Review Article

A Brief Review on Polymeric Nanoparticles for Drug Delivery and Targeting

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ABSTRACT

For the past few decades, there has been a considerable research interest in the area of drug delivery using particulate delivery systems as carriers for small and large molecules. Various polymers have been used in the formulation of nanoparticles for drug delivery research to increase therapeutic benefit, while minimizing side effects. Polymeric nanoparticles with a size in the nanometer range protect drugs against \textit{in vitro} and \textit{in vivo} degradation; it releases the drug in a controlled manner and also offers the possibility of drug targeting. The use of polymeric drug nanoparticles is a universal approach to increase the therapeutic performance of poorly soluble drugs in any route of administration. Here, we review various aspects of nanoparticles formulation, characterization, effect of their characteristics and their applications in delivery of drug molecules and therapeutic uses.

\textbf{Key words:} Nanoparticles, drug delivery, targeting, drug release

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\textbf{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\textsuperscript{1, 2}. Colloidal drug delivery systems offer a number of advantages over conventional dosage forms. Due to their small particle size, colloidal preparations lend themselves to parenteral preparations and may be useful as sustain release injections for the delivery to a specific organ or target site\textsuperscript{3}. The major colloidal drug delivery systems include liposome and nanoparticles\textsuperscript{4}.

The nano delivery systems mainly include nanoemulsions, lipid or polymeric nanoparticles, and liposomes. Nanoemulsions are primarily used as vehicles of lipophilic drugs following intravenous administration. On the other hand, the ultimate objective of the other nanodelivery systems is to alter the normal biofate of potent drug molecules in the body following their intravenous administration to markedly improve their efficacy and reduce their potential intrinsic severe adverse effects\textsuperscript{5}.

Ongoing efforts are being made to develop polymeric nanocarriers capable of rendering energetic molecules particularly to the intentional end organ. This approach involves modifying the pharmacokinetic profile of several beneficial module of drugs during their inclusion into nanodelivery systems. These respective liberation systems permit an successful
Drug targeting offers enormous advantages but is highly challenging and extremely complicated. Increased knowledge on the cellular internalization mechanisms of the nanocarriers is crucial for improving their efficacy, site-specific delivery, and intracellular targeting. Optimal pharmacological responses require both spatial placement of the drug molecules and temporal control at the site of action. Many hurdles still need to be overcome through intensive efforts and concentrated interdisciplinary scientific collaborations to reach the desired goals. There are various techniques to prepare drug-loaded nanoparticles, the selection of which depends on the physicochemical properties of the bioactive molecule and the polymer. The nanoparticle drug delivery field is complex and requires considerable interdisciplinary knowledge.

Pharmaceutical nanoparticles are submicron-sized, colloidal vehicles that carry drugs to the target or release drugs in a controlled way in the body. After preparation, nanoparticles are usually dispersed in liquid. Such a system can be administered to humans for example by injection, by the oral route, or used in ointments and oculair products. Alternatively, nanoparticles can be dried to a powder, which allows pulmonary delivery or further processing to tablets or capsules. Nanoparticles in pharmaceutical applications have gained plenty of research attention during recent decades. Although the research concerning formulation of nanoparticles into drug delivery devices has been extensive, only a few polymeric nanoparticulate products have reached the market. Among the drugs used in nanoparticle formulations, particularly cancer therapeutics is widely studied because the formulation might reduce toxicity of the drug while improving efficacy of the treatment. In addition to drug molecules, other candidates to be encapsulated in or coupled with nanoparticles include macromolecules like proteins, peptides and genes (nucleic acids). These kinds of molecules tend to be inactivated in the body by enzymatic degradation. In terms of controlled release, nanoparticles provide protection against the body conditions resulting in sustained release and maintenance of bioactivity before the drug reaches the target.

After intravenous administration, nanosized particles are mainly taken up by the macrophages of the mononuclear phagocyte system (MPS) and, thus, can be localized in the liver, spleen and lungs. By modifying particle surface, e.g., by coating, defense mechanisms of the body can be avoided to some extent leading to longer circulation times of nanoparticles in the blood.

History
Nanotechnology is an emerging and dynamic field. It is multidisciplinary in nature. Several ancient practices have been developing nanoparticles through the traditional processes but they were not identified as nanosystems/nanoparticles. Ayurveda, the ancient traditional system of medicine in India has described several “Bhasmas” which have particles with sizes in nano range and have been used traditionally. Nanotechnology has immense applications in almost all the fields of science and human life. As generally acknowledged, the modern nanotechnology originated in 1959. However, the actual term “nanotechnology” was not coined until 1974 by Norio Taniguchi from Japan. First polymer nanoparticles for pharmaceutical application were prepared in the late 1960’s and early 1970’s. Since the last two decades, studies have particularly focused on the stealth-type carriers which are undetectable by mononuclear phagocytes system (MPS). These stealth nanoparticles have shown a prolonged half-life in the blood. Such long-circulating nanoparticles are supposed to be able to directly target tumors located outside the MPS regions.

Definition
Nanoparticles are colloidal polymeric particles of size below 1µm with a therapeutic agent either dispersed in polymeric matrix or encapsulated in polymer. 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 seen in Fig: 1.

![Figure 1: Structure of the polymeric nanospheres and nanocapsules](image)

Depending on the type of material or carrier used, four broad classes of nanoparticles are recognized as:

- Polymeric nanoparticles
- Lipid based nanoparticles
- Metal based nanoparticles and
- Biological nanoparticles.

Advantages
Nanoparticles have received considerable attention over the past 20 years due to their advantages compared to other drug delivery systems.
These advantages include⁴,¹⁰:
- Targeted delivery of drugs to the specific site to minimize toxicity.
- Improved bioavailability by reducing fluctuations in therapeutic ranges.
- Improved stability of drugs against enzymatic degradation.
- Sustained and controlled release effect that reduces dosing frequency with improved patient compliance.
- The ease of administering through various routes including oral, nasal, pulmonary, intraocular, parenteral and transdermal.
- The small particle size also reduces potential irritant reactions at the injection site.

Advantages over Microparticles⁴
- They have higher intracellular uptake compared to micro particles.
- They are better suited for I.V. delivery since the smallest blood capillaries in the body is about 5-6 μm.

Advantages over Liposomes⁴
- They have better stability in biological fluids and during storage.
- Their preparation is more amenable to scale-up.
- They have the unique ability to create a controlled release product.

Limitations of Nanoparticles¹⁰
- As nanoparticles contain superior plane region as equate to their quantity, resistance and clustering of the nanoparticles into a bigger arrangement is expected, which may change their role as a drug deliverance scheme.
- In addition, small particle bulk and huge exterior vicinity willingly effect in partial drug load and rupture let loose.
- Once the nanoparticles entered into the human body, they must be forbidden by an peripheral direct, forestall them from causing adverse effects.

Materials used for preparation of nanoparticles
Nanoparticles can be made as a mixture of resources such as metals (silver, gold, platinum, silicon), as well as polymers and lipids. Researchers have developed virus based nanoparticles for tissue-specific targeting and imaging agents in vivo⁴. Biodegradable polymers are advantageous in many ways over other materials for use in drug delivery systems such as nanoparticles. By selecting the appropriate polymer type, molecular weight, and copolymer blend ratio, the degradation/erosion rate of the nanoparticles can be controlled to achieve the desired type and rate of release of the encapsulated drug⁷. Polymeric materials can be classified broadly as natural polymers and synthetic polymers. The selection of materials for preparing nanoparticles depends upon consideration of the following factors⁴,¹⁰:
- Size and surface characteristics of the particle desired.
- Aqueous solubility and stability of drugs or active ingredients.
- Antigenicity of the polymer.

A broad range of synthetic and natural polymers available for nanoparticle formation, but their biocompatibility and biodegradability are the major limiting factors for their use in the drug delivery area. Synthetic polymers, on the other hand, offer better reproducibility of the chemical characteristics of the synthesized nanoparticles as compared to the natural polymers¹².

Most widely used materials for preparing nanoparticles in drug delivery were given in the table: 1.

<table>
<thead>
<tr>
<th>Material</th>
<th>Full name</th>
<th>Abbreviation or commercial names</th>
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<tbody>
<tr>
<td>Synthetic homopolymers</td>
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<tr>
<td>Poly lactide</td>
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<td>Poly lactide-co-glycolide</td>
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<td>Poly(epsilon-caprolactone)</td>
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<td>Poly(isobutylcyanoacrylate)</td>
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<td>Poly(isohexylecyanoacrylate)</td>
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<td>Poly(n-butylicyanoacrylate)</td>
<td>PBBC</td>
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<td>Poly(acrylate) and poly(methacrylate)</td>
<td>Eudragit</td>
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<td>Natural polymers</td>
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<td>Chitosan</td>
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<td>Alginate</td>
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<td>Gelatin</td>
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<td>Albumin</td>
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<td>Copolymers</td>
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<td>Poly(lactide)-poly(ethyleneglycol)</td>
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<td>Poly(lactide-co-glycolide)poly(ethyleneglycol)</td>
<td>PLGA-PEG</td>
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<tr>
<td>Poly(epsilon-caprolactone)poly(ethyleneglycol)</td>
<td>PCL-PEG</td>
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<tr>
<td>Poly(hexadecylcyanoacrylate-co-poly(ethylene glycol)cyanoacrylate)</td>
<td>Poly(HDCA-PEGCA)</td>
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<td>Colloid stabilizers</td>
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<td>Dextran</td>
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<td>Pluronic®68</td>
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<td>Poly(vinyl alcohol)</td>
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<td>Tween20 and Tween80</td>
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Table 1: Most widely used polymers for preparing nanoparticles in drug delivery⁴.

Biodegradable Polymers Used In The Fabrication of Nanoparticles
Biodegradable polymers are advantageous in many ways over other materials used in drug delivery systems such as nanoparticles⁴. By selecting the appropriate polymer type, molecular weight, and copolymer blend ratio, the degradation/erosion rate of the nanoparticles can be controlled to achieve the desired type and rate of release of the encapsulated drug⁷. The common biodegradable polymers used in drug delivery include⁴,⁵.
• Polyesters, such as lactide and glycolide copolymers, polycaprolactones, poly(-hydroxybutyrates),
• Polyamides, which includes natural polymers such as collagen, gelatin, and albumin, and semisynthetic pseudo-poly(amino acids) such as poly(N-palmitoyl hydroxyproline ester),
• Polyurethanes,
• Polyphosphazenes,
• Polyorthoesters,
• Polyanhydrides and Poly (alkyl cyanoacrylates).

Degradation and biocompatibility of biodegradable polymers

Natural polymers

Various polymers have been used in the formulation of nanoparticles for drug delivery research to increase therapeutic benefit, while minimizing side effects. Natural polymers have been classified into polysaccharides and proteins. Proteins are gelatin; albumin, lecithin, legumin and vicillin. Polysaccharides are alginate, dextran, chitosan and pollulan.

Chitosan

Chitosan is a modified natural carbohydrate polymer prepared by the partial N-deacetylation of chitin, a natural biopolymer derived from crustacean shells such as crabs, shrimps and lobsters. Chitosan is also found in some microorganisms, yeast and fungi. After cellulose chitin is the second most abundant polysaccharide in nature. It is physically protected, non poisonous, biocatalyst and eco-friendly polysaccharide. The primary unit in the chitin polymer is 2-deoxy-2- (acetylamino) glucose\textsuperscript{13,14}. These units combined by 1,4-glycosidic linkages, forming a long chain linear polymer. Although chitin is insoluble in most solvents, chitosan is soluble in most organic acidic solutions at pH less than 6.5 including formic, acetic, tartaric, and citric acid\textsuperscript{15}.

Gelatin

Gelatin is one of the protein materials that can be used for the production of nanoparticles. It is obtained by controlled hydrolysis of the fibrous, insoluble protein, collagen, which is widely found as the major component of skin, bones and connective tissue. The interest was based on the facts that gelatin is biodegradable, non-toxic, easy to crosslink and to modify chemically and has therefore an immense potential to be used for the preparation of colloidal drug delivery systems such as microspheres and nanoparticles. These properties, combined with the high potential of nano-sized delivery systems make gelatin-based nanoparticles a promising carrier system for drug delivery\textsuperscript{13, 14}.

Albumin

Albumin is a good-looking macromolecular shipper and extensively use to arrange nanospheres and nanocapsules, due to its availability in pure form and its biodegradability, nontoxicity and nonimmunogenicity. on the other hand, albumin nanoparticles are biodegradable, easy to prepare in defined sizes, and carry reactive groups (thiol, amino, and carboxylic groups) on their surfaces that can be used for ligand binding and/or other surface modifications and also albumin nanoparticles offer the advantage that ligands can easily be attached by covalent linkage. A number of studies have shown that albumin compiles in hard tumors creation it a budding macromolecular transporter for the site-directed relief of antitumor drugs\textsuperscript{13, 17}.

Alginates

Alginate, a naturally occurring copolymer of glucuronic acid and manuronic acid, is widely used for pharmaceutical applications. Specifically, the simple aqueous-based gel formation of sodium alginate in the presence of divalent cations such as Ca\textsuperscript{2+} has been used for drug delivery\textsuperscript{13,14}. Alginate is anionic polysaccharide that it has been widely used in drug delivery. Alginate (a natural polymer) based nanoparticulate delivery system was developed for frontline ATDs (Rifampicin, Isoniazid, Pyrazinamide and Ethambutol). High drug encapsulation efficiency was achieved in alginate nanoparticles, ranging from 70%-90%. Its merits for making particles of less than 100 nm for gene delivery\textsuperscript{17}.

Synthetic polymers

Synthetic polymers, offer better reproducibility of the chemical characteristics of the synthesized nanoparticles as compared to the natural polymers. Common classes of polymers used to encapsulate drugs in colloidal systems include polyamides, poly (amino acids), polyesters, polyorthoesters and polyanhydrides.

Lactide and Glycolide copolymers

One of the most popular biodegradable polymers used in drug delivery are aliphatic polyester copolymers based on lactic and glycolic acids. Poly (d,l-lactic-glycolic acid) (PLGA) is used for the manufacture of implants and internal sutures and is known to be biocompatible, degrading to produce the natural products lactic acid and glycolic acid\textsuperscript{1,5}.

Bio degradation

PLGA nanoparticles undergo homogenous hydrolytic degradation, which is modulated by various factors such as chemical composition, porosity, hydrophilicity/hydrophobicity, morphology (crystalline/ amorphous), and molecular weight and molecular weight distribution. Owing to the presence of methyl groups in the lactide polymers, they are more hydrophobic, than the glycolide polymers\textsuperscript{2}. The half-life of these linear polyesters can be increased by
cobbling with more hydrophobic comonomers such as polycaprolactone\(^5\).

**Biocompatibility**

The evaluation of the biocompatibility of biodegradable polymers takes into consideration the incidence of the inflammatory and healing responses of the injected and implanted materials\(^4\).

**Poly (\(\varepsilon\)-Caprolactones)**

**Biodegradation**

PCL is a water-permeable polymer with hydrophobic and high crystalline properties. It undergoes bulk erosion by random hydrolytic chain cleavage in the first phase, resulting in a decrease in the molecular weight of the polymer. This is followed by the second phase; in which these low molecular weight fragments undergo phagocytosis or solubilization in the body fluids\(^6,11\).

**Polyanhydrides**

**Biodegradation**

These hydrophobic and crystalline materials have been shown to undergo erosion by surface hydrolysis, minimizing water diffusion into the bulk of the delivery device. The monomeric anhydride bonds have extreme reactivity toward water and undergo hydrolysis to generate the dicarboxylic acids. Although hydrolysis is catalyzed by both acid and base, an increase in pH enhances the rate of hydrolytic degradation. At low pH, oligomeric products formed at the surface of the matrix have poor solubility; this hinders the degradation of the core\(^1\).

**Production of polymeric nanoparticles**

Biodegradable nanoparticles for pharmaceutical use are prepared from a variety of synthetic and natural polymers\(^4\). Synthetic polymers such as polyacrylates, polycaprolactones, polylactides and its copolymers with polyglycolides are widely used and discussed. Several methods exist for the preparation of nanoparticles. When synthetic polymers are used, they are typically dissolved in a convenient solvent followed by precipitation in a liquid environment leading to nanoparticle formation. The drug intended to be encapsulated in the particles is usually incorporated in the process during the polymer solvation and precipitation\(^1\).

The two main procedures can be followed to form polymeric nanoparticles, namely top-down and bottom-up techniques. The top-down methods use size reduction to obtain controlled-size nanoparticles. This size reduction is based on the application of strong shear stress by wave sound emission (sonication), high pressure (microfluidization), and high speed agitation (homogenization)\(^12\). The ultimate physicochemical properties of the particles are critically influenced by the conditions of manufacturing\(^18\). The bottom-up methods start from individual molecules to form nanoparticles, by polymerization. The polymerization methods commonly used are emulsion polymerization (water-in-oil, oil-in-water, and polymerization in bicontinuous structures), dispersion polymerization, and interfacial polymerization. Monomers, initiators, additives, and solvent are the basic chemical components used in the polymerization methods\(^19\).

The main drawbacks of the bottom-up methods are the presence of residual sub-products in the final nanoparticles that can impart toxicity to the nanoparticles, the difficulty in the prediction of polymer molecular weight, affecting the biodistribution and release behavior of the drug from the nanoparticle; and the possibility for drug inhibitions due to interactions, or cross reactions of the drug with activated monomers and H\(^+\) ions present during polymerization. To overcome these limitations, top-down methods were developed using natural and synthetic polymers. The emulsion evaporation, salting out, nanoprecipitation, and emulsion diffusion are the main top-down methods used to form polymeric nanoparticles\(^13\).

**Laboratory-scale production of nanoparticles**

Nanoparticles have been prepared most frequently by three methods\(^10\):

- Dispersion Of Preformed Polymers
- Polymerization Of Monomers And
- Ionic Gelation or Coacervation of Hydrophilic Polymers.

**Dispersion of preformed polymers**

Dispersion of 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). Each synthesis method has advantages and disadvantages. This technique can be used in various ways as described below\(^10\).

**Emulsification–solvent diffusion**

The emulsification solvent diffusion is the widely used method for preparing nanoparticles. The drug and polymer usually PLA, PLGA, PCL or Eudragit are dissolved in a partially water soluble solvent. Commonly used solvents are propylene carbonate, benzyl alcohol, ethyl acetate, isopropyl acetate, methyl acetate, methyl ethyl ketone, butyl lactate or isovaleric acid. The organic phase is saturated with water and is then diluted with an extensive amount of pure water to facilitate diffusion of the organic solvent from the organic phase droplets leading to the precipitation of the polymer as presented in Figure: 2. The aqueous phase may contain surfactants such as Pluronic, PVA and sodium taurocholate while the organic phase sometimes contains soy lecithin as the emulsifier. Finally, the solvent is eliminated by evaporation.
Advantages
- The use of non highly toxic solvents (i.e. benzyl alcohol).
- Reduced energy consumption because it only requires mild stirring. The process does not require high stress shear (i.e. sonication or microfluidization).
- Suitable for hydrophobic active components.

Disadvantages
- The requirement of large amounts of water for nanoparticles formation, large time of emulsion agitation.
- The size is highly sensitive to polymer concentration if the process does not use shear stress for size reduction (high speed agitation or sonication).

Figure 2: Schematic representation of the emulsification-solvent diffusion method

Nanoprecipitation (Solvent diffusion/interfacial deposition method)
One of the easiest and reproducible techniques for preparing nanospheres was the solvent displacement (also called nanoprecipitation) method developed by Fessi et al and has been widely used to prepare nanoparticles. The method is based on the precipitation of preformed polymer following displacement of a semipolar solvent miscible with water in the presence or absence of surfactant. Thus, the process is often called solvent displacement or interfacial deposition. Three basic ingredients are needed for this method: polymer, polymer solvent and non-solvent for the polymer. In brief, both the polymer and drug are dissolved in a water miscible organic solvent (polymer solvent phase) of intermediate polarity (e.g. acetone and ethanol). The resulting organic phase is injected into a stirred aqueous phase (non-solvent phase) containing a surfactant as stabilizer. The nanoparticles are formed instantaneously during the rapid diffusion of the organic phase into the aqueous phase as shown in Fig: 3, typically, this method is used for hydrophobic drug entrapment, but it has been adapted for hydrophilic drugs as well. Finally, the solvent is removed under reduced pressure.

Advantages
- The use of non highly toxic solvents (i.e. acetone).
- Reduced energy consumption because it only requires regular stirring.

Disadvantages
- The main drawback is the requirement of drugs that are highly soluble in polar solvents (Acetone, ethyl acetate), but they should be slightly soluble in water to minimize losses during solvent diffusion.
- Nanoparticle size is very much affected by the polymer concentration; higher nanoparticle sizes are obtained at higher polymer concentrations.

Emulsion evaporation/extraction method
Emulsion evaporation is the oldest method used to form polymeric nanoparticles from preformed polymers. The method is based on the emulsification of an organic solution of the polymer in an aqueous phase followed by the evaporation of the organic solvent. The polymer is dissolved in a suitable solvent (e.g., ethyl acetate, chloroform, methylene chloride). The organic phase or aqueous phase is poured into the continuous phase (aqueous or organic phase) in which a surfactant is dissolved to impart stability to the emulsion. Emulsification is carried out under high-shear stress to reduce the size of the emulsion droplet (directly related with the final size of the nanoparticles). The process of emulsification is followed by evaporation of the organic solvent under vacuum, which leads to polymer precipitation and nanoparticle formation. The o/w emulsion is used for entrapment of hydrophobic compounds, whereas w/o/w double emulsion is used for the entrapment of hydrophilic compounds.

Advantages
- The use of non highly toxic solvents (i.e. ethyl acetate).
- Additives can be used for nanoparticle size reduction.
- Suitable for hydrophilic (double emulsions) and hydrophobic active components.

Disadvantages
- High consumption of energy by the necessity of high stress shear (i.e. sonication or microfluidization).
The solvent is removed by evaporation (energy consumption), but the process time for solvent removal is reduced (special with fast evaporation with vacuum).

**Oil in water emulsion method (single emulsion)**

The method is based on the emulsification of an organic solution which contains the polymer and the active component in an aqueous phase, followed by the evaporation of the organic solvent. Different surfactants such as PVA, SDS, Pluronic F68 can be dissolved in the aqueous phase. The size reduction of the emulsion droplet is done by sonication or microfluidization for miniemulsion formation. The evaporation step is required to eliminate the organic solvent present in the organic phase. This leads to the precipitation of the polymer as nanoparticles with a diameter in the nanometers range.

**Double emulsion (w/o/w) method**

The first step of the double emulsion method is the formation of a water in oil (w/o) emulsion where the aqueous solution contains the hydrophilic active component and the organic phase contains PLGA and a suitable surfactant (Span 80, pluronic F- 68, and others) with a low HLB. The miniemulsion is formed under strong shear stress. Next, the water in oil in water (w/o/w) emulsion formation is sonicated for droplet size reduction. This second size reduction should be controlled to minimize the hydrophilic active component diffusion to the external aqueous phase. Evaporation, the final step, is used to remove the organic solvent. Evaporation is done under vacuum to avoid polymer and active component damage, and to promote final nanoparticle size reduction.

**Salting out method**

Bindschaedler and co workers patented this technique in 1988. The salting-out procedure can be considered as a modification of the emulsification/solvent diffusion method. Contrary to the emulsion diffusion method, there is no diffusion of the solvent due to the presence of salts. The fast addition of pure water, to the o/w emulsion, under mild stirring, reduces the ionic strength and leads to the migration of the water-soluble organic solvent to the aqueous phase inducing nanosphere formation.

The separation of a water miscible solvent from aqueous solution is achieved via a salting-out effect.

**Polymerization method**

In this method, monomers are polymerized to form nanoparticles in an aqueous solution. Drug is incorporated either by being dissolved in the polymerization medium or by adsorption onto the nanoparticles after polymerization completed. The nanoparticle suspension is then purified to remove stabilizers and surfactants employed for polymerization by ultracentrifugation and re-suspending the particles in an isotonic surfactant-free medium.

The polymerization method can be classified into Emulsion polymerization

**Emulsion polymerization**

This method is the fastest and scalable method of producing nanoparticles. It can be classified into two categories, depending on the use of the continuous phase; include

- continuous organic phase or
- continuous aqueous phase methodology

In general, the monomer is dissolved into an organic or aqueous continuous phase. Additional monomer molecules are then emulsified into the emulsion droplets that are stabilized by surfactant. In the continuous phase, chain growth starts when the initiated monomer ion or monomer radical collide with each other and forms aggregates which are stabilized by polymeric emulsifier particles. This mechanism is known as anionic polymerization. A schematic diagram for preparation of Poly (alkyl-cyanoacrylate) nanoparticles by anionic polymerization is presented in Fig: 6.

**Interfacial polymerization**

This method is generally used to prepare nanocapsules using oily components such as benzyl benzoate or migliol along with an organic solvent. In this case, polymerization occurs at the interface between the oily and aqueous phase to produce nanocapsules spontaneously. The nanocapsules are stabilized with the help of surfactant added in the aqueous phase. The technique is advantageous from the standpoint of producing nanocapsules with high drug entrapment efficiency with hydrophilic insulin.
Coacervation or phase separation method
Much research has been focused on the preparation of nanoparticles using biodegradable hydrophilic polymers such as chitosan, gelatin and sodium alginate. Calvo and co-workers developed a method for preparing hydrophilic chitosan nanoparticles by ionic gelation. The method involves a mixture of two aqueous phases, of which one is the polymer chitosan, a di-block co-polymer ethylene oxide or propylene oxide (PEO-PPO) and the other is a polyanion sodium tripolyphosphate. In this method, positively charged amino group of chitosan interacts with negative charged triplyphosphate 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. This method includes
Phase separation in aqueous system
This technique depends on the precipitaiton of the drug-entrapping polymer either by the addition of a third compound to the polymer solution or by some other physical means. Briefly, two steps are involved in the process: (i) the formation of liquid droplets of the polymer from the complete solution phase, which depends on the solubility parameters of the polymer, and (ii) subsequent hardening of the polymer droplets due to extraction or evaporation of the polymer solvent. A number of organic solvents, such as dichloromethane, isopropanol, and heptanes, have been used as solvent, coacervating agent, and hardening agent. Both hydrophilic and hydrophobic drugs can be entrapped by this principle.
Phase separation in nonaqueous system
Unlike the single o/w and double w/o/w emulsion techniques, this process can be used to encapsulate both hydrophilic and lipophilic drugs. In this method, hydrophilic drugs are solubilized in water and added to an organic solution of the polymer (w/o emulsion), whereas lipophilic drugs can be dissolved/dispersed in the polymer solution. Subsequently, an organic nonsolvent (e.g., silicone oil), which is miscible with the organic solvent (e.g., dichloromethane) but does not dissolve either the drug or the polymer, is added to the emulsion system with stirring; this gradually extracts the organic polymer solvent. With the loss of the solvent, there is a reduction in the polymer solubility, and the coating polymer in the solution undergoes phase separation, with the coacervate phase containing the polymer coacervate droplets. The polymer coacervate adsorbs on to the drug particle surface, resulting in the encapsulation of the drug by the precipitated polymer.
Important modifications of traditional methods
The methods detailed above are the main methods extensively employed in the synthesis of nanoparticles for different purposes. There is a continuous effort to improve the nanoparticle size (size reduction), to reduce the polydispersity index, to better entrap the active components (hydrophilics and hydrophobics), and to reduce the potential toxicity of the different components involved. These efforts stimulated research and discovery of new methods, based on slight modifications of standard methods, and the application of new synthesis steps in the nanoparticles formation. Some of modified methods were named below.
Membrane emulsion evaporation method
Spray dry method for water in oil
Sprayer solvent displacement with dialysis and freeze dryer stabilization
Double emulsion with emulsion diffusion
Dialysis method for modified PLGA
Large-scale pilot production of nanoparticles
Spray drying
Some of the challenges faced by this technique include the production of small-sized nanoparticles and the need for innovative methods to increase the drug-entrainment efficiency. However, when compared with other methods, it provides a relatively rapid and convenient production technique that is easy to scale up, involves mild processing conditions, and has relatively less dependence on the solubility characteristics of the drug and the polymer. In this method, a solution or dispersion (w/o) of a drug in an organic solvent containing the polymer is sprayed from the sonicating nozzle of a spray dryer and subsequently dried to yield nanoparticles.

Figure 6: Schematic for the production of nanoparticles by spray-drying.

Supercritical fluid technology
Conventional methods such as solvent extraction-evaporation, solvent diffusion and organic phase separation methods require the use of organic solvents which are hazardous to the environment as well as to physiological systems. Therefore, the supercritical fluid technology has been investigated as an alternative to prepare biodegradable micro-
nanoparticles because supercritical fluids are environmentally safe. A supercritical fluid can be generally defined as a solvent at a temperature above its critical temperature, at which the fluid remains a single phase regardless of pressure. Supercritical CO$_2$ (SC CO$_2$) is the most widely used.

The most common processing techniques involving supercritical fluids are:

- Supercritical anti-solvent (SAS)
- Gas antisolvent method (GAS)
- Rapid expansion of critical solution (RESS).

**Supercritical antisolvent method**

In the supercritical antisolvent method, both the drug and the polymer are dissolved in a suitable organic solvent and are atomized through a nozzle into supercritical CO$_2$. The dry, micronized powder is then collected following the depressurization of CO$_2$.

**Gas antisolvent method**

In the gas antisolvent method, antisolvent CO$_2$ is introduced into the organic solution containing the solutes of interest. Supercritical CO$_2$ is miscible with the solvent but does not solubilize the solutes. This causes the solvent concentration to be significantly lowered, resulting in the precipitation of the drug inside the polymer matrix. Later, the solid product is flushed with fresh CO$_2$ to strip the residual solvent.

The rate of addition of CO$_2$ to the organic solution affects the final particle size. A major challenge of this process is the need to filter the precipitate from the organic solvent solution without particle growth and aggregation.

**Rapid expansion of supercritical solutions technique**

In the rapid expansion of supercritical solutions technique, the solute is dissolved in supercritical CO$_2$ and this solution is atomized through a nozzle into a collection chamber at atmospheric conditions. When expanded, CO$_2$ immediately evaporates and the solute precipitates as a co-precipitate of the drug embedded in the polymer matrix. The disadvantages of this method include the use of higher temperatures to form homogenous precipitates (thus degrading thermally labile drugs) and the limited solubility of the polymers and drugs that result in low drug loading.

**Characterization of nanoparticles**

**Particle size and surface morphology**

Electromagnetic radiation of shorter wavelengths must be used to observe the structures smaller than 1µm. Electron beams present this possibility. The development of electron microscopes has resulted in instruments that are able to routinely achieve magnifications of the order of 1 million and that can disclose details with a resolution of up to about 0.1 nm.

**Scanning Electron Microscopy (SEM)**

The scanning electron microscope (SEM) is one of the most versatile instruments widely applied to surface microstructure imaging. SEM is a type of electron microscopy that images the sample surface of a solid specimen by using a focused beam of high-energy electrons.

**Instrumentation**

The major components of an SEM include the electron gun, electron lenses, sample stage, detectors, data output devices, and the vacuum system. Fig: 11 show a structure of a conventional SEM.
Application
SEM is routinely used to analyze shapes and surface topography of samples. It is also used to analyze spatial variation in chemical compositions by using elemental maps, and spot chemical analysis. It is also used to identify the microfabric and crystalline orientation of materials. Though there are few limitations associated with SEM such as its applicability only for solid sample which are stable under vacuum, inability to detect very light elements (H, He, Li), and extensive sample preparation for nonconductive materials.

Transmission Electron Microscopy (TEM)\textsuperscript{4,5}
Transmission Electron Microscope (TEM) is a type of microscopy technique which operates on the same basic principle as the light microscope except TEM uses a beam of electrons, instead of light. The image is formed by the interaction of the sample specimen when electron beams are transmitted through it. Due to the small de Broglie wavelength of electrons, it is possible to get significantly higher resolution down to 0.1 nm in TEM over light microscopy.

![Schematic diagram of the cross section of a Transmission Electron Microscope](image)

**Figure 11:** Schematic diagram of the cross section of a Transmission Electron Microscope\textsuperscript{4}

Instrumentation
The schematic diagram for a conventional TEM with its major components is depicted in Figure:11. In TEM, the emission source of electrons is a tungsten filament or lanthanum hexaboride source. They are also known as electron gun. Electromagnetic lenses are used to accelerate and focus the electrons into a very thin beam by varying the magnetic field of electromagnetic lenses. The interior of the microscope is evacuated to low pressure typically 10–4 Pa in order to minimize scattering of the electrons by air molecules and to increase the mean free path of the electron gas interaction. Depending on the density of the sample specimen used, some of the electrons will be scattered while some will be unscattered and hit at the bottom on to a fluorescent screen or on a layer of photographic film. The image can be detected by a sensor.

Preparation of sample
Preparation of samples for TEM analysis is specific to the material under study. For pharmaceutical and material sciences, the powder in the solid state is dissolved or dispersed in solvent and deposited onto a support mesh known as “grid”. Usually a grid is 2.5–3 mm in diameter, with a 50–400 mesh and made up of copper, molybdenum, gold or platinum. Biological samples can be fixed onto the grid using a negative staining material such as uranyl acetate or by plastic embedding.

Application
Combined with good spatial resolution, ultra high magnification, TEM is widely used to obtain structural and compositional information of various materials. Recently, High resolution TEM (HRTEM) has been used to obtain a resolution of 0.2 nm.

Surface charge of the nanoparticles
The Zeta potential of the nanoparticles was determined by laser Doppler anemometry using a Malvern Zetasizer also called Doppler Electrophoretic Light Scatter Analyzer\textsuperscript{11}. The zeta potential, an important parameter when considering the stability of the nanoparticles in vitro\textsuperscript{12}. The more negative or positive values of zeta potential are related to more stable particles; more repulsion between particles reduce the particle aggregation\textsuperscript{3}. Mucoadhesion, on the other hand, can be promoted by a positive zeta potential value. The mucus layer itself is at a neutral pH value an anionic polyelectrolyte. Consequently, the presence of the positively charged groups on the particles could lead to electrical charge interactions between the mucus and the particles\textsuperscript{21}.

The most widely-used theory for calculating zeta potential was developed by Smoluchowski in 1903. The theory is based on electrophoresis and can be expressed as\textsuperscript{4}:

\[
\mu = \frac{\zeta \varepsilon}{\eta}
\]

(\(\mu\)) is the electrophoretic mobility, (\(\varepsilon\)) is the electric permittivity of the liquid, (\(\eta\)) is the viscosity and (\(\zeta\)) is the zeta potential.

<table>
<thead>
<tr>
<th>Zeta potential (mV)</th>
<th>Stability behavior of the colloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to ±5</td>
<td>Rapid coagulation/flocculation</td>
</tr>
<tr>
<td>from±10 to ±20</td>
<td>Incipient instability</td>
</tr>
<tr>
<td>from±20 to ±40</td>
<td>Moderate instability</td>
</tr>
<tr>
<td>from±40 to ±60</td>
<td>Good stability</td>
</tr>
<tr>
<td>More than ±61</td>
<td>Excellent stability</td>
</tr>
</tbody>
</table>

**Table 2:** Zeta potential for colloids in water and their stability\textsuperscript{4}

Basic principle and Instrumentation\textsuperscript{4}
Laser Doppler Electrophoresis (LDE) is based on the combination of electrophoresis and Laser Doppler Anemometry (LDA). It is used to measure velocities and thereby zeta potential of colloid particles. The
technique is based on the measurement of light scattering to the determine particle size for diluted dispersions or suspensions when particles flow through a series of interference fringes. Most widely used instrument available is Zetasizer® with standard cell.

Figure: 12 Schematic of a Laser Doppler Electrophoresis instrument

Fourier Transform Infrared Spectroscopy (FTIR)

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

Entrapment and loading efficiency

Drug entrapment or encapsulation efficiency is a percentage value that describes the quantity of the drug material in the nanoparticles out of the total amount used in the process. The drug content (or drug loading) percentage is the drug amount compared to the nanoparticle mass. The entrapment into the nanoparticles is described by two important parameters: theoretical drug loading, which is the ratio between mass of drug used in synthesis and mass of polymer used in synthesis, and nanoparticle recovery, which is the ratio between mass of nanoparticles recovered and mass of polymer and drug used in synthesis. The drug content is calculated by the ratio of mass of drug in nanoparticles to mass of nanoparticles recovered, and the drug entrapment by the ratio of mass of drug in nanoparticles to mass of drug used in synthesis. The quantitative determination of active component entrapped in nanoparticles is done by centrifugation method.

The redispersed nanoparticles suspension was centrifuge to separate the free drug in the supernatant. Concentration of free drug in the supernatant was determined by UV-Vis spectrophotometringly at desired wave length after suitable dilution if necessary. The encapsulation efficiency was determined by using the following formula:

\[
\text{Encapsulation efficiency} \, (%) = \frac{1-(\text{Drug in supernatant liquid} \div \text{Total drug added})}{1} \times 100
\]

The percentage drug loading capacity was determined using the following formula:

\[
\% \, \text{Drug loading} = \frac{[(\text{Total amount of drug} - \text{Amount of free drug}) \div \text{Nanoparticles weight}]}{100}
\]

Drug release study

Nanoparticles exhibit their special drug delivery effects in most cases by direct interaction with their environment, i.e., their biological environment. Drug release may occur by diffusion of surface bound drug, Desorption of surface bound drug, Dissolution through the nanoparticle matrix.

Methods of Measurement of Drug Release

For characterization purposes and for quality control reasons, the determination of the in vitro release of drug from nanoparticles is important. United States Pharmacopoeia (USP) methods are generally used to evaluate drug release profiles of conventional and novel drug delivery systems of macro size by using any of the USP-recommended dissolution test apparatus. In case of micro and nanoparticulate systems, these apparatus are not usable due to the following reasons:

- Difficulty to achieve sink conditions with nanoparticles having a very high surface area in the existing USP methods.
- Difficulty to separate dissolved drug from undissolved particulates while sampling.
- Need for specific enzymes to release the drug from biodegradable polymeric particulates (colon-specific microparticulates).
- Need for unconventional conditions of pH or temperature for specialized nanoparticulates.

The following methods for the determination of the in vitro release have been used:

1. Side by side diffusion cells with artificial or biological membranes
2. Dialysis bag diffusion technique
3. Reverse dialysis sac technique
4. Ultracentrifugation
5. Ultra filtration (Centrifugal) technique

The dialysis technique is generally preferred.

Various researchers have proposed different methods with one common strategy of using synthetic
membrane bag with specified porosity to hold the sample. The bag containing the sample is immersed in the recipient fluid, which is stirred at a specified rpm. The samples are withdrawn at regular intervals and are analyzed for the drug content.

**Factors affecting drug release**

Besides polymer erosion, a number of interrelated factors govern rate of release from particles. Mainly the physicochemical properties associated with particles such as size, shape, porosity, morphology etc. These, in turn are influenced by a variety of factors, for example; method of preparation, formulation parameters to name a few.

**Drug release kinetics - model fitting of the release data**

In order to analyze the drug release mechanism, in vitro release data were fitted into a zero-order, first order, Higuchi, Korsmeyer-peppas model. Drug dissolution has been described by kinetic models in which the dissolved amount of drug (Q) is a function of the test time, t or Q=f(t). Some analytical definitions of the Q(t) function are commonly used, such as zero order, first order, Higuchi, Korsmeyer–Peppas models.

**Zero order kinetics**

The zero order rate Equation describes the systems where the drug release rate is independent of its concentration.

\[
Q_t = Q_0 + K_0 t
\]

Where;

- \( Q_t \) = the amount of drug dissolved in time \( t \),
- \( Q_0 \) = the initial amount of drug in the solution (most times, \( Q_0 = 0 \))
- \( K_0 \) = the zero order release constant.

\[
ft = K_0 t
\]

Where;

- \( ft = 1-(W_t/W_o) \) and \( ft \) represents the fraction of drug dissolved in time \( t \)
- \( K_0 \) = the apparent dissolution rate constant or zero order release constant.

In this way, a graphic of the drug dissolved fraction versus time will be linear if the previously established conditions were fulfilled.

**Use:**

This relation can be used to describe the drug dissolution of several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems, as well as matrix tablets with low soluble drugs, coated forms, osmotic systems, etc.

**First order kinetics**

The first order Equation describes the release from a system where the release rate is concentration dependent.

Kinetic equation for the first order release is as follows:

\[
\log Q_t = \log Q_0 + K_1 t/2.303
\]

Where \( Q_t \) is the amount of drug released in time \( t \), \( Q_0 \) is the initial amount of drug in the solution and \( b \) is the first order release constant.

In this way a graphic of the decimal logarithm of the released amount of drug versus time will be linear. The pharmaceutical dosage forms following this dissolution profile, such as those containing water soluble drugs in porous matrices, release the drug in a way that is proportional to the amount of drug remaining in its interior, in such way, that the amount of drug released by unit of time diminishes.

**Higuchi model**

Higuchi describes drug release as a diffusion process based in the Fick’s law, square root time dependent.

\[ Qt = K_H t^{1/2} \]

Where \( K_H \) is the Higuchi dissolution constant treated sometimes in a different manner by different authors and theories. This relation can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems and matrix tablets with water-soluble drugs.

**Korsmeyer–Peppas model**

To find out the drug release mechanism first 60% drug release data can be fitted in Korsmeyer–Peppas model which is often used to describe the drug release behavior from polymeric systems when the mechanism is not well-known or when more than one type of release phenomena is involved.

\[ \log \left( \frac{M_t}{M_{\infty}} \right) = \log K_{KP} + n \log t \]

Where,

- \( M_t \) = the amount of drug release at time \( t \)
- \( M_{\infty} \) = the amount of drug release after infinite time
- \( K_{KP} \) = a release rate constant incorporating structural and geometrical characteristics
- \( n \) = the release exponent indicative of the mechanism of drug release.

**Applications of nanoparticulate delivery systems**

Tumor targeting using nanoparticulate delivery systems

The rationale of using nanoparticles for tumor targeting is based on:

1) Nanoparticles will be able to deliver a concentrate dose of drug in the vicinity of the tumor targets via the enhanced permeability and retention effect or active targeting by 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 glycoprotein (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 absorptive 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 cellspecific 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 lecin and tomato lecin, 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.

Hedley et al. reported that following their intracellular uptake and endolysosomal escape, nanoparticles could release DNA at a sustained rate resulting in sustained gene expression.

**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.

**REFERENCES**