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POLYMERIC NANOPARTICLES PREPARATION TECHNIQUES AND APPLICATIONS FOR DRUG DELIVERY SYSTEM- A BRIEF REVIEW.

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ABSTRACT

The most compelling branch in pharmaceutical sciences 'Pharmaceutical nanotechnology' presents new implement, chance and scope, which have revealing usage in disease diagnostic and treatment. Nanotechnology is essential part of pharmaceutical drug delivery system. Diverse number of polymer have been used in formulation of nanoparticle to improve therapeutic effect of drugs and minimizing their side effects. The polymeric nanoparticles approaches to increase therapeutic performance of poorly soluble drugs via any route of administration. Present article is a review of various aspect of polymeric nanoparticles formulation, characterization and application in drug delivery system.

KEYWORDS: Nanoparticles, Drug delivery, Polymer.

INTRODUCTION

During last two decades, considerable attention has been given to the development of novel drug delivery systems (NDDS). The rational for control drug delivery is to alter the pharmacokinetics and pharmacodynamics of drug substance in order to improve the therapeutic efficacy and safety. Besides more traditional matrix or reservoir drug delivery systems, colloidal drug delivery system has gained in more popularity.[1] Colloidal drug delivery systems offer a number of advantages over conventional dosage forms. The major colloidal drug delivery systems include liposome and nanoparticles.[2] Nanoparticles are colloidal polymeric particles of size below 1µm with a therapeutic agent either dispersed in

polymeric matrix or encapsulated in polymer.[1, 4] The term "polymeric nanoparticle" encompasses nanospheres and nanocapsules. Nanospheres are defined as a polymeric matrix in which the drug is uniformly dispersed and nanocapsules are described as a polymeric membrane that surrounds the drug in the matrix core as shown in figure.[3]

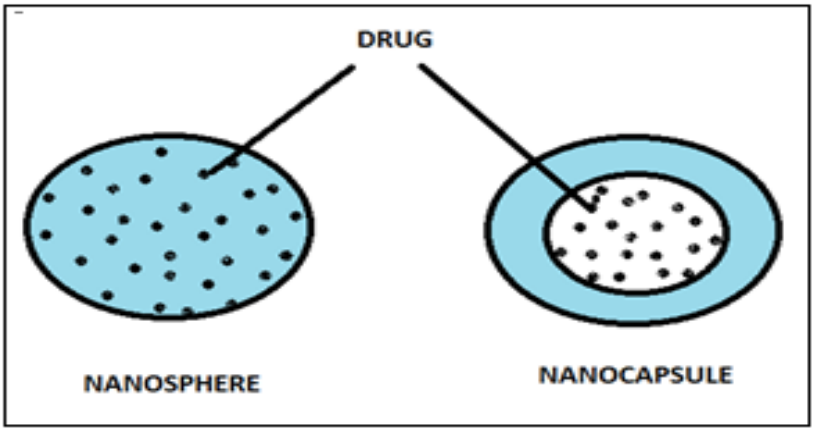

Figure 1: Difference between the nanosphere and nanocapsule.

The field of polymer nanoparticles (PNPs) is quickly expanding and playing an important role in a wide spectrum of areas ranging from electronics, photonics, conducting materials, sensors, medicine, biotechnology, pollution control and environmental technology.[5-13] Polymer-based nanoparticles effectively carry drugs, proteins, and DNA to target cells and organs. Their nanometer-size promotes effective permeation through cell membranes and stability in the blood stream. Polymers are very convenient materials for the manufacture of countless and varied molecular designs that can be integrated into unique nanoparticle constructs with many potential medical applications.[14]

Advantages of polymeric nanoparticles[15, 16, 17]

- Increases the stability of any volatile pharmaceutical agents, easily and cheaply fabricated in large quantities by a multitude of methods.
- They offer a significant improvement over traditional oral and intravenous methods of administration in terms of efficiency and effectiveness.
- Delivers a higher concentration of pharmaceutical agent to a desired location.
- The choice of polymer and the ability to modify drug release from polymeric nanoparticles have made them ideal candidates for cancer therapy, delivery of vaccines, contraceptives and delivery of targeted antibiotics.

- Polymeric nanoparticles can be easily incorporated into other activities related to drug delivery, such as tissue engineering.

Polymer Used in the Preparation of Nanoparticles

Table 1: Most widely used polymers for preparing nanoparticles in drug delivery[3]

Material	Full Name	Commercial Name
Synthetic homopolymers	Poly lactide	PLA
	Poly (lactidecoglycoloide)	PLGA
	Poly(epsiloncaprolactone)	PCL
	Poly (isobutylcyanoacrylate)	PIBCA
	Poly (isohexylecyanoacrylate)	PIHCA
	Poly (n butylcyanoacrylate)	PBCA
	Poly(acrylate) and poly(methacrylate)	Eudragit
Natural polymers	Chitosan	
	Alginate	
	Gelatin	
	Albumin	
Copolymers	Poly (lactide)-poly (ethyleneglycol)	PLA-PEG
	Poly(lactide-co-glycolide)poly(ethyleneglycol)	PLGA-PEG
	Poly(epsilon-caprolactone) poly(ethyleneglycol)	PCL-PEG
	Poly(hexadecylcyanoacrylate-co- poly(ethyleneglycol) cyanoacrylate	Poly(HDCA- PEGCA)
Colloid stabilizers	Dextran	
	PluronicF68	F68
	Poly(vinyl alcohol)	PVA
	Tween20 and Tween80	

Technique of preparation

The properties of PNPs have to be optimized depending on the particular application. In order to achieve the properties of interest, the mode of preparation plays a vital role. Thus, it is highly advantageous to have preparation techniques at hand to obtain PNPs with the desired properties for a particular application. Different techniques like polymerization, preformed

polymers or ionic gelation etc are used. In figure general method of preparation of polymeric nanoparticles and their principle involved in the mechanism are described.[18, 19]

1-Methods for preparation of nanoparticles from dispersion of preformed polymer[20]

Dispersion of drug in preformed polymers is a common technique used 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). These can be accomplished by different methods described below.

a) Solvent evaporation

b) Nanoprecipitation

c) Emulsification/solvent diffusion

d) Salting out

e) Dialysis

f) Supercritical fluid technology (SCF)\

2-Methods for preparation of nanoparticles from polymerization of monomers

a) Emulsion

b) Mini emulsion

c) Micro emulsion

d) Interfacial polymerization

e) Controlled/Living radical polymerization(C/LRP)

Solvent evaporation

Solvent evaporation was the first method developed to prepare PNPs from a. In this method, polymer solutions are prepared in volatile solvents and emulsions are formulated. In the past, dichloromethane and chloroform preformed polymer were widely used, but are now replaced with ethyl acetate which has a better toxicological profile. The emulsion is converted into a nanoparticle suspension on evaporation of the solvent for the polymer, which is allowed to diffuse through the continuous phase of the emulsion. In the conventional methods, two main strategies are being used for the formation of emulsions, the preparation of single-emulsions, e.g., oil-in-water (o/w) or double-emulsions, e.g., (water-in-oil)-in-water, (w/o)/w. These methods utilize high-speed homogenization or ultrasonication, followed by evaporation of the solvent, either by continuous magnetic stirring at room temperature or under reduced pressure. Afterwards, the solidified nanoparticles can be collected by ultracentrifugation and

washed with distilled water to remove additives such as surfactants. Finally, the product is lyophilized.[21, 22]

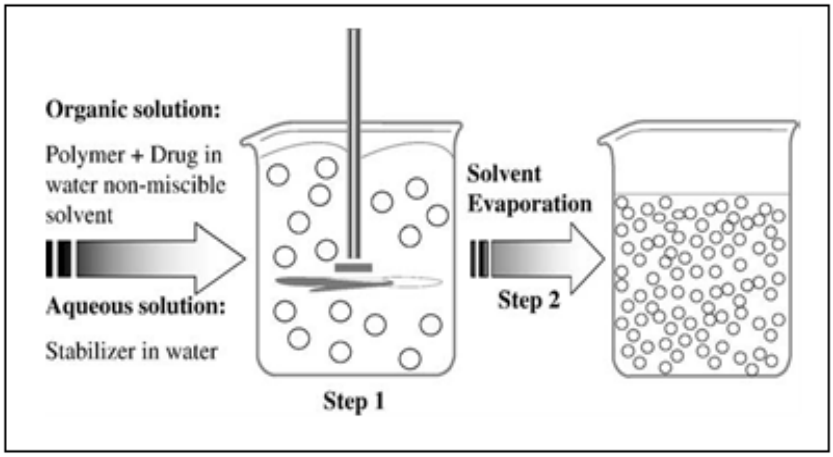

Figure 2: Schematic representation of the solvent-evaporation technique.

Nanoprecipitation

Nanoprecipitation is also called solvent displacement method. It 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 a surfactant.[23-27] The polymer generally PLA, is dissolved in a water-miscible solvent of intermediate polarity, leading to the precipitation of nanospheres. This phase is injected into a stirred aqueous solution containing a stabilizer as a surfactant. Polymer deposition on the interface between the water and the organic solvent, caused by fast diffusion of the solvent, leads to the instantaneous formation of a colloidal suspension.[28] To facilitate the formation of colloidal polymer particles during the first step of the procedure, phase separation is performed with a totally miscible solvent that is also a non solvent of the polymer.[29] The solvent displacement technique allows the preparation of nanocapsules when a small volume of nontoxic oil is incorporated in the organic phase. Considering the oil-based central cavities of the nanocapsules, high loading efficiencies are generally reported for lipophilic drugs when nanocapsules are prepared. The usefulness of this simple technique[28] is limited to water-miscible solvents, in which the diffusion rate is enough to produce spontaneous emulsification. Then, even though some water-miscible solvents produce a certain instability when mixed in water, spontaneous emulsification is not observed if the coalescence rate of the formed droplets is sufficiently high.[30] Although, acetone/dichloromethane (ICH, class 2) are used to dissolve and increase the entrapment of drugs, the dichloromethane increases the mean particle size and is considered toxic. 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.[31]

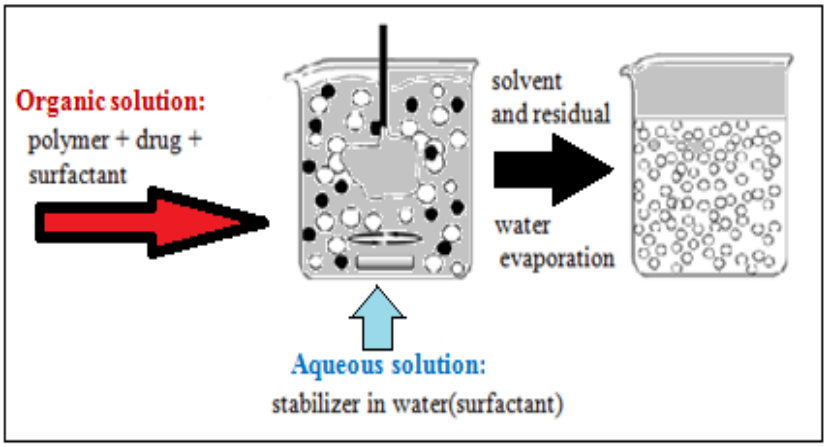

Figure 3: Schematic representation of the nanoprecipitation technique. Surfactant is optional.

Emulsification/solvent diffusion (ESD)

This is a modified version of solvent evaporation method. The encapsulating polymer is dissolved in a partially water soluble solvent such as propylene carbonate 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, leading to solvent diffusion to the external phase and the formation of nanospheres or nanocapsules, according to the oil-to-polymer ratio. Finally, the solvent is eliminated by evaporation or filtration, according to its boiling point. The procedure is illustrated in figure 4. This technique presents several advantages, such as high encapsulation efficiencies (generally >70%), no need for homogenization, high batch-to-batch reproducibility, ease of scale-up, simplicity, 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.[21,32,33]

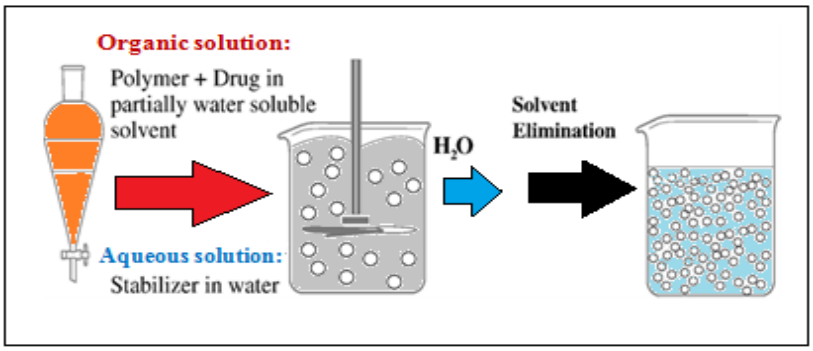

Figure 4: Schematic representation of the emulsification/solvent diffusion technique.

Salting out

Salting out is based on the separation of a water miscible solvent from aqueous solution via a salting out effect. The salting out procedure can be considered as a modification of the emulsification/solvent diffusion. 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. This technique used in the preparation of PLA, poly (methacrylic) acid, nanospheres leads to high efficiency and is easily scaled up. The main advantage of salting out is that it minimizes stress to protein encapsulants. Salting out does not require an increase of temperature and therefore, may be useful when heat sensitive substances have to be processed. The greatest disadvantages are exclusive application to lipophilic drugs and the extensive nanoparticle washing steps.[34,35,36]

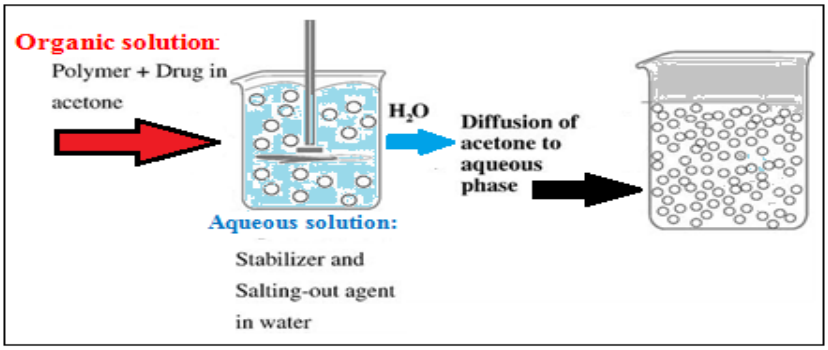

Figure 5: Schematic representation of the salting out technique.

Dialysis

Dialysis offers a simple and effective method for the preparation of small, narrow-distributed PN. Polymer is dissolved in an organic solvent and placed inside a dialysis tube with proper molecular weight cut off. Dialysis is performed against a non-solvent miscible with the former miscible. The displacement of the solvent inside the membrane is followed by the progressive aggregation of polymer due to a loss of solubility and the formation of homogeneous suspensions of nanoparticles. The mechanism of PNP formation by dialysis method is not fully understood at present. It is thought that it may be based on a mechanism similar to that of nanoprecipitation. A number of polymer and copolymer nanoparticles, were obtained by this technique.[37, 38, 39, 40]

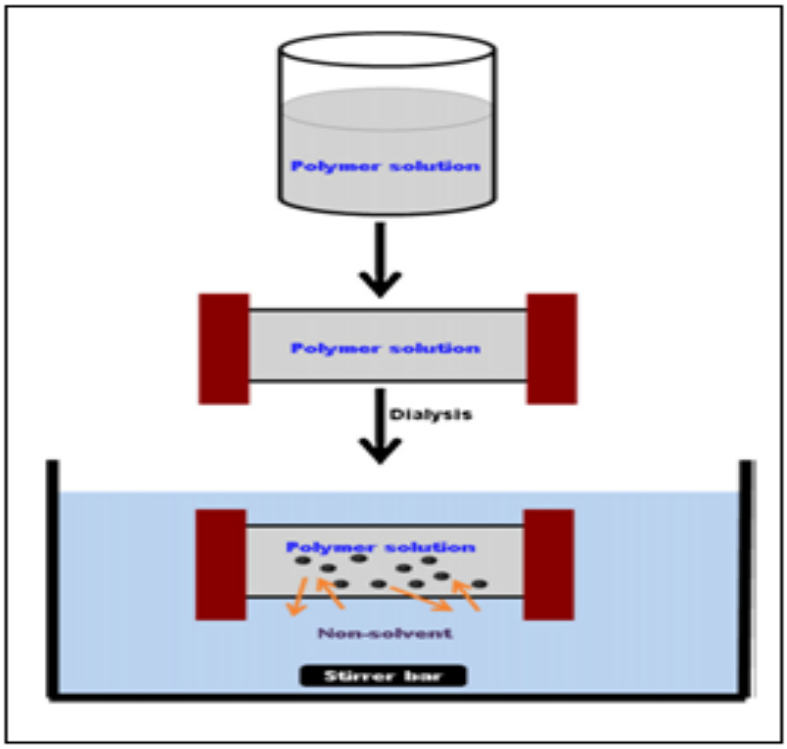

Figure 6: Schematic representation of osmosis based method for preparation of polymer nanoparticles.[41]

Supercritical fluid technology

The need to develop environmentally safer methods for the production of PNP has motivated research on the utility of supercritical fluids as more environmental friendly solvents, with the potential to produce PNPs with high purity and without any trace of organic solvent. Supercritical fluid and dense gas technology are expected to offer an interesting and effective technique of particle production, avoiding most of the drawbacks of the traditional methods.

Two principals have been developed for the production of nanoparticles using supercritical fluids[42, 43]

1. Rapid expansion of supercritical solution (RESS)
2. Rapid expansion of supercritical solution into liquid solvent (RESOLV).

Rapid expansion of supercritical solution

In traditional RESS, the solute is dissolved in a supercritical fluid to form a solution, followed by the rapid expansion of the solution across an orifice or a capillary nozzle into ambient air. The high degree of super saturation, accompanied by the rapid pressure reduction in the expansion, results in homogenous nucleation and thereby, the formation of well-dispersed particles. Results from mechanistic studies of different model solutes for the RESS process indicate that both nanometer and micrometer-sized particles are present in the expansion jet [43]. A few studies were carried out on the production of PNPs using RESS. Poly (perfluoropolyetherdiamide) droplets produced from the rapid expansion of CO2 solutions. The RESS experimental apparatus consists of three major units: a high-pressure stainless steel mixing cell, a syringe pump, and a pre-expansion unit. A solution of polymer in CO2 is prepared at ambient temperature. Before the solution leaves the nozzle, using syringe pump, it is pumped to the pre-expansion unit and is heated isobarically to the pre-expansion temperature. The supercritical solution is now allowed to expand through the nozzle, at ambient pressure. The concentration and degree of saturation of the polymer have a considerable effect on the particle size and morphology of the particles for RESS.[22, 44-47]

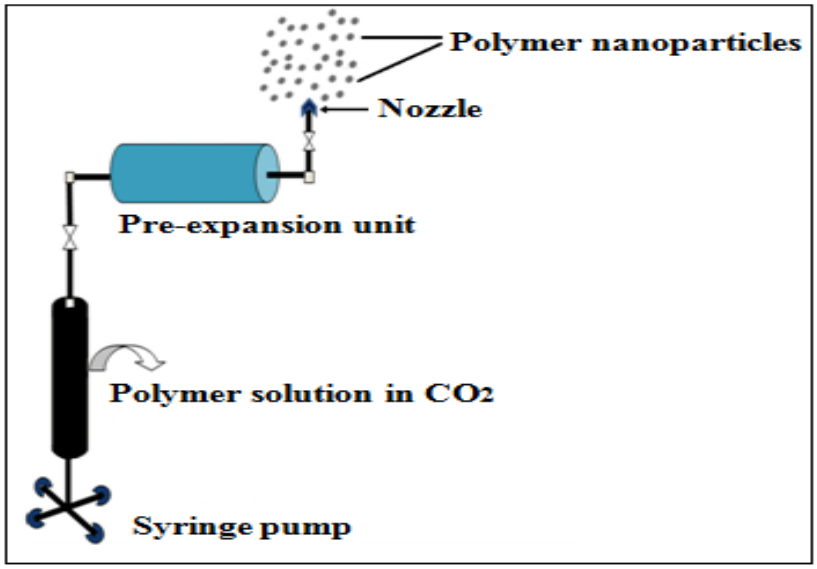

Figure 7: Experimental set-up for preparation of polymer nanoparticles by rapid expansion of supercritical fluid solution.

Rapid expansion of supercritical solution into liquid solvent

A simple, but significant modification to RESS involves expansion of the supercritical solution into a liquid solvent instead of ambient air, termed as RESOLV.[22, 48] Meziani *et al*[49] reported the preparation of Poly (heptadecafluorodecyl acrylate) nanoparticles having an average size of less than 50 nm. Even though in RESS technique no organic solvents used for the formation of PNPs, the prime products obtained using this technique are microscaled rather than nanoscaled, which is the main drawback of RESS. In order to overcome this drawback a new supercritical fluid technology known as RESOLV has been developed. In RESOLV the liquid solvent apparently suppresses the particle growth in the expansion jet, thus making it possible to obtain primarily nanosized particles.[49-51]

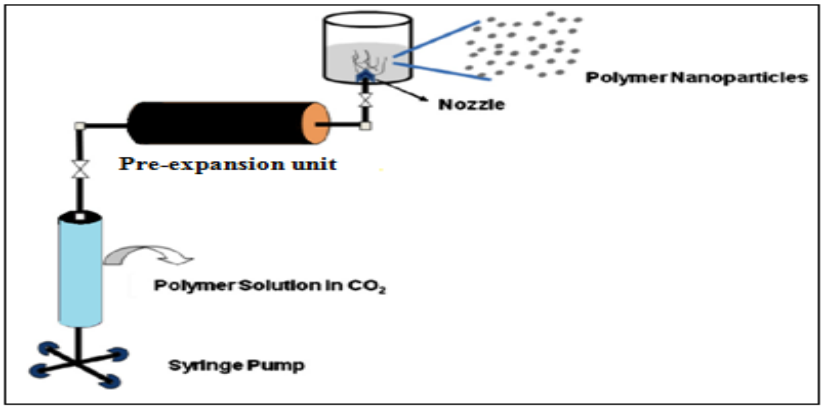

Figure 8: Experimental set-up for the rapid expansion of supercritical fluid solution into liquid solvent process[22]

Preparation of nanoparticles by polymerization of a monomer

To attain the desired properties for a particular application, suitable polymer nanoparticles must be designed, which can be done during the polymerization of monomers. Processes for the production of PNPs through the polymerization of monomers are discussed below.

Emulsion polymerization

Emulsion polymerization is one of the fastest methods for nanoparticle preparation and is readily scalable. The method is classified into two categories, based on the use of an organic or aqueous continuous phase. The continuous organic phase methodology involves the dispersion of monomer into an emulsion or inverse microemulsion, or into a material in which the monomer is not soluble (nonsolvent).[23] Polyacrylamide nanospheres were produced by this method.[51, 52] In the aqueous continuous phase the monomer is dissolved in a continuous phase that is usually an aqueous solution, and the surfactants or emulsifiers are

not needed. The polymerization process can be initiated by different mechanisms. Initiation occurs when a monomer molecule dissolved in the continuous phase collides with an initiator molecule that might be an ion or a free radical. Alternatively, the monomer molecule can be transformed into an initiating radical by high-energy radiation, including g-radiation, or ultraviolet or strong visible light. Chain growth starts when initiated monomer ions or monomer radicals collide with other monomer molecules according to an anionic polymerization mechanism. Phase separation and formation of solid particles can take place before or after termination of the polymerization reaction.[23, 29, 53]

Mini-emulsion polymerization

A typical formulation used in mini- emulsion polymerization consists of water, monomer mixture, co-stabilizer, surfactant, and initiator. The key difference between emulsion polymerization and mini-emulsion polymerization is the utilization of a low molecular mass compound as the co-stabilizer and also the use of a high-shear device (ultrasound, etc.). Mini-emulsions are critically stabilized, require a high-shear to reach a steady state and have an interfacial tension much greater than zero.[22] The various polymer nanoparticles were prepared by using Mini-emulsion method as discussed in the literature.[55-57]

Micro-emulsion polymerization

Micro-emulsion polymerization is a new and effective approach for preparing nanosized polymer particles and has attracted significant attention. Although emulsion and micro-emulsion polymerization appear similar because both methods can produce colloidal polymer particles of high molar mass, they are entirely different when compared kinetically. Both particle size and the average number of chains per particle are considerably smaller in micro-emulsion polymerization. In micro-emulsion polymerization, an initiator, typically water-soluble, is added to the aqueous phase of a thermodynamically stable micro-emulsion containing swollen micelles. The polymerization starts from this thermodynamically stable, spontaneously formed state and relies on high quantities of surfactant systems, which possess an interfacial tension at the oil/water interface close to zero. Furthermore, the particles are completely covered with surfactant because of the utilization of a high amount of surfactant. Initially, polymer chains are formed only in some droplets, as the initiation cannot be attained simultaneously in all microdroplets. Later, the osmotic and elastic influence of the chains destabilize the fragile micro- emulsions and typically lead to an increase in the particle size, the formation of empty micelles, and secondary nucleation. Very small latexes, 5–50nm in

size, coexist with a majority of empty micelles in the final product. The types of initiator and concentration, surfactant, monomer and reaction temperature are some of the critical factors affecting the micro-emulsion polymerization kinetics and the properties of PNP.[58, 59]

Interfacial polymerization

It is one of the well-established methods used for the preparation of polymer nanoparticles[60-64] It involves step polymerization of two reactive monomers or agents, which are dissolved respectively in two phases (i.e., continuous- and dispersed-phase), and the reaction takes place at the interface of the two liquids.[65] Nanometer-sized hollow polymer particles were synthesized by employing interfacial cross-linking reactions as polyaddition and polycondensation.[66-68] or radical polymerization.[69, 70] Oil-containing nanocapsules were obtained by the polymerization of monomers at the oil/water interface of a very fine oil-in-water micro- emulsion.[71] The organic solvent, which was completely miscible with water, served as a monomer vehicle and the interfacial polymerization of the monomer was believed to occur at the surface of the oil droplets that formed during emulsification.[72, 73] To promote nanocapsule formation, the use of aprotic solvents, such as acetone and acetonitrile was recommended. Protic solvents, such as ethanol, n-butanol and isopropanol, were found to induce the formation of nanospheres in addition to nanocapsules.[74] Alternatively, water-containing nanocapsules can be obtained by the interfacial polymerization of monomers in water-in-oil micro-emulsions. In these systems, the polymer formed locally at the water-oil interface and precipitated to produce the nanocapsule shell.[75, 76]

Controlled/living radical polymerization (C/LRP)

The most important factors contributing to this trend of the C/LRP process are increased environmental concern and a sharp growth of pharmaceutical and medical applications for hydrophilic polymers. These factors have given rise to “green chemistry” and created a demand for environmentally and chemically benign solvents such as water and supercritical carbon dioxide. Industrial radical polymerization is widely performed in aqueous dispersed systems and specifically in emulsion polymerization. The primary goal was to control the characteristics of the polymer in terms of molar mass, molar mass distribution, architecture and function. Implementation of C/LRP in the industrially important aqueous dispersed systems, resulting in the formation of polymeric nanoparticles with precise particle size and size distribution control, is crucial for future commercial success of C/LRP.[77] Among the available controlled/living radical polymerization methods successful and extensively studied

methods are 1) nitroxide-mediated polymerization (NMP)[78-81], 2) atom transfer radical polymerization (ATRP)[82-86] and 3) reversible addition and fragmentation transfer chain polymerization (RAFT).[87-91]

Ionic gelation or coacervation of hydrophilic polymers

Polymeric nanoparticles are prepared by using biodegradable hydrophilic polymers such as chitosan, gelatin and sodium alginate. 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 poly anion 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.[92]

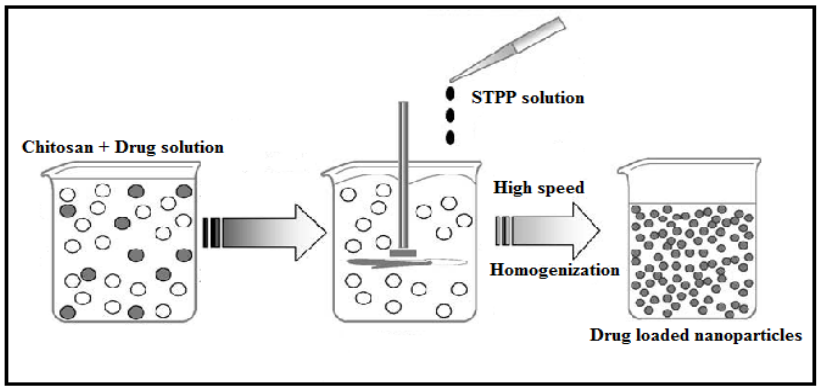

Figure 9: Schematic representation of ionic gelation method

Characterization

Characterization of nanoparticles is based on the size, morphology and surface charge, using such advanced microscopic techniques as atomic force microscopy (AFM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Properties such as the size distribution, average particle diameter, charge affect the physical stability and the in vivo distribution of the nanoparticles.

Properties like surface morphology, size and overall shape are determined by electron microscopy techniques. Features like physical stability and redispersibility of the polymer dispersion as well as their *in vivo* performance are affected by the surface charge of the nanoparticles. Different characterization tools and methods for nanoparticles are mentioned

in Table. Therefore it's very important to evaluate the surface charge during characterization of nanoparticles.[93]

Table 2: Various characterization tools and methods for nanoparticles.

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

Particle Size

Characterizations of nanoparticles are primarily evaluated by the particle size distribution and morphology. With the aid of electron microscopy it's now possible to ascertain the morphology as well as the size of nanoparticles. Application of nanoparticles in drug release and drug targeting can be conveniently determined by various tools. It has already been reported that particle size of nanoparticles has profound effect on the drug release. Smaller the size of nanoparticles larger surface area, which results in to fast drug release. Loaded drug when exposed to the particle surface area causes significant drug release. In contrast, inside the nanoparticles drugs slow diffusion of larger particles occurs. Consequently smaller particles tend to aggregate during storage and transportation of nanoparticle dispersion. Therefore there is a mutual compromise between maximum stability and small size of nanoparticles. In addition degradation of the polymer can also be affected by the particle size e.g. the extent of poly (lactic-co-glycolic acid) degradation was found to increase with increasing particle size *in vitro*. With the advancement in analytical tools various techniques are now available for determining nanoparticle size as discussed below.[93, 94]

Scanning Electron Microscopy (SEM)

This electron microscopy based technique determines the size, shape and surface morphology with direct visualization of the nanoparticles. Therefore scanning electron microscopy offer several advantages in morphological and sizing analysis. However they provide limited information about the size distribution and true population average. During the process of SEM characterization, solution of nanoparticles should be initially converted into a dry powder. This dry powder is then further mounted on a sample holder followed by coating with a conductive metal (e.g. gold) using a sputter coater. Whole sample is then analyzed by scanning with a focused fine beam of electrons. Secondary electrons emitted from the sample surface determine the surface characteristics of the sample. This electron beam can often damage the polymer of the nanoparticles which must be able to withstand vacuum. Average mean size evaluated by SEM is comparable with results obtained by dynamic light scattering. In addition these techniques are time consuming, costly and frequently need complementary information about sizing distribution.[95, 96]

Transmission Electron Microscope

Experimental difficulties in studying nanostructures stem from their small size, which limits the use of traditional techniques for measuring their physical properties. Transmission electron microscopy techniques can provide imaging, diffraction and spectroscopic information, either simultaneously or in a serial manner, of the specimen with an atomic or a sub-nanometer spatial resolution. 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. High-resolution TEM imaging, when combined with nanodiffraction, atomic resolution electron energy-loss spectroscopy and nanometer resolution X-ray energy dispersive spectroscopy techniques, is critical to the fundamental studies of importance to nanoscience and nanotechnology. During the TEM characterization nanoparticles dispersion is deposited onto support grids or films. After dispersion they are fixed using either a negative staining material (phosphotungstic acid or derivatives, uranyl acetate, etc., or by plastic embedding). This is done to make nanoparticles withstand against the instrument vacuum and facilitate handling. Alternatively nanonoparticles sample can also be exposing to liquid nitrogen temperatures after embedding in vitreous ice. When a beam of electrons is transmitted through an ultra-thin sample it interacts with the sample as it passes through[96]

Atomic Force Microscopy

This technique is also known as scanning force microscopy (technique that forms images of surfaces using a probe that scans the specimen), very high resolution type of scanning probe microscopy, with reported resolution on the order of fractions of a nanometre, more than 100 times better than the optical diffraction limit. The atomic force microscopy is based on a physical scanning of samples at sub-micron level using a probe tip of atomic scale and offers ultra-high resolution in particle size measurement. Depending upon properties, samples are usually scanned in contact or noncontact mode. During 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. One of the prime advantage of AFM is its ability to image non-conducting samples without any specific treatment. This feature allows the imaging of delicate biological and polymeric nano and microstructures. Moreover AFM (without any mathematical calculation) provides the most accurate description of size, size distribution and real picture which helps in understanding the effect of various biological conditions.[97, 98, 99]

Surface Charge

Surface charge and intensity determines the interaction of nanoparticles with the biological environment as well as their electrostatic interaction with bioactive compounds. Stability of colloidal material is usually analyzed through zeta potential of nanoparticles. Zeta potential is an indirect measure of the surface charge. It can be obtained by evaluating the potential difference between the outer Helmholtz plane and the surface of shear. Thus zeta potential of colloidal based dispersion assists in directly evaluating its storage stability. Zeta potential values (high zeta potential values, either positive or negative) are achieved in order to ensure stability and avoid aggregation of the particles. Zeta potential values can be utilized in evaluating surface hydrophobicity and the nature of material encapsulated within the nanocapsules or coated onto the surface.[100]

Surface Hydrophobicity

Techniques such as hydrophobic interaction chromatography, biphasic partitioning, adsorption of probes, contact angle measurements etc. can be utilized for the determination of surface hydrophobicity. Recent advancement in research offers several sophisticated analytical tools for surface property analysis of nanoparticles. Modern technique such as X-

ray photon correlation spectroscopy not only determine surface hydrophobicity but also permits the identification of specific chemical groups on the surface of nanoparticles.[101]

Drug Release

It's very essential to determine extent of the drug release and in order to obtain such information most release methods require that the drug and its delivery vehicle be separated. Drug loading capacity of the nanoparticles is defined as the amount of drug bound per mass of polymer or in another term it is the moles of drug per mg polymer or mg drug per mg polymer or it could also be given as percentage relative to the polymer. Various techniques such as UV spectroscopy or high performance liquid chromatography (HPLC) after ultracentrifugation, ultra filtration, gel filtration, or centrifugal ultrafiltration are used to determine this parameter. Methods that are employed for drug release analysis are also similar to drug loading assay which is more often assessed for a period of time to evaluate the drug release mechanism.[102, 103]

Applications of nanoparticulate delivery systems[104, 105]

Tumor targeting using nanoparticulate delivery systems

The rationale of using nanoparticles for tumour targeting is based on

1) Nanoparticles will be able to deliver a concentrate dose of drug in the vicinity of the tumour targets via the enhanced permeability and retention effect or active targeting by ligands on the surface of nanoparticles

2) Nanoparticles will reduce the drug exposure of healthy tissues by limiting drug distribution to target organ.

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.

Reversion of multidrug resistance in tumour cells

Multidrug resistance (MDR) is one of the most serious problems in chemotherapy. MDR occurs mainly due to the over expression of the plasma membrane pglycoprotein (Pgp), which is capable of extruding various positively charged xenobiotics, including some anticancer drugs, out of cells. In order to restore the tumoral cells' sensitivity to anticancer

drugs by circumventing Pgp-mediated MDR, several strategies including the use of colloidal carriers have been applied.

Nanoparticles for oral delivery of peptides and proteins

Significant advances in biotechnology and biochemistry have led to the discovery of a large number of bioactive molecules and vaccines based on peptides and proteins. 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.

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 M- cells display cell-specific 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.

Nanoparticles for gene delivery

Polynucleotide vaccines work by delivering genes encoding relevant antigens to host cells where they are expressed, producing the antigenic protein within the vicinity of professional antigen presenting cells to initiate immune response. Such vaccines produce both humoral and cell-mediated immunity because intracellular production of protein, as opposed to extracellular deposition, stimulates both arms of the immune system. Nanoparticles loaded with plasmid DNA could also serve as an efficient sustained release. Gene delivery system due to their rapid escape from the degradative endo-lysosomal compartment to the cytoplasmic compartment.

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. Strategies for nanoparticle targeting to the brain rely on the presence of and nanoparticle

interaction with specific receptor-mediated transport systems in the BBB. 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.

CONCLUSION

Polymeric nanoparticles are the most effective for prolonged drug delivery system. They may overcome stability issues for certain drug and minimize drug induced side effects. Drug encapsulation and drug release profile of polymeric nanoparticles depends on the type of polymer and its physiochemical properties, particle size and its morphology. The choice of polymer and the ability to modify drug release from polymeric nanoparticles have made them ideal candidates for cancer therapy, delivery of vaccines, contraceptives and delivery of targeted antibiotics. Polymeric nanoparticles can be easily incorporated into other activities related to drug delivery, such as tissue engineering. Importance of polymeric nanoparticles acquiring good market which will grow further in future.

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