkylcyanoacrylates)
Solvent extraction evaporation	Hydrophilic and Hydrophobic Soluble in polar solvent	**Polyesters** Poly (lactic acid), poly(caprolactone)
Solvent displacement	Hydrophilic and Hydrophobic Soluble in polar solvent	**Polyesters** Poly (lactic acid), Poly (lactide-co-glycolide),
Salting out	Soluble in polar solvent	**Polyesters** Poly (lactic acid), Poly (lactide-co-glycolide).

1. Dispersion of preformed polymers

For the preparation of biodegradable nanoparticles from polymers such as poly (lactic acid) (PLA); poly (D, L-glycolide), PLG; poly (D, L-lactide-co-glycolide) (PLGA) and Poly-(cyanoacrylate) (PCA), dispersion of preformed polymer method is used [13]. This technique can be used in various ways as described below.

Solvent evaporation method

In this method, there is conventional formation of o/w emulsion between a partially water miscible solvent containing the polymer and the drug, and an aqueous phase containing the stabilizer. In this polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate. Oil in water (o/w) emulsion is prepared by emusification of drug and polymer mixture in aqueous solution which contain emulsifying agent, which result in formation of stable emulsion. After that by using pressure reduction method or continuous stirring, organic solvent is evaporated. The homogenizer speed, nature and stabilizer concentration along with the property of polymer effect size of nanoparticle. Usually high speed homogenizer or ultrsonication had been used to reduce the size of nanoparticle to an optimum size [14].

Spontaneous emulsification or solvent diffusion method

Also known as modified version of solvent evaporation method. In this method, two phase solvent is used, one is water miscible and other is water immiscible i.e. organic in nature which act as oil phase.

In this method interfacial turbulence is created, by immediate diffusion between two solvents (which are differing in phase) which lead to the formation of small particles. A reduction in

particle size can be gained by increasing the concentration of water miscible solvent both the above described method can be used for preparation of hydrophilic and hydrophobic drugs.

Salting out

It is one of commonly used method used for preparation of nanoparticle. This method involves the mixing of saturated aqueous solution of polyvinyl alcohol (PVA) into an acetone solution of the polymer under magnetic stirring resulting in the formation of o/w emulsion. The precipitation of the polymer occurs when sufficient amount of water is added to external phase to allow complete diffusion of the acetone from internal phase into aqueous phase.

2. Polymerization method

Polmerization of monomers in an aqueous solution form the basis of this method. Two different techniques are used for the preparation in aqueous solution.

a) Emulsion polymerization: - this method involves emulsification of monomer in non-solvent phase.
b) Dispersion polymerization: - this method involves dispersion of monomer in non-solvent phase.

Incorporation of drug in nanoparticle can be achieved either by dissolving the drug in polymerization medium or by adsorption onto nanoparticle. Suspension of nanoparticles is formed, which contain surfactants and stabilizers that are used in polymerization which has to be removed by method like ultracentrifugation or by suspending them in isotonic medium which is free of surfactant. Polybutylcyanoacrylate or poly (alkylcyanoacrylate) nanoparticles are been prepared by this method. The polmer particle size had been affected by concentration of stabilizer and surfactant involved in preparation. [15].

3. Coacervation or ionic gelation method

Chitosan, sodium alginate and gelatin are hydrophilic biodegradable polymers which are used for the preparation of nanoparticles by coacervation method. Preparation of hydrophilic chitosan nanoparticles by ionic gelation was developed by Calvo and Co-worker [16]. This method involves a preparation of two aqueous phases, of which one is the polymer chitosan, adi-block co-polymer ethylene oxide or propylene oxide (PEO-PPO) and the other is a polyanion sodium tripolyphosphate which are mixed, due to mixing positively charged amino group of chitosan interacts with negative charged tripolyphosphate to form coacervates with a size in the range of nanometer. when electrostatic interaction take place between two aqueous phases coacervates are formed, and when two molecules interact due to ionic force, resulting in transition from liquid phase to gel phase at room temperature this is known as ionic gelation method.

4. Production of nanoparticles using supercritical fluid technology

Various conventional approaches like solvent diffusion, solvent extraction-evaporation and organic phase separtion require the use of organic solvent are hazardous to the environment as well as the physiological systems. Supercritical fluid technology thus has been invested as an alternative to prepare biodegradable micro and Nanoparticles [17]. Solvent which remain fluid in a single phase regardless of pressure above critical temperature are known as supercritical fluid [17].Super critical CO_2 is the most widely used supercritical fluid. The most common processing

techniques involves supercritical fluids are supercritical Antisolvent (SAS) and rapid expansion of critical solution (RESS). Formation of hydrophilic drug Dexamethsone phosphate by the use of modified SAS had been reported by Thote and Gupta (2005) [18]. RESS diffuse from SAS process in that its solute in dissolved in super critical fluid .Thus with solvent power of super critical fluid decrease and the solute eventually precipitate.

Characterization of nanoparticles

To understand synthesis and application of nanoparticle, characterization of nanoparticle is necessary [19].Size determination is the primary parameter for characterization of nanoparticle. Various techniques had been used for this purpose.

Particle size

Most of the properties of nanoparticle like drug loading and release pattern , in vivo distribution, tissue targeting, toxicity and biological fate are concerned with the size and size distribution of Nanoparticles so they had become an important parameter in characterization of product. It has been reported that micro particles are less effective drug delivers than particle having size ranging in between nanometers for e.g Nanoparticles having size range greater than 230 nm acquire in the spleen shown by body distribution studies [20]. Drug release is depend upon surface area larger the surface area more is the diffusion and less the surface area less is diffusion and surface area depend upon particle size i.e smaller the size greater is the surface area and vice –versa. Also large particle has large core which fills more drug and they diffuse out slowly [21]. It has been seen that aggregation occurs with small particle size. So it was considered that large particles will assist fast drug release and polymer degradation [22].

Method of determining particle size is by [22]

1. Photon-correlation spectroscopy.
2. Dynamic light scattering.
3. Brownian motion and light scattering properties [23].
4. Scanning or transmission electron microscopy (SEM or TEM).

Surface properties of nanoparticles

The nature and intensity of the surface charge of nanoparticle is very important as it determine their interaction with its biological environment as well as their electrostatic interaction with bioactive compounds. After the intravenous administration of nanoparticles, body immune system recognizes these, followed by phagocytic removal from body by blood circulation. After the recognization by body, delivered to the mono nuclear phagocytes system (MPS) of body which degrade them .The MPS system of body involves parts such as liver, spleen, lungs and bone marrow. And if once, surface non-modified nanoparticles (conventional nanoparticles) reached in the blood stream they undergo rapid opsonization and cleared by the macrophages of MPS rich organs [24].

Phagocytes can be prevented by-

(1) Coating the surface of nanoparticles with by using hydrophilic polymers/surfactants which coat the surface of nanoparticle.

(2) With the help of biodegradable copolymers having hydrophilic segments like polyethylene glycol (PEG), polyethylene oxide, poloxamine and polysorbate 80 (Tween 80) which are used to prepare Nanoparticles.
For the characterization of surface property of nanoparticle determination of zeta potential is commonly employed [25]. Zeta potential reflects the electrical potential hold by particle and factor which affect its value are composition of particle and solvent in which it is dispersed.

Drug loading [26]
A high drug- loading capacity is the measure of successful nanoparticulate system because it reduces the amount of matrix material for administration. Drug loading can be done by two methods:
a) Incorporation method: - In this drug is incorporated during the formation of nanoparticle.
b) Adsorption/absorption method: - In this method drug is made to be adsorbed on nanoparticle. In this formed nanoparticle is kept in concentrated solution of drug and adsorption phenomenon take place.

Drug release [4]
Another Factor for a formulation of successful nanoparticulate system, study of parameter such as both drug release profile and polymer biodegradation is concern. In general, drug release rate depends on:
(a) Solubility of drug.
(b) How far the Drug is diffused through the nanoparticle matrix.
(c) Combination of erosion/diffusion process.
(d) Degree of material matrix erosion/degradation and
(e) Time taken by the drug for desorption through surface.

Loading of drug by incorporation method produce system which has small burst effect and good sustained release characteristics [27]. Coating the nanoparticle with polymer, release is affected by movement of drug from core across the polymeric membrane. In this case polymeric membrane becomes release determing factor because it affects the solubility and diffusivity of drug. A number of methods can be used to determine in vitro release of drug [28, 29].
(a) Reverse dialysis bag technique
(b) Dialysis bag diffusion technique.
(c). centrifugal ultra-filtration techniques
(d) Agitation.
(e) Using biological or artificial membrane i.e. Side-by-side diffusion of cells.

To summarize different parameters to be characterized along with their characterization method are presented in (table no.2)

Applications of nanoparticles [30]
A list of some of the applications of nanoparticles to biology or medicine is given below
1. Fluorescent biological labels [31, 32 33].
2. Delivery of drug and gene [34, 35].
3. Pathogens can be detected [36].
4. Proteins can be detected [37].

5. Probing of DNA structure [38].
6. Helps in tissue engineering [39, 40].
7. Destruction of tumour via heating (hyperthermia) [41].
8. Biological molecules and cells can be separated and purified [42].
9. Phagokinetic studies can be done [43].
10. Contrast of MRI can be enhancement.

Table. 2: Different parameters and characterization methods for nanoparticles [2].

Parameter	Characterization method
Particle size and distribution	Photon correlation spectroscopy(PCS) Laser defractometry Transmission electron microscopy Scanning electron microscopy Atomic force microscopy
Surface hydrophobicity	Water contact angle measurement Rose Bengal(dye) binding X-ray photoelectron spectroscopy
Charge determination	Laser Doppler Anemometry Zeta potentiometer
Carrier-drug interaction	Diffential scanning calorimetry
Chemical analysis of surface	Static secondary ion mass spectrometry Sorptometer
Nanoparticle dispersion stability	Critical flocculation temperature(CFT)
Release profile	In vitro release characteristics under physiologic and sink conditions
Drug stability	Bioassay of drug extacted from Nanoparticles Chemical analysis of drug

Some of the examples of nanoparticles which are being used currently are [44]:

- The Magnetic Resonance Imagining scan can be enhanced by Iron oxide nanoparticle.
- Breakdown of volatile organic compounds in air can be catalyst by gold embedded nanoparticle in porous manganese oxide.
- Coating the nanoparticles with protein is used as drug deliver to damage regions of arteries in cardiovascular disease treatment.
- Cancer cell can be removed before establishing new tumours from blood cell using Magnetic nanoparticles.
- Palladium nanoparticles are used for detection of hydrogen.
- Presences of cancer cell are located by Quantum Dots (crystalline nanoparticles).
- NOMFET (Nanoparticle Organic Memory Field-Effect Transistor) Gold nanoparticle are combined with organic molecules used for delivery of chemotherapy drugs directly into tumour cells.
- For the removal of pollutant like carbon tetrachloride from water Iron nanoparticles can be used.
- To increase power of battery and reducing it recharge time anodes of lithium-ion batteries are coated with Silicon nanoparticles.

Marketed products: some of the products which are used are described in (table. No.3) [45].

Table. 3:-list of some marketed products [45]

Company	Trade name	Composition	Indication	Administration
Enzon	Abelect	Liposomal amphotericin B	Fungal infection	Intravenous
Berna Biotech	Epaxal	Liposomal IRIV vaccine	Hepatitis A	Intramuscular
Novavax	Estrasorb	Micellular estradiol	Menopausal therapy	Topical
Nektar, Hoffmann-La Roche	Pegasys	PEG–a-interferon 2a	Hepatitis B, Hepatitis C	Subcutaneous
Genzyme	Renagel	Poly(allylamine hydrochloride)	End-stage renal disease	Oral
Elan, Merck	Emend	Nanocrystalline aprepitant	Antiemetic	Oral
Elan, Abbott	Tricor	Nanocrystalline fenofibrate	Anti-hyperlipidemic	Oral
Elan, Wyeth	Rapamune	Nanocrystalline sirolimus	Pharmaceuticals Immunosuppressant	Oral

Future prospect of nanoparticles in drug delivery

Therapeutic agents such as oral insulin, clotting factors, growth factors, hormones and anticoagulants agents face problem like decrease bioavailability, less stable, decrease permeability across biological membrane and some time are immunogenic this problem can be overcome by nanoparticulate delivery system. Nanoparticles see can be seen in future of antitumour therapy, gene therapy or vaccines preparation. Nanoparticles can be used in diagnosing purpose for eg. can be used detecting mutated genes, damaged tissue also high level of hormones can be detected nanoparticle finds the solution for old problems such as bioavailability, solubility, release rate, immunogenicity etc[46]. A nanoparticle had got valuable future for therapeutic agents.

CONCLUSION

The foregoing review shows that therapeutic agents which are poorly soluble absorbed poorly and that are labile can be converted into promising forms which can be delivered through different routes of administration. It has various advantages simultaneously and also has some limitation such as can be immunogenic and others. The physio-chemical parameters of the drug play an important role in the selection of the nanoparticle material that has to be employed. Various methods can be used for the preparation of nanoparticles depending upon need. Characterization of them is must for better therapeutic response. Currently application of Nanoparticles is widely spread which are providing their service to humans.

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