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Review Article

DIFFERENT TECHNIQUES FOR PREPARATION OF POLYMERIC NANOPARTICLES- A REVIEW

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ABSTRACT

Polymeric nanoparticles (PNPs) are defined as particulate dispersions or solid particles with size in the range of 10-1000nm. There has been a considerable research interest in the area of drug delivery using particulate delivery systems as carriers for small and large molecules. Particulate systems like nanoparticles have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules. Polymeric nanoparticles have been extensively studied as particulate carriers in the pharmaceutical and medical fields, because they show promise as drug delivery systems as a result of their controlled and sustained release properties, subcellular size, biocompatibility with tissue and cells. Several methods to prepare polymeric nanoparticles have been developed and these techniques are classified according to whether the particle formation involves a polymerization reaction or nanoparticles form directly from a macromolecule or preformed polymer. In this review the different techniques for preparation of polymeric nanoparticles are described.

Keywords: Polymeric nanoparticles, nanospheres, nanocapsules.

INTRODUCTION

The polymeric nanoparticles (PNPs) are prepared from biocompatible and biodegradable polymers in size between 10-1000 nm where the drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. Depending upon the method of preparation nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed [1,2]. 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 [3–11]. PNPs are promising vehicles for drug delivery by easy manipulation to prepare carriers with the objective of delivering the drugs to specific target, such an advantage improves the drug safety[12]. 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[13]. Several methods have been developed during the last two decades for preparation of PNPs, these techniques are classified according to whether the particle formation involves a polymerization reaction or nanoparticles form directly from a macromolecule or preformed polymer or ionic gelation method.

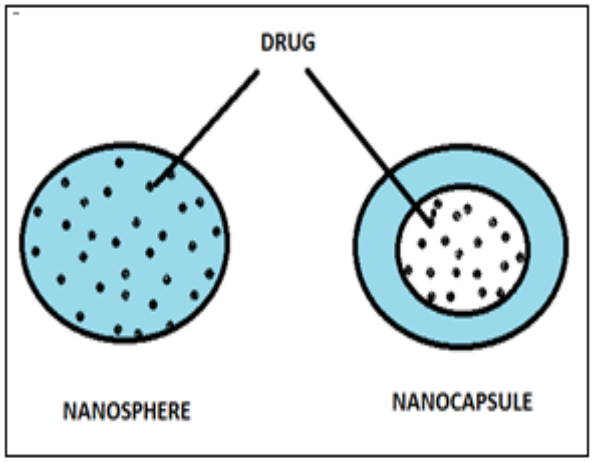


Fig 1:Difference between the nanosphere and nanocapsule

Advantages of polymeric nanoparticles [14, 15]

- 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.

Polymers used in preparation of nanoparticles

The polymers should be compatible with the body in the terms of adaptability (non-toxicity) and (non-antigenicity) and should be biodegradable and biocompatible[16].

Natural polymers: The most commonly used natural polymers in preparation of polymeric nanoparticles are [17-20].

- Chitosan
- Gelatin
- Sodium alginate
- Albumin

There are many synthetic polymers like[12, 16, 21-30]

- Polylactides(PLA)
- Polyglycolides(PGA)
- Poly(lactide co-glycolides) (PLGA)
- Polyanhydrides
- Polyorthoesters
- Polycyanoacrylates
- Polycaprolactone
- Poly glutamic acid
- Poly malic acid
- Poly(N-vinyl pyrrolidone)
- Poly(methyl methacrylate)
- Poly(vinyl alcohol)
- Poly(acrylic acid)
- Poly acrylamide
- Poly(ethylene glycol)
- Poly(methacrylic acid)

Mechanisms of drug release [16]

The polymeric drug carriers deliver the drug at the tissue site by any one of the three general physico-chemical mechanisms.

1.By the swelling of the polymer nanoparticles by hydration followed by release through diffusion.

2.By an enzymatic reaction resulting in rupture or cleavage or degradation of the polymer at site of delivery, there by releasing the drug from the entrapped inner core.

3.Dissociation of the drug from the polymer and its de-adsorption/release from the swelled nanoparticles.

Techniques 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.

Methods for preparation of nanoparticles from dispersion of preformed polymer

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)

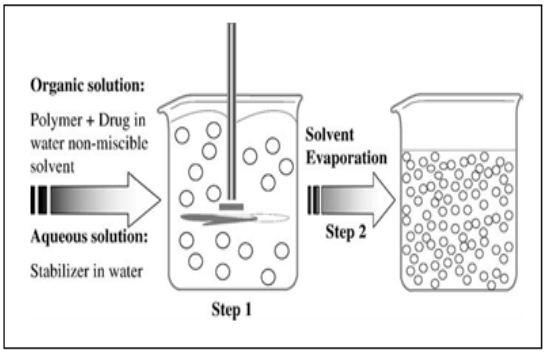
Methods for preparation of nanoparticles from polymerization of monomers

a) Emulsion
b) Mini emulsion
c) Micro emulsion
d) Interfacial polymerization
e) Controlled/Living radical polymerization(C/LRP)

Ionic gelation or coacervation of hydrophilic polymers

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[31]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[31,32]. Lemoine *et al* [33] prepared PLGA nanoparticles of about 200nm by utilizing dichloromethane 1.0% (w/v) as the solvent and PVA or Span 40 as the stabilizing agent. Song *et al.*[34] prepared nanoparticles of PLGA with a typical particle size of 60–200nm by employing dichloromethane and acetone (8:2, v/v) as the solvent system and PVA as the stabilizing agent. 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.

FIG 2:Schematic representation of the solvent-evaporation technique [Ref: Catarina Pinto Reis *et al* [32]].

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[35-38]. 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[39]. 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[40]. 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[39] 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[41]. Although, acetone/dichloromethane (ICH, class 2) are used to dissolve and increase the entrapment of drugs, the dichloromethane increases the mean particle size[42] 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. This method has been applied to various polymeric materials such as PLGA[36], PLA[43], PCL[44], and poly (methyl vinyl ether-comaleic anhydride) (PVM/MA)[45,46]. This technique was well adapted for the incorporation of cyclosporin A, because entrapment efficiencies as high as 98% were obtained[47]. Highly loaded nanoparticulate systems based on amphiphilic h-cyclodextrins to facilitate the parenteral administration of the poorly soluble antifungal drugs Bifonazole and Clotrimazole were prepared according to the solvent displacement method[48].

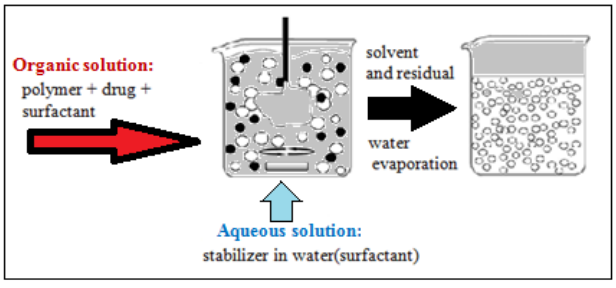


Fig 3:Schematic representation of the nanoprecipitation technique. Surfactant is optional.

Emulsification/solvent diffusion (ESD)

This is a modified version of solvent evaporation method[49]. 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[2,32]. As with some of the other techniques, this one is efficient in encapsulating lipophilic drugs[39]. Several drug-loaded nanoparticles were produced by the ESD technique, including mesotetra(hydroxyphenyl)porphyrin-loaded PLGA (p-THPP) nanoparticles[50,51], doxorubicin-loaded PLGA nanoparticles[52], plasmid DNA-loaded PLA nanoparticles[53], coumarin-loaded PLA nanoparticles [54], indocyanine [55], cyclosporine (Cy-A)-loaded gelatin and cyclosporin (Cy-A)-loaded sodium glycolate nanoparticles [56].

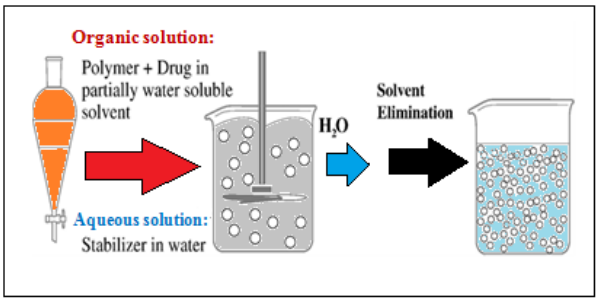


Fig 4: Schematic representation of the emulsification/solvent diffusion technique

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[32]. 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[57]. Salting out does not require an increase of temperature and therefore, may be useful when heat sensitive substances have to be processed[58]. The greatest disadvantages are exclusive application to lipophilic drugs and the extensive nanoparticle washing steps[59].

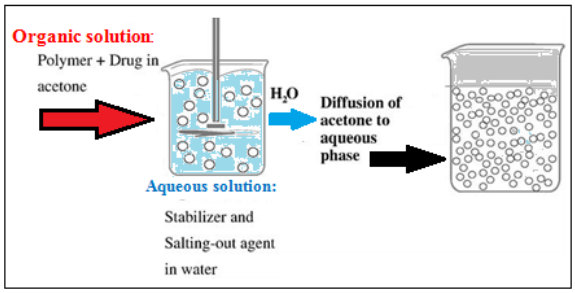


Fig 5:Schematic representation of the salting out technique

Dialysis

Dialysis offers a simple and effective method for the preparation of small, narrow-distributed PN[31,35,60-62]. 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 proposed by the Fessi *et al.*[35] A number of polymer and copolymer nanoparticles[63–72] were obtained by this technique. Poly(benzyl-l-glutamate)-b-poly(ethylene oxide), Poly(lactide)-b-poly(ethylene oxide) nanoparticles were prepared using DMF as the solvent[73,74]. The solvent used in the preparation of the polymer solution affects the morphology and particle size distribution of the nanoparticles. Chronopoulou *et al.*[75] reported a novel osmosis based method (Fig. 6) for the preparation of various natural and synthetic PNP. It is based on the use of a physical barrier, specifically dialysis membrane or common semi permeable membranes that allow the passive transport of solvents to slow down the mixing of the polymer solution with a non solvent; the dialysis membrane contains the solution of the polymer.

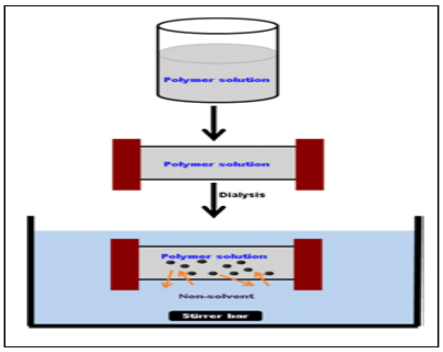


Fig 6:Schematic representation of osmosis based method for preparation of polymer nanoparticles *[31].*

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[31,76,77]. 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 principles have been developed for the production of nanoparticles using supercritical fluids:

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 [78]. A few studies were carried out on the production of PNPs using RESS. Poly (perfluoropolyetherdiamide) droplets produced from the rapid expansion of CO_2 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 CO_2 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 [79-82].

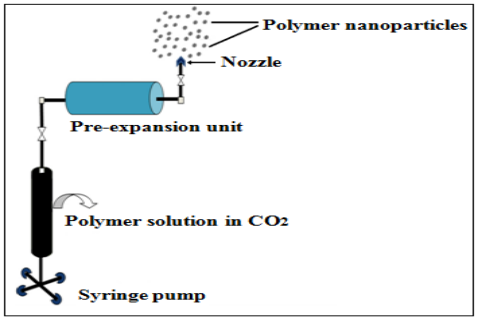


Fig 7:Experimental set-up for preparation of polymer nanoparticles by rapid expansion of supercritical fluid solution [ref: prasad rao. J *et al* [31]].

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[31,83]. Meziani *et al* [84] 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 [84-86].

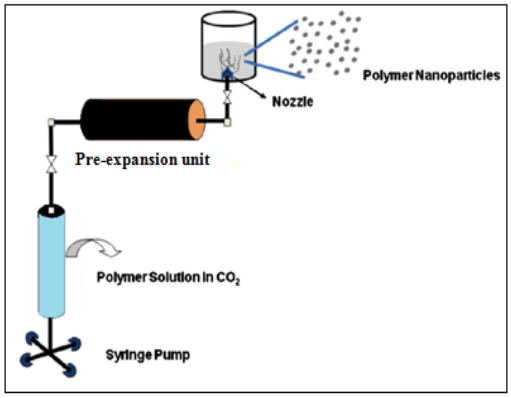


Fig 8:Experimental set-up for the rapid expansion of supercritical fluid solution into liquid solvent process [ref: prasad rao. J *et al* [31]].

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)[32]. Polyacrylamide nanospheres were produced by this method[87,88]. As one of the first methods for production of nanoparticles, surfactants or protective soluble polymers were used to prevent aggregation in the early stages of polymerization. This procedure has become less important, because it requires toxic organic solvents, surfactants, monomers and initiator, which are subsequently eliminated from the formed particles. As a result of the non biodegradable nature of this polymer as well as the difficult procedure, alternative Approaches are of greater interest. Later, poly(methylmethacrylate) (PMMA), poly(ethylcyanoacrylate) (PECA), and poly(butylcyanoacrylate) nanoparticles were produced by dispersion via surfactants into solvents such as cyclohexane (ICH, class 2), n-pentane (ICH, class 3), and toluene (ICH, class 2) as the organic phase . 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 [32,40,89].

II.b) Mini-emulsion polymerization

Publications on the mini-emulsion polymerization and the development of a wide range of useful polymer materials have recently increased substantially. 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[31].The various polymer nanoparticles were prepared by using Mini-emulsion method as discussed in the literature [90-92].

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. Further more, 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 [35,93].

Interfacial polymerization

It is one of the well-established methods used for the preparation of polymer nanoparticles [94-98]. 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[99]. Nanometer-sized hollow polymer particles were synthesized by employing interfacial cross-linking reactions as polyaddition and polycondensation [100-102] or radical polymerization[103,104]. Oil-containing nanocapsules were obtained by the polymerization of monomers at the oil/water interface of a very fine oil-in-water micro- emulsion[105]. 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[40,106.107]. 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 [108]. 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 [109,110].

Controlled/living radical polymerization (C/LRP)

The primary limitations of radical polymerization include the lack of control over the molar mass, the molar mass distribution, the end functionalities and the macromolecular architecture. The limitations are caused by the unavoidable fast radical–radical termination reactions. The recent emergence of many so-called controlled or 'living' radical polymerization (C/LRP) processes has opened a new area using an old polymerization technique [31,111-113]. 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 [114]. Among the available controlled/living radical polymerization methods[115,116] successful and extensively studied methods are 1) nitroxide-mediated polymerization (NMP)[117-121], 2) atom transfer radical polymerization (ATRP)[122-128] and 3) reversible addition and fragmentation transfer chain polymerization (RAFT)[129-131]. The nature and concentration of the control agent, monomer, initiator and emulsion type (apart from temperature) are vital in determining the size of PNPs. Of these, the nature of the control agent is critical in determining the particle size of the final product.

Ionic gelation or coacervation of hydrophilic polymers

Polymeric nanoparticles are prepared by 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 [132,133]. Amir Dustgani *et al* [134] prepared Dexamethasone Sodium Phosphate loaded chitosan nanoparticles by ionic gelation method. 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.

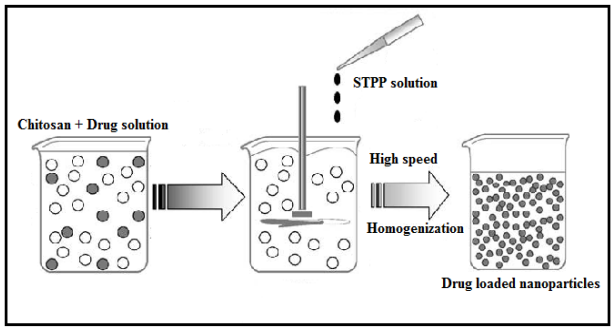


Fig 9:Schematic representation of ionic gelation method

CONCULSION

The main goal of this review was to describe the different preparation techniques available for production of polymeric nanoparticles. It was observed that preparing PNPs is a state-of-art technology that requires a suitable technique among the various possible methods. The drug-loaded nanospheres or nanocapsules now can be produced by simple, safe, and reproducible techniques available. Depending on the physicochemical characteristics of a drug, it is possible to choose the best method of preparation and the polymer to produce nanoparticles with desired size range with good entrapment efficiency of the drug. Nanoparticle preparation methods have been marked by three aspects: 1) need for less toxic reagents 2) simplification of the procedure to allow economic scale-up and 3) optimization to improve yield and entrapment efficiency. The limitations like one particular process or technique is not suitable to all drugs, post preparative steps, such as purification and preservation, incomplete or discontinuous film, inadequate stability of certain active components are remained to solve. Despite these technological challenges, nanoparticles have been showed great promise for the development of drug delivery system.

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