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REVIEW ARTICLE

Synthesis and characterization methods of polymeric nanoparticles

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ABSTRACT

This review provided a detailed overview of the different synthesis and characterization methods of polymeric nanoparticles. Nanoparticles are defined as solid and colloidal particles of macromolecular substances ranging in size under 100 nm. Different types of nanoparticles are used in many biological fields (bio-sensing, biological separation, molecular imaging, anticancer therapy, etc.). The new features and functions provided by nano dimensions are largely different from their bulk forms. High volume/surface ratio, improved resolution and multifunctional capability make these materials gain many new features.

Keywords: Nanoparticle; Polymer; Synthesis Methods; Characterization Methods; Particle Size

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1. Introduction

Nanoparticles are defined as solid and colloidal particles of macromolecular substances ranging in size under 100 nm[1,2]. There are different application fields that led to the exploration of different nano compositions. For instance, they are able to polymerize NPs, biological NPs, lipid-based NPs and metal-based NPs[3-6]. Biocompatible and biodegradable polymers are used for preparation of the polymeric nanoparticles. Polymers are used as biomaterials because of their useful properties such as good biocompatibility, biodegradability, easy preparation and design, various chemical structures and interesting biological imitation character[7].

Most polymeric NPs are biodegradability and biocompatible, and over the accomplished few decades, researches had accepted sample absorption in developing biodegradable NPs as a drug-delivery system[8]. These biodegradable polymeric nanoparticles coated with hydrophilic polymers known as long period of time circulating particles, have been used as potential drug delivery vehicles for their ability to control drug release for a long time. Polymeric nanoparticles have the ability to deliver drugs, proteins, peptides, and antigens and they can be targeted to particular organ. Additionally, they can be used as DNA transporters in gene therapy[9-11].

There are many biodegradable polymers that can be produced from proteins such as milk proteins and gelatin; polysaccharides such as starch, chitosan and sodium alginate; and synthetic polymers such as polymethylmethacrylate, poly (cyanoacrylate) PCA, poly-ε-caprolactone (PCL), poly (lactic acid) (PLA), poly (D, L-glycolic acid) (PGA), and their copolymer of poly (lactide-co-glycolide) PLGA are used in preparing nanoparticular systems[12]. Nanoparticles which ob-

tained by using natural or synthetic polymers have two major advantages for targeting of proteins, peptides and genes, as well as drugs.

The first property is that the nanoparticles have small particle sizes. In this way, they pass more easily than small capillaries, which are easier to enter intracellular and extracellular spaces[13], and release effective active substance in the target region[14,15].

The second property is to use biodegradable materials in the preparation of nanoparticles. Biodegradable materials provide controlled release of active substance in the target tissue for days to further weeks.

In addition, nanoparticles have a good deal of advantages that can be:

1) Nanoparticles have a protective effect against the enzymatic degradation of active substances such as drugs, proteins or peptides in the biological system. They can also increase their stability while reducing side effects[13,14].

2) Beside this, drugs can be encapsulated to the nanoparticle systems without any chemical reaction; this is important for protecting the biological activity of drug[9].

3) Site-specific targeting of nanoparticles can be accomplished by adhering targeting ligands to particles surface or use of magnetic guidance[9].

4) They allow rapid-formulation development. The nanoparticle systems can be used for different routes of administration including oral, parenteral, intra-ocular, nasal etc.[9].

2. Preparation methods of polymeric nanoparticles

Polymeric nanoparticles can be prepared by dispersing performed polymers, using different methods such as solvent evaporation, nanoprecipitation, salting-out, dialysis and supercritical fluid technology.

These methods have similar properties that they involve an organic phase containing the nanoparticle components and a water phase containing stabilizers. The other similarity is the poor encapsulation efficiency of partially water soluble and freely water-soluble drugs (involving proteins and peptides), which escape from the organic phase to the aqueous phase[16].

2.1 Solvent evaporation

The most widely used method to prepare the polymeric nanoparticles for the delivery of active substance is solvent evaporation[17]. This method is used for producing polymeric nanoparticles by biodegradable polymers which have been applied in the drug delivery systems. In this method, the polymer and drug are dissolved in an organic solvent and emulsions are formulated using the aqueous solution of surfactants. In the past, dichloromethane or chloroform were used as the solvent for dissolving the hydrophobic drug. But now, ethyl acetate is used instead of dichloromethane and chloroform because of their toxicological profile[9,17].

Solvent evaporation method has two main strategies that are used for the formation of emulsions. The first one is the preparation of single-emulsions [(oil-in-water, (o/w)], and the second one is dou-ble-emulsions [(water-in-oil-in-water, (w/o/ w)].

In this method, high-speed homogenization or ultra-sonication are used for preparation of emulsion. After that, the organic solvent is evaporated and the nanoparticles are collected by ultracentrifugation. For removing of surfactants, the nanoparticles are washed with distilled water. Nanoparticles can be lyophilized after all steps.

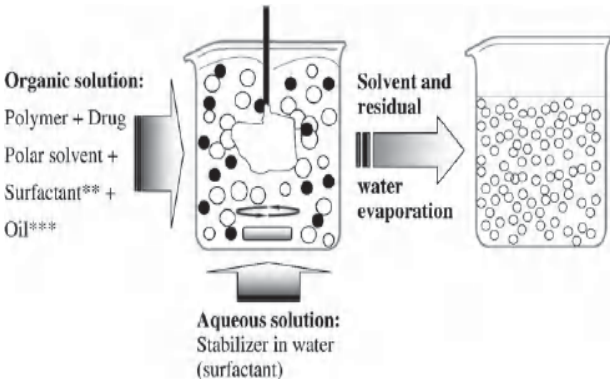

Figure 1. Schematic representation of the solvent-evaporation method[18].

While solvent evaporation method (**Figure 1**) is a simple method for the preparation of polymeric nanoparticles, it is needed to the external energy. The power of external energy, time consuming and pos-sible agglomeration of the nanodroplets, during the evaporation process, may affect the particle size and morphology of nanoparticles[17].

2.2 Salting out

Salting out is another method for preparation of polymeric nanoparticles (**Figure 2**). This method is based on the separation of a water miscible solvent as acetone by using salting out effect. In other words, salting out technique is a modification of the emulsification/solvent diffusion by beginning with an emulsion[19,20]. The organic phase is prepared by dissolving the polymer in a solvent that is totally miscible in water such as acetone, tetrahydrofuran and ethanol. Then, this organic phase is added to the aqueous phase that is constituted by water, the salting-out agent and a stabilizer. The choice of salting out agent that can be electrolytes, such as calcium chloride, magnesium chloride or non-electrolytes, such as sucrose, which is so important for encapsulation efficiency of drug. In the following procedure, an sufficient volume of water is added to the mixture and the resulting nanoparticles are collected via cross-flow filtration[20].

Salting out method has some advantages, of which the most important is reducing stress to protein encapsulants. Another advantage is that it does not lead to an increase of temperature. Therefore, heat sensitive molecules can be processed by this method. On the contrary, this method has some disadvantages that lie in specific application to lipophilic drugs and extensive washing steps[19].

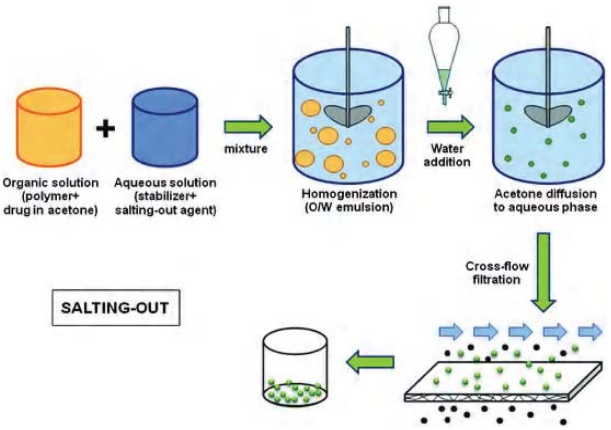

Figure 2. Schematic representation of the salting-out method[20].

2.3 Nanoprecipitation

This method is also called solvent displacement (**Figure 3**), which is a simple, rapid and reproducible method largely used for the preparation of nanocapsules and nanosphere, besides the meaning of solvent displacement[17,21,22].

The basic principle is the interfacial collapse of the substitutional polymer of the splitting solvent which can be miscible with the water in the lipophilic solution. The polymer (synthetic, semi synthetic, or natural), organic solvent of the polymer and the water (non-solvent for the polymer) are three basic components of nanoprecipitation system. Organic solvent should be miscible in water and easy to remove by evaporation. Thus, in the nanoprecipitation method, acetone is the most widely used solvent. Nanoprecipitation occurs when the polymer solution is added to the non-solvent[22].

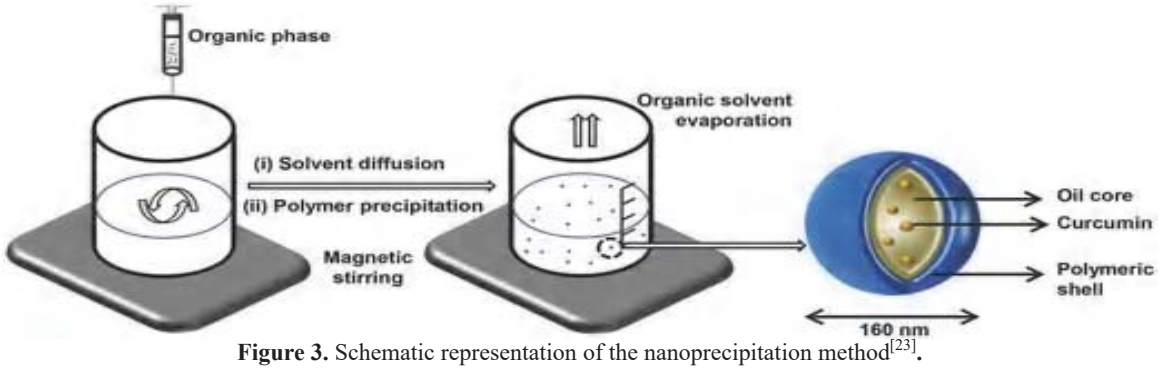

Figure 3. Schematic representation of the nanoprecipitation method[23].

2.4 Dialysis

Dialysis is a preparation method that provides a basic and effective way to prepare small PNPs (**Figure 4**).

Firstly, the polymer is dissolved in the organic solvent and then put inside a dialysis tube while producing the nanoparticle by dialysis. It is an important point that the dialysis tube is suitable for the molecular weight of the nanoparticle. During dialysis, the solvent loses its solubility as a result of displacement. In this way, progressive aggregation of the polymer occurs and a homogeneous suspension

of nanoparticles is obtained[14,17].

2.5 Super critical fluid technology

Supercritical fluids are generally defined as fluids that do not change in phase despite the change of pressure (**Figure 5**). Supercritical CO_2, which is the most widely used supercritical fluid, because it is compatible with the critical state (Tc = 31.1°C, Pc = 73.8 bar), is nontoxic, nonflammable, and inexpensive[15,24].

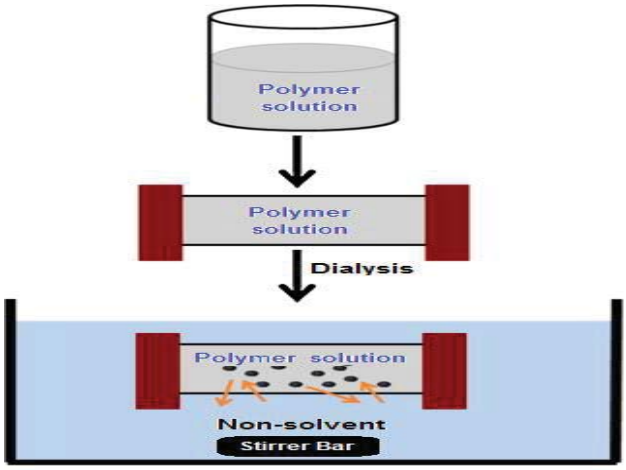

Figure 4. Schematic representation of dialysis method[17].

Supercritical fluid technology is expected to offer an interesting and effective particle production technique, avoiding many of the disadvantages of traditional methods[17]. The greatest advantage of the supercritical fluid technology method used to prepare polymeric nanoparticles is that the precipitated product does not contain solvent[15,24].

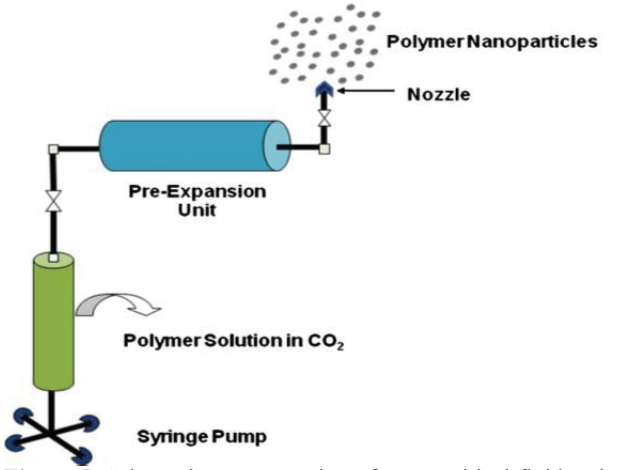

Figure 5. Schematic representation of super critical fluid technology method[17].

The most widely used methods are rapid expansion of supercritical solution (RESS) and supercritical anti-solvent method (SAS). In the RESS method, the drug substance is dissolved in the organic solvent and then released to the supercritical fluid. The organic phase rapidly dissolves in the supercritical solvent and remains nanoparticles that can be filtered back. In the SAS method, the active substance and polymer are dissolved in the supercritical solvent at high pressure[25,26].

2.6 Mini-emulsion

Mini-emulsion polymerization is based on a typical formulation of water, a monomer mixture, surfactant, co-stabilizer and initiator. It is used of a low molecular weight co-stabilizer compound in this method. Mini-emulsions are critically balanced. Also, it requires high-shear stress to achieve a steady state, and interface strain is much bigger than zero[19].

3. Characterization methods of polymeric nanoparticles

Characterization of nanoparticles is based on analysis of particle size, zeta potential, polydispersity index (PDI), morphology, surface area and composition. The most important characteristics of na- noparticles are particle size and size distribution which can influence drug loading, drug release, and stability of nanoparticles (**Figure 6**). The other important characteristic of nanoparticles is zeta potential that is used to characterize the surface charge property[1].

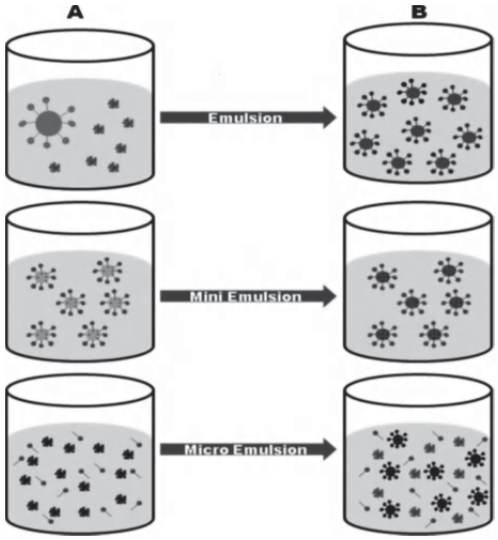

Figure 6. (A) Differences among various hetero-phase polymerization methods before and **(B)** after polymerization[17].

There are some advanced microscopic techni-

ques such as atomic force microscopy (AFM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM), which are used for characterization of nanoparticles. Laser light scattering (LLS), especially dynamic light scattering (DLS) is used for measurement of size and size distribution; Atomic force microscopy (AFM) and scanning electron microscopy (SEM) are used for morphological studies; X-Ray photoelectron spectroscopy (XPS) is used for surface chemistry analysis of nanoparticle suspension[1].

3.1 Transmission electron microscopy (TEM)

Transmission electron microscopy is a useful technique for the investigation shape and size of nanoparticles. In other words, imaging, diffraction and spectroscopic information can be provided by TEM. TEM and SEM give the same kind of information despite different study principles. Sample preparation for the TEM is complicated and time-consuming due to the need to be very thin sample for electron affinity. Nanoparticle sample is precipitated in the films during TEM characterization, and then immobilized with staining material. This process provides withstanding against vacuum. At the point, when an electron bar is transmitted through an ultra-fine, it contacts with the sample and the surface characteristics of the sample are obtained[27].

3.2 Scanning electron microscopy (SEM)

Scanning electron microscopy works on the principle of scanning the surface with high-energy electrons that focus on a very small area. Especially it is based on the principle of creating images by reflecting electrons from the sample. SEM has some advantages in morphological and dimensional analysis, it gives limited information about size distribution and actual population averages[28].

Electrons produced by an electron gun are accelerated by an anode and are made parallel to each other by magnetic lenses. Thus, the electron beam to be used for the measurement is prepared. For this purpose, the electron beam that focused by the objective lens and is picked up by the condenser electromagnetic lens performs the scanning process on the sample surface by the electromagnetic deflector coils[29].

3.3 Atomic force microscopy (AFM)

The AFM creates an image based on the surface forces that occur between the sample surface and the tip attached to an arm. The sharp tip used in this technique is 1-2 microns long and less than 100 Å in diameter. The forces that occur between the surface of material and the tip that are inspected during a probe travel cause to diverge the position of the tip. The surface topography is created by measuring this deviation. Atomic force microscope can be operated with 3 different ways.

The first of these is called the “contact” type. In this method, the sharp tip makes soft physical contact with the surface and the changes that are happening in the position of the arm are recorded. In the “non-contact” type, the arm vibrates in a position close to the sample surface (50-150 Å). In this method, the attractive Van der Waals forces acting and depending on the changes in the surface and the changes occurring in the frequency of the vibration of the arm are measured. The final “tapping” method is similar to the method of contact. This potent technique allows high resolution topographic imaging of sample surfaces[30-32].

3.4 X-Ray diffraction (XRD)

X-ray diffraction (XRD) is an important technique used in crystal structure determination of nanoparticles. In this technique, the types of atoms at lattice point, crystal planes and plane distances can be determined without damaging the sample. Different diffraction patterns occur depending on the structure of the crystal and the wavelength of the light interacting with the crystal. The diffraction occurs as a result of the interference of reflected rays from different layers of the material in the periodic structure. The condition for the occurrence of the diffraction is given by the Law of Bragg[33,34].

3.5 Fourier transform infrared spectroscopy (FT-IR)

The Fourier transform infrared spectroscopy is used for the chemical functional group analysis on the surface of nanoparticles. IR radiation is sent onto the sample in the Fourier transform infrared spectroscopy (FT-IR). While some of this radiation is

absorbed by the sample, some of it also passes and thus, absorption and transmission spectra of the molecules come out. These absorption/transmission spectra are the characteristic spectra of the molecules in the sample and define the absorption/transmission peaks of the material. These peaks correspond to the vibrational frequencies of the bonds between the atoms in the material. The intensity of the peaks gives information about the amount of the material as well as the wavelengths at which the peaks appear in the spectrum to define the bonds between the atoms. For this reason, FT-IR spectroscopy is a useful method to characterize the material[33,35].

3.6 Dynamic light spectroscopy (DLS)

Dynamic light spectroscopy (DLS) or other known photon correlation spectroscopy is used to determine particle size distribution and particle size characterization. DLS calculates the size of the nanoparticle in a solvent based on the Brownian motion. The hydrodynamic diameter of the diffusion factor in a homogeneous solution is determined by Stokes Einstein equation. DLS measurement is suitable for monodisperse and polydisperse materials, but DLS measurement is not suitable for high-dimensional samples. In addition, large size samples are dispersed in a solvent for measurement. DLS has the most common and fairly simple measuring technique thanks to its large measuring range[29]. The main advantage of the DLS technique is that measurements can be made in a short time and the cost of the apparatus is low[36].

3.7 Electrophoretic light scattering

Electrophoretic light scattering (ELS), is by far the best methods for zeta potential determination of suspended particles, due to its sensitivity, accuracy, and versatility[37]. Particles contained in aqueous colloidal solutions carry electrical charge. Zeta potential is varying depending on the nature of the material and the environment e.g., pH, ionic strength, and even the type of ions in the particle suspension. The most important mechanisms of surface charge are ionization of surface groups, dissolution and adsorption of charged species.

Zeta potential measurements are based on the "electrophoresis" technique. Accordingly, when an electric field is applied to the liquid phase, the charged particles suspended in the liquid move toward the charged electrode opposite to their own charge. The viscous forces that acting on the particle try to prevent this movement. When the two opposing forces are balanced, the particles move at a constant speed. This speed depends on the following factors:

1) The power of the electric field;
2) The dielectric constant of the medium;
3) Viscosity of the medium;
4) Zeta potential;

The value of the zeta potential also provides information about the stability of the colloidal solution. The stability of colloidal particles varies with the type and rate of interaction between the particles. If all the particles in the suspension have a large negative or positive zeta potential, they push each other, agglomeration or precipitation will not occur. When the zeta potential is low, there is not enough force to keep the particles away from each other and agglomeration occurs. This value is accepted as +/- 30mV[30, 38].

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