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REVIEW ARTICLE

NANOPARTICULATE DRUG DELIVERY SYSTEMS: PROMISING APPROACHES FOR DRUG DELIVERY

Srinivas Lankalapalli*, Krishna Chaitanya Routhu, Shruthi Ojha, V S Vinai Kumar Tenneti

GITAM Institute of Pharmacy, GITAM University, Rushikonda, Visakhapatnam, Andhra Pradesh, India

**Corresponding Author's Email: lsrinivas2001@gitam.edu*

ABSTRACT

Advanced drug delivery technologies can increase safety and efficacy, extend patent lives and provide competitive differentiation for biopharmaceuticals by helping drug manufacturers in differentiating new therapeutics from existing products that enable novel treatments. It provides improved therapy through increased efficacy and duration of drug activity, decreased dosing frequency, convenient routes of administration and improved targeting of drug to specific sites reducing unwanted side effects. The role of advanced drug delivery based on Nanoparticles is discussed herein. The growing range of nanoparticle based drug delivery methods is assured of changing the formulation characteristics of new compounds and extending the lifecycle of existing compounds. In order to achieve this, article deals with the fundamental understanding of their properties, types, manufacturing, characterization, challenges and emerging trends in the field of drug delivery.

Keywords: Nanoparticles, Biopharmaceuticals, Nanoparticulate drug delivery.

INTRODUCTION

Nanotechnology is defined as the production and application of materials at nanometer scale. It involves the study of control of matter on an atomic and molecular scale which is usually below 100 nm or in the range of 0.2 nm to 100 nm[1]. In simple terms, it is one billionth of a meter i.e., 10^{-9} meters[2]. The primary goals for research in nanotechnology include:

- Specific and targeted drug delivery
- Decreased toxicity and maintenance of therapeutic effects
- Increased safety and biocompatibility
- Faster development of new and safer medicines[3]

The active pharmaceutical ingredient and its release mechanism from drug delivery system plays a critical role in determining the safety and efficacy of therapeutics[4]. The active pharmaceutical ingredient in nanoparticulate drug delivery system is either dissolved, entrapped, encapsulated or attached to a matrix and depending on this nanoparticles, nanospheres or nanocapsules may be obtained[5].

- Nanoparticles are solid colloidal particles consisting of macromolecular substances that vary in size from 10-1000 nm.
- Nanospheres are matrix systems in which the drug is physically and uniformly dispersed.
- Nanocapsules are vesicular systems in which a drug is confined to a cavity surrounded by a polymer membrane[6].

Large size materials in drug delivery systems pose problems such as poor bioavailability, solubility, intestinal absorption, plasma fluctuations and in vivo stability[1]. Nanoparticles by virtue of their unique physicochemical properties such as ultra small size, large surface area to mass ratio etc can be used to overcome the limitations of conventional drug delivery systems[7]. Therefore the development and fabrication of nanoparticles have reported the following advantages over conventional systems:

- Protection of drugs from gastric environment degradation
- Targeted drug delivery
- Prevention of first pass metabolism
- Increased bioavailability and longer circulating time due to special absorptive mechanisms such as endocytosis
- Controlled release of the drug
- Minimized plasma fluctuations and side effects
- Easy penetration and absorption through tissues and cells
- Enhanced performance of drugs that are unable to pass clinical trials
- Carrier for challenging drugs for diseases such as Cancer, HIV, diabetes etc.
- Improved acceptability of dosage form by increased efficacy, safety, patient adherence and reduced health care costs[1].

Properties of Nanoparticles

In pharmaceutics, ~90% of all medicines, the active ingredient is in the form of solid particles. The evolution of nanotechnology made it possible to produce drug

nanoparticles that can increase drug efficacy and reduce side effects by utilizing the new drug delivery pathways.

1. Nanoparticle size:

Size matching is an important parameter in carrying out the activity. The basic unit of the biological processes is the cell and the biochemical reactions inside it. It is believed that to treat the nanometer scaled component cells of human cells, nanoparticles drug delivery, which aims at influencing the cellular process, is of much interest.

2. ***Nanoparticle surface:***

As the particle size decreases, the number of molecules present on the particle surface increases. For a spherical solid particle of diameter d and the molecular diameter is σ, then the percentage of molecules on the surface monolayer is given as

$$\% \text{ Surface molecules} = \frac{\left(\frac{4}{3}\right)\pi[d^3-(d-\sigma)^3]}{\left(\frac{4}{3}\right)\pi d^3} 100$$

$$= 100[(\frac{\sigma}{d})^3 - 3(\frac{\sigma}{d})^2 + 3(\frac{\sigma}{d})] \quad 1$$

For a 10μm particle sized material, a very small percentage of molecules are present on the surface. Hence, the dissolution rate is much lower for the microparticles than the nanoparticles. Lamprecht et al[8] observed adhesion of polystyrene particle to inflamed colonic mucosa, with the deposition 5.2%, 9.1%, and 14.5% for 10-mm, 1000-nm, and 100-nm particles, respectively which shows that nanoparticle can show strong adhesion because of the increased contact area for van der Waals attraction.

3. ***Nanoparticle suspension and settling:***

Since the particle size of the nanoparticle is small, the gravitational force is smaller on it so they can be easily suspended in a liquid. The particle settling velocity v, is given by stokes law as

$$v = \frac{d^2 g\,(\rho_s-\rho_l)}{18\mu_l} \quad 2$$

where g is the gravitation acceleration (9.8 m/sec), ρ_l is liquid density (997 kg/m^3 for water at 25°C), μ_l is viscosity.

Brownian fluctuations resist the particle settlement. According to Einstein's fluctuation –dissipation theory, average Brownian displacement x in time t is given as

$$x = \sqrt{\frac{2k_bTt}{\pi\mu d}} \quad 3$$

Where k_b is the Boltzman constant (1.38 x 10^{-23} J/K), and T is the temperature in Kelvin.

For nanoparticles the gravitational pull is not stronger than the random thermal motion of the particles. Hence nanoparticles do not settle which provides a long shelf-life.

4. ***Magnetic and optical properties***

Small nanoparticles exhibit unique magnetic and optical properties. For example, ferromagnetic materials become superparamagnetic below about 20 nm, i.e., the particles do not retain the magnetization because of the lack of magnetic domains.Such materials are useful for targeted delivery of drug and heat. For example, interaction of electromagnetic pulses with nanoparticles can be utilized for enhancement of drug delivery in solid tumors[9]. The particles can be attached to antibodies directed against antigens in tumor vasculature and selectively delivered to tumor blood vessel walls.

Gold and silver nanoparticles show size-dependent optical properties[10]. The intrinsic color of nanoparticles changes with size because of surface plasmon resonance. Such nanoparticles are useful for molecular sensing, diagnostic, and imaging applications.

5. Hydrophobicity

It gives the extent and type of hydrophobic interactions of nanoparticulates with blood components and determines their bio fate .It also plays a role in drug release profile by impacting the degradation kinetics of polymeric shell[11]. It can be evaluated by methods such as hydrophobic interaction chromatography, two phase partition, contact angle measurement etc[12].

6. Crystallinity

It affects the solubility and dissolution characteristics of the drug. In case of polymeric nanoparticles, degradation first occurs in amorphous regions followed by a slow rate in crystalline regions. Therefore it affects degradation rate and drug release kinetics[11].

7. Drug loading

High drug loading capacity of a system reduces the quantity of matrix materials for administration. It can be done either by incorporation at the time of nanoparticle production or by absorption by incubation of carrier with concentrated drug solution after nanoparticle production. It depends upon the solid state solubility of drug in the matrix or polymer used which in turn depends upon the composition, molecular weight, interactions and end functional groups of the polymer[5].

8. Drug release

Drug release and polymer biodegradation are important considerations for a nanoparticulate drug delivery system. Release rate depends upon factors such as drug solubility, surface bound/ adsorbed drug desorption, drug diffusion through matrix, erosion/ degradation of the matrix and combination of erosion/diffusion process[5].

Types of Nanoparticles[13]

Different types of nanoparticles along with description, materials used and applications are shown in below table 1.

Table 1: Different Types of Nanoparticles

S.N.	Type of Nanoparticle	Description	Materials used	Applications	Ref
1.	Lipid Based	Submicron particles made of oily lipid core surrounded by soli or semi solid shell	Phospholipids, triglycerides and cholesterol	Systemic gene delivery, transdermal delivery of high molecular weight and poorly soluble drugs, drug delivery to lungs by nebulization	14
2.	Polymeric Micelles based	Formed by association of amphiphilic surfactants or polymeric molecules spontaneously in aqueous medium as core shell structures or vesicles	Amphiphilic block copolymers, such as poly(ethylene oxide)-poly(_-benzyl-Laspartate) and poly(N-isopropylacrylamide)-polystyrene,	Targeted delivery of chemotherapeutics, sustained release , parenteral delivery	15, 16
3.	Polymer Based	Colloidal solid particles with a size range of 10-1000 nm which can be spherical, branched or shell structures	Polymers such as chitosan, alginate gelatin, polyacrylates	Increased uptake by immune cells, targeting of anti cancer drug to liver, oral delivery of insulin, brain targeting for neurodegenerative disorders	17-19
a.	Hydrogels	Association of hydrophobic moieties with soluble macromolecules	Collagen, Gelatin, Starch, Poly(*n*-vinyl pyrrolidine), Methacrylates, Poly(vinyl alcohol)	Encapsulation and delivery of proteins, antigens and anti cancer agents	20, 21
b.	Dendrimers	Nanostructures highly branched with an inner core, size range is 1-100 nm but mostly less than 10 nm	Macromolecules such as polyamidoamine (PAMAM), polypropyleneimine and polyaryl ether	Attachment of drug molecules and targeting groups, coating agent protecting drugs, DNA delivery, diagnostic utilization for cancer treatment	22, 23
c.	Calcium carbonate based	Incorporation of drug into solid calcium carbonate	Calcium carbonate	Sustained release of drugs	24
d.	Protamine based	Composed of protamine, a peptide associated with DNA or therapeutic agents	Protamine- non antigenic, non toxic peptide	DNA and oligonucleotide delivery	25
e.	Chitosan based	Composed of chitosan, a biocompatible polymer associated with therapeutic agents	Chitosan- polycationic polymer compring of d-glucosamine and *N*-acetyl-dglucosamine linked by b-(1,4)-glycosidic bonds	Carriers for gene delivery, ocular drug delivery	26
f.	Silicone nanopore membrane Based	Nanopore membranes which consist of arrays of parallel rectangular channels of 7-50 nm		Increase stability of proteins which are unstable in aqueous solution at body temperature	27
g.	Polymeric nanocapsules	Spherical hollow structures in which drug is confined to a cavity and surrounded by poymer membrane	poloxamer, PEG 400, polysorbate 80, propylene glycol, and citric acid	Confined reaction vessels, protective shell for cells or enzymes, transfection vectors in gene therapy, dye dispersants, carriers in heterogenous catalysis, imaging and drug carrier	28, 29

Production of Nanoparticles

Developing the nanoparticles of size range <100nm will help in exhibiting some unique physical and biological properties. But inorder to achieve nanoparticles of size range <100nm is possible with hard materials rather than using soft materials like drug and polymer.

For hard materials, such as silica, metal oxides, and diamonds with melting points above 1000°C, nanoparticles are prepared in the size range of 1–100nm. For drugs that are usually soft materials with melting point below 300°C particles in the 1–100nm size range are more difficult to prepare, so they are prepared at <300nm size.

To obtain nanoparticles in the 50–300nm range, for soft materials, for drug delivery, one requires of the order of 10^4–10^8 molecules in each particle. This size has

to be achieved from either solution phase (single molecule) or millimeter-size particle (10^{18} molecules).

The two general approaches for the production of drug nanoparticles are

a. The particle is broken down to nano size,
b. The particle will be built up from molecules.

Manufacturing techniques of Nanoparticles

Milling and Homogenization Techniques:

There are specific and non specific approaches for the improvement of solubility and bioavailability. Specific approaches can only be applied to certain drug molecules i.e., with in case of cyclodextrins (CDs) to molecules that fit into the respective CD ring. Whereas, the nonspecific formulation approaches are applicable to almost any drug molecule. Such a nonspecific formulation approach since many years is micronization, which means converting relatively coarse drug particles to micrometer crystals with a mean diameter in the range of approximately 2–5 µm, and a corresponding size distribution approximately between 0.1 and 20 µm[30]. Here, the increase in the surface area leads to an increase in the dissolution velocity. That means micronization is a formulation approach to overcome the bioavailability problems of drugs of the biopharmaceutical specification class II (BSC II), where the rate limiting step is a too low dissolution velocity.

Nowadays however, many of the new compounds are so poorly soluble that micronization is not sufficient to overcome a too low oral bioavailability. Consequently, the next step taken was to move from micronization to nanonization, which leads to further increase in surface area and thus there is an increase in dissolution velocity.

Even highly water-sensitive drugs can be reduced to drug nanocrystals, even stored in the form of an aqueous nanosuspension (drug nanocrystals dispersed in aqueous surfactant/stabilizer medium). Drug nanocrystals can be produced by bottom-up or topdown technologies. In the case of bottom-up technologies, one starts with the molecules in solution and moves via association of these molecules to the formation of solid particles, i.e., it is a classical precipitation process[31]. In the case of top-down technologies, one starts with a coarse material and applies forces to disintegrate into the nanosize range. The diminution technologies can be categorized into two principal classes:

1. Pearl/ball milling.

2. High-pressure homogenization, and other processes.

1. Pearl/ball-milling technology for the production of drug nanocrystal:

Traditional equipment like Jet milling leads to a drug powder with a size range of roughly between 0.1 and 20 mm, containing only a very small fraction of about 10% in the nanometer range[30]. Hence the traditional equipments used for micronization of drug powders such as rotor–stator colloid mills (Netzsch) or jet mills (Retsch) are of limited use for the production of nanocrystals.

When a pearl mill is run over a sufficiently long milling time, drug nanosuspensions can be obtained. These mills consist of a milling container filled with fine milling pearls or larger-sized balls. The container can be static and the milling material is moved by a stirrer; alternatively, the complete container is moved in a complex movement leading consequently to movement of the milling pearls. Surfactants or stabilizers have to be added for the physical stability of the produced nanosuspensions. In the production process the coarse drug powder is dispersed by high-speed stirring in a surfactant/stabilizer solution to yield a macrosuspension. The choice of surfactants and stabilizers depends on the properties of the particles to be suspended and on the physical principles (electrostatic and steric stabilization) and the route of administration.

Steric stabilization is recommended as the first choice because it is less susceptible to electrolytes in the gut or blood. Electrolytes reduce the zeta potential and subsequently impair the physical stability, especially of ionic surfactants. In many cases an optimal approach is the combination of a steric stabilizer with an ionic surfactant, i.e., the combination of steric and electrostatic stabilization. Adsorption onto the particle surface leads to high zeta potential values providing good physical stabilities.

In case of parenteral drug nanocrystals, the choice is limited; for eg. For intravenous injection, accepted are lecithins, Poloxamer 188, Tween1 80, low molecular weight polyvinylpyrrolidone (PVP), sodium glycocholate (in combination with lecithin).

Production of parenteral drug nanosuspension using pearl mills is much more tedious compared to producing oral drug nanosuspensions. The equipment needs to be sterilized and the product needs to be separated from the milling pearls by a preferentially aseptic separation process.

One advantage of the pearl mills, apart from being low-cost products, is their ability for scaling up.

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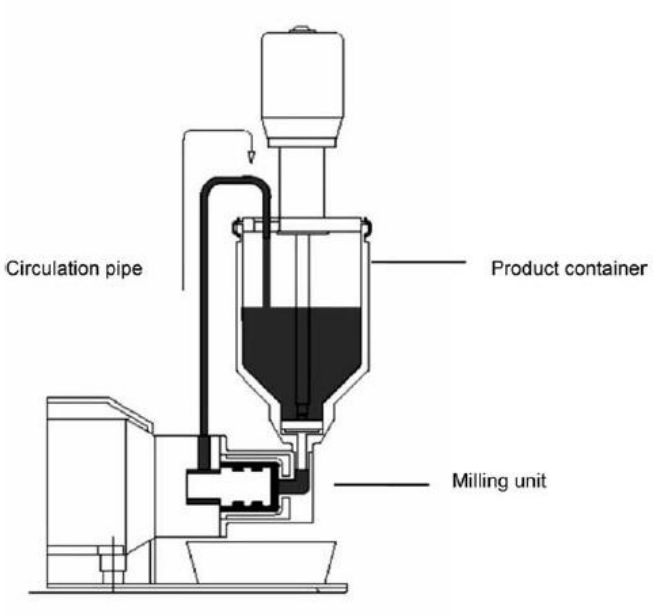

Figure 1: Schematic view of a bed mill using recirculation method[32] DISPERMAT® SL

2. Drug Nanocrystals produced by High- Pressure Homogenization

High-pressure homogenization has been applied for many years in various areas for the production of emulsions and suspensions and is currently used in food industry like homogenization of milk. A distinct advantage of this technology is its ease for scaling up, even to very large volumes. In the pharmaceutical industry parenteral emulsions are produced by this technology.

Most of the homogenizers used are based on the piston-gap principle; an alternative is the jetstream technology. The Microfluidizer is based on the jet-stream principle. Two streams of liquid collide, diminution of droplets or crystals is achieved mainly by particle collision, but occurrence of cavitation is also considered.The Microfluidizer has also been described for the production of drug nanosuspensions; and requires 10-50 cycles [33].

The Microfluidizer can be used for the production of drug nanosuspensions in the case of soft drugs. In the case of harder drugs, a larger fraction of particles in the micrometer range remain, which do not exhibit the increase in saturation solubility because of their too large size.

For many years cavitation was considered as the major force leading to particle diminution in the high-pressure homogenization process. So, mostly water was used as the dispersion medium. In the piston-gap homogenizer the liquid is forced through a tiny homogenization gap, typically in the size range of 5–20 µm. According to the Bernoulli equation, the streaming velocity and dynamic pressure increase extremely, the static pressure in the gap falls below the vapor pressure of water at room temperature.

A liquid boils when its vapor pressure is equal to the static pressure, which means water starts boiling in the gap at room temperature leading to the formation of gas bubbles. The formation of gas bubbles leads to pressure waves disrupting oil droplets or disintegrating crystals. When leaving the homogenization gap, the static pressure increases to normal air pressure, which means the water does not boil anymore and the gas bubbles collapse. Collapsing of the gas bubbles (implosion) leads again to shock waves contributing to diminution.

At the end of the 1990s it was found that similar efficient particle diminution can be achieved by homogenization in nonaqueous media such as oils and liquid polyethylene glycols. Preparation of drug nanocrystals in PEGs or oils leads to nanosuspensions that can directly be filled into capsules[34,35]. It is also possible to homogenize in melted nonaqueous matrices, which are solid at room temperature. Solidification of such a matrix leads to a fixation of drug nanocrystals in the solid matrix, thus minimizing or avoiding crystal contact and subsequent crystal fusion/growth.

Preparation of drug nanosuspensions in water–ethanol mixtures is favorable for producing dry products, because later the spray drying can be performed under milder conditions when using such a mixture. Homogenization in water–glycerol mixtures (2.25% of water-free glycerol) leads to isotonic drug nanosuspensions for parenteral administration.

3. Production of Drug Nanocrystal Compounds by Spray-Drying:

For the production of tablets, an aqueous nanosuspension can be used as granulation fluid. Starting from an aqueous macrosuspension containing the original coarse drug powder, surfactant, and water-soluble excipient, the homogenization process can be performed in an easy one step yielding a fine aqueous nanosuspension. In a subsequent step the water has to be removed from the suspension to obtain a dry powder. One method of removing the water from the formulation is freeze drying, but it is complex and cost-intensive leading to a highly sensitive product[36,37] and another

method is spray drying, which is simple and most suitable method for industrial production.

The drug nanosuspension can directly be produced by high-pressure homogenization in aqueous solutions of water-soluble matrix materials. Afterward the aqueous drug nanosuspension can be spray dried under adequate conditions; the resulting dry powder is composed of drug nanocrystals embedded in a water-soluble matrix[38].

One aim of a solid nanoparticulate system is releasing the drug nanocrystals after administration in the gastrointestinal tract (GI) as a fine nonaggregated suspension; the other is to increase the physical stability for long-term storage.

Production in hot-melted matrices:

A further possibility for the production of drug nanocrystals in solid matrices is high-pressure homogenization in hot melts. It offers advantages over production in aqueous solution and subsequent spray drying. The process is completely anhydrous, avoiding possible drug degradation or instabilities. The production can directly be performed by hot high pressure homogenization in melted material[39,40]. The homogenizers Micron Lab 40, batch and continuous, were equipped with temperature control jackets placed around the sample/product containers. Working temperatures up to 100°C (heated with water) or higher (heated with silicon oil) can be selected depending on the melting temperature of the used matrix material. For batch operation, solidification has to be averted between each homogenizing cycle. For homogenizers working in the continuous mode, the product containers must be also heated.

As the first production step, a presuspension has to be formed consisting of a melted matrix with the addition of the drug powder and surfactant. In the following production step, the hot presuspension can be directly homogenized in the temperature-controlled homogenizer. After reaching the envisaged particle size and size distribution, the suspension can be solidified at room temperature by applying controlled cooling. Subsequently, the solid nanodispersions obtained can be processed to granulate by milling, for filling capsules or tablet compaction. Alternatively, the hot melt can directly be filled into hard gelatine or hydroxypropyl methylcellulose (HPMC) capsules. The absence of water during the whole production process as well as the short processing times and the one-step process to the final product are especially to be noted using the hot melt method.

Pelletization Technique:

The most commonly used pelletization techniques are the extrusion–spheronization and the drug layering onto sugar spheres. Irrespective of the pelletization technique applied, a multiparticulate dosage form will be obtained. Multiparticulate dosage forms show a faster and more predictable gastric emptying and more uniform drug distribution in the GI tract with less inter- and intraindividual variability in bioavailability[41]. A broad distribution of the pellets in the gut lumen can enhance the complete redispersion of the nanoparticles from the final solid dosage form.

1. Matrix pellet preparation

Aqueous nanosuspensions can be mixed with matrix materials like MCC, lactose etc., Also the nanosuspension works as a binder and wetting fluid for the extrusion process[42-45]. Binders like gelatine, HPMC, chitosans, or other polymers can be added to the nanosuspension before the high-pressure homogenization, which simplifies the production process. Alternatively, they can be dispersed in the produced nanosuspension after the highpressure homogenization. The binders which are used are necessary for the extrusion process but they can also positively influence the properties of the nanosuspension or the nanoparticles.

2. Pellet preparation by Nanosuspension Layering

An alternative way to transfer a prior produced nanosuspension into a pellet formulation is the suspension layering onto sugar spheres[46]. The binders that are necessary for this process can also be added before the high-pressure homogenization process resulting in the improved nanosuspension properties.

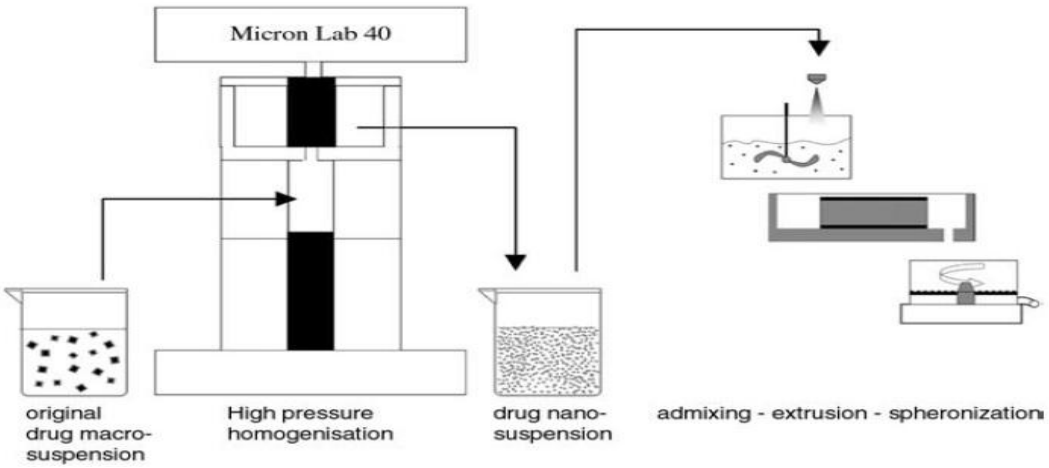

Figure 2: Schematic Production of drug nanocrystal-loaded matrix cores

Supercritical fluid Technology:

A fluid is supercritical when it is compressed beyond its critical pressure (Pc) and heated beyond its critical temperature (Tc). Super Critical Fluid (SCF) technology has emerged as an important technique for particle manufacturing. The critical constant values of different super critical solvents are shown in table 2 below.

Table 2: Critical Constant for Various Supercritical Solvents

SCF	T_c(°C)	P_c(bar)
Ethylene	9.3	50.3
Trifluoromethane (fluoroform)	25.9	47.5
Chlorotrifluoromethane	28.9	39.2
Ethane	32.3	48.8
Carbon dioxide	31.1	73.7
Dinitrogen monoxide (laughing gas)	36.5	72.6
Sulfur hexafluoride	45.5	37.6
Chlorodifluoromethane (HCFC 22; R 22)	96.4	49.1
Propane	96.8	43.0
Ammonia	132.4	112.7
Dimethyl ether (wood ether)	126.8	52.4
Trichlorofluoromethane (CFC 11, R 11)	198.0	44.1
Isopropanol	235.2	47.6
Cyclohexane	280.3	
Toluene	318.6	41.1
Water	374.0	220.5

Much attention has been given to supercritical carbon dioxide for pharmaceutical particle formation since it is nontoxic, inexpensive, and has mild critical temperature. Carbon dioxide due to its quadrupole moment it is a nonpolar molecule with a small polarity. Hence, nonpolar or light molecules (e.g., menthol, methanol, acetone, toluene, and hexanes) easily dissolve in CO_2, whereas the polar or heavy molecules (e.g., griseofulvin, paclitaxel, tetracycline, and dexamethasone phosphate) have a very poor solubility.

Three important factors that govern drug solubility in supercritical CO2 are

- Vapor pressure of drug,
- Drug–CO_2 interaction
- Density of CO_2.

Drug vapor pressure is a function of temperature (T), and CO2 density is a function of pressure (P) and T.

Studies reveal that solubility of pharmaceutical compounds is highly dependent on CO_2 pressure. As the pressure is reduced, solubility decreases because of a reduction in the CO_2 density, which is closely related to its solubility power[47-50]. At a high pressure, the drug can be dissolved in CO2 and if the pressure is reduced to ambient, the drug precipitates out as fine particles. The fast depressurization results in a very fast rate of precipitation providing small drug particles. This process is termed as rapid expansion of supercritical solution (RESS). Most of the drug particles produced by RESS have been in the 1–5 mm-size range. The rapid expansion of supercritical CO_2 does produce nuclei 5–10nm in diameter, but these nuclei grow because of coagulation and condensation to produce the final micrometer-size particle.

Recently, Thakur and Gupta[51] the challenges of RESS (low solubility and growth by coagulation) are overcomed by utilizing a cosolvent that is solid at the nozzle exit conditions. The solid cosolvent (SC) enhances the solubility in supercritical carbon dioxide and provides a barrier for coagulation in the expansion chamber. The SC is later removed from the solute particles by lyophilization (sublimation). The new process is termed as RESS–SC. The choice of a proper SC is the key for successful RESS-SC.

Various requirements for the selection of the SC are

- Good solubility in supercritical CO_2
- Solid at nozzle exit condition (5–30 °C)
- Good vapor pressure for easy removal by sublimation
- Should be nonreactive with drugs or CO2
- Inexpensive.

Menthol satisfies the requirements mentioned above. It has appreciable solubility in CO2 and can easily sublime under vacuum.

Polymer or Protein Stabilized Nanoparticles from Emulsions

Poorly water-soluble drugs have a challenge in their delivery. Such drugs can be given by nanoparticle delivery, which can avoid the allergic side effects due to the use of cremaphors (e.g., polyethyoxylated castor oil) in conventional formulations. However, for drugs with crystal forming habits, there is always the hazard of the formation of large microparticles (>10–15 mm) from aggregation/ bonding of nanoparticles; this can lead to infarction or blockage of the capillaries, resulting in ischemia or oxygen deprivation and possible tissue death. Hence, the nanoparticles need to be stabilized using biocompatible proteins (e.g., human serum albumin) or polymers (e.g., polylactide, polycaprolactone).

Emulsification Solvent Evaporation process

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In this process, polymers like poly(d,l-lactide-co-glycolide) (PLGA), poly(lactic acid) (PLA), polymethacrylate and the drug is mixed in a water-immiscible solvent like methylene chloride, chloroform, ethyl acetate and is added dropwise to aqueous phase containing a surface stabilizer (e.g., polysorbate, polyvinyl alcohol, methyl cellulose, genatin, albumin, poloxomar)[52,53]. A high shear is provided using a homogenizer, which reduces the droplet size of the organic dispersed phase. The evaporation of solvent hardens the nanoparticles. Formed nanoparticles are harvested from the aqueous slurry by lyophilization.

For the water-soluble drugs, a double-emulsion (water/oil/water) variation of the process is utilized. First, the drug is dissolved in water and then emulsified in water to obtain drug/ water as the dispersed phase and organic solvent as the continuous phase. Then, this emulsion is added to the large aqueous phase with emulsifier to create double emulsion. As the droplet size of the first emulsion needs to be much smaller than in the second outer emulsion, the emulsifier amount is much higher in the first emulsion than in the second emulsion. In emulsification, shear forces help create more surface and hence smaller droplet size emulsion, whereas the surface tension opposes the formation of more surface.

If a smaller droplet size is desired, then high shear energy is needed. This energy requirement can be reduced if the surface tension is reduced which can be done by adding a surfactant or surface stabilizing agent such as albumin, poly vinyl alcohol (PVA), poly acrylic acid (Carbopol®), poly(oxyethylene-b-oxypropylene-b-oxyethylene) (Poloxamer or Pluronic®). Both Carbopol and Poloxamer show mucoadhesive properties which may be beneficial in oral drug delivery applications.

Once the droplets are created, it is then important to solidify them to avoid coalescence. The final particle size is directly proportional to emulsion droplet size and the coalescence during hardening. For creating fine emulsion for obtaining nanoparticles, the use of a high amount of surface stabilizer is avoided to reduce the high load of the polymer exipients.

Sonication

Sonication generates emulsions through ultrasound-driven mechanical vibrations, which causes cavitation. Rarefaction and compression cycles of sonication create vapor bubbles, which grow with time. Once a critical size is achieved, the bubble collapses violently, releasing the energy creating hot spots and hydroxyl free radicals. The duration and intensity of sonication can be used to create varying emulsion droplet sizes.

Homogenization

Homogenization is similar to sonication based on emulsification efficiency, but is relatively more effective in emulsifying viscous solutions. Ambient pressure homogenizers use rotor–stator types of mixers, which can go to very high rotational speeds. High-pressure homogenization uses high pressure to force the fluid into microchannels of a special configuration and initiates emulsification via a combined mechanism of cavitation, shear, and impact, exhibiting excellent emulsification efficiency. Sonication usually generates more heat, and hence is less suitable for heat-sensitive materials. Homogenization is generally more effective in making fine emulsions. Usually, multiple passes are needed to achieve the desired emulsion droplet size. The emulsion droplet size decreases with increasing homogenization intensity[54]. Using a rotor–stator homogenizer, the emulsion droplet size was found to be viscosity (μ) dependent and proportional to $\mu^{0.11}$ of the dispersed phase and $\mu^{-0.43}$ of the continuous phase.

Nanoparticle Hardening

Particle hardening due to solvent evaporation plays an important role in the growth of the particle during coalescence. The particle stickiness comes from the solvent associated with the polymer and drug. In the beginning of the process, the droplets are liquid and coalesce if they come any closer than about 1 nm. When part of the solvent is removed, the droplets are still sticky, but the particle bridging is slowed down owing to the increased viscosity of the drop interior. Once most of the solvent is removed, the particles become hard and now they can start to bounce off from other colliding particles. Wang and Schwendeman[55] measured the removal rate of the solvent from particles with respect to time as shown in Figure 3.

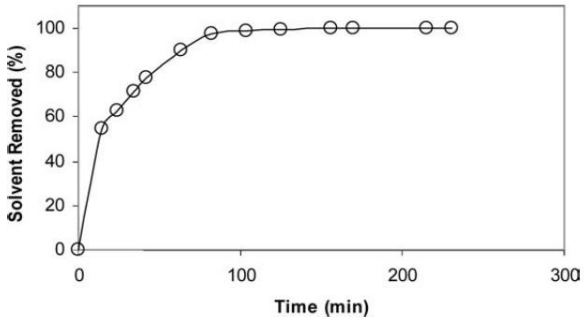

Figure 3: Methylene chloride removal profile from encapsulation of triamcinolone acetonide in PLGA particles[55].

Initially, the solvent removal is fast, owing to the high diffusivity of solvent and the dissolution of the solvent in the aqueous media. With time the droplets become hard on the surface due to polymer precipitation, which slows down the solvent diffusion. due to polymer precipitation, which slows down the solvent diffusion. Particle growth continues as a result of coalescing for the duration in which the solvent is not completely removed to the point when particles are not sticky.

Residual Solvent and Emulsifier

Residual solvent in pharmaceutical preparations, including nanoparticles, is a growing concern because of the toxicological risks associated with such residuals. If proper evaporation and lyphilization is not carried out, then the final nanoparticle may retain the solvent. The interfacial PVA influences particle size, zeta potential, polydispersity index, surface hydrophobicity, and drug loading. Both residual solvent and emulsifier can be reduced by cross-flow microfiltration. Cross-flow microfiltration is particularly attractive for the processing of large volumes of

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nanoparticulate suspension, as the membrane surface can be easily increased. Other methods such as evaporation under reduced pressure or ultracentrifugation usually only treat small batch volumes.

Protein Stabilized Nanoparticles

Owing to the concerns of residual emulsifier in the final product, several researchers have utilized albumin protein stabilizer because of its complete compatibility with even the injectable formulations. The choice of organic solvent and the extent of homogenization can be used for further variation, for example the aqueous phase was presaturated with the organic solvent and a small amount of ethanol was added to the organic phase. In this variation, smaller nanoparticles, 140–160 nm, are obtained. To form a solid and stable layer of albumin onto drug nanoparticles, the protein needs to be cross-linked (or denatured) onto the particle surface. Typically albumin crosslinking can be achieved by heat, use of cross-linker such as gluteraldehyde, or high shear. Fortunately, in the emulsification solvent evaporation process high shear is already in use, hence it can also be used for cross-linking protein stabilizers. High-shear cross-linking works for the protein-bearing sulfhydryl or disulfide groups (e.g. albumin). The high-shear conditions produce cavitation in the liquid, which causes tremendous local heating and results in the formation of hydroxyl radicals that are capable of cross-linking the polymer, for example, by oxidizing the sulfhydryl residues (and/or disrupting the existing disulfide bonds) to form new, cross-linking disulfide bonds[56-58].

Physical Characterization of Nanoparticles

Sizing methods are frequently classified (shown in table 3 below) according to the manner in which they extract information from the sample. Counting methods, such as microscopy or single-particle optical sensing (SPOS), measure the size of individual particles to compile a histogram reflecting the overall distribution.

Table 3: Physical characterization of Nanoparticles

Sno.	Property	Analytical method(s)	References
1.	Presence size	Dark field optical microscopy Size Dynamic light scattering, Static light scattering, Ultrasonic spectroscopy, Turbidimetry, NMR, Single particle optical sensing, FFF Hydrodynamic fractionation, Filtration	59,60-66
2.	Morphology	TEM, SEM, Atomic force microscopy	67–75
3.	Surface charge	Electrophoretic light scattering, U-tube electrophoresis, Electrostatic-FFF	64,76 -81
4.	Surface hydrophobicity	Hydrophobic interaction chromatography	76,71,82
5.	Surface adsorbates	Electrophoresis	70,83
6.	Density	Isopycnic centrifugation, sedimentation-FFF	70,84
7.	Interior structure	Freeze-fracture SEM, DSC, X-ray diffraction, NMR	75,77,85,86-88

Dynamic Light Scattering

DLS, also known as photon correlation spectroscopy (PCS) or quasi-elastic light scattering (QELS) records the variation in the intensity of scattered light on the microsecond time scale[60,61]. This variation results from interference of light scattered by individual particles under the influence of Brownian motion, and is quantified by compilation of an autocorrelation function. This function is fit to an exponential, or some combination or modification thereof, with the corresponding decay constant(s) being related to the diffusion coefficient(s).

Static light Scattering/Fraunhofer Diffraction

Static light scattering (SLS) is an ensemble method in which the pattern of light scattered from a solution of particles is collected and fit to fundamental electromagnetic equations in which size is the primary variable[61,62]. The method is fast and rugged, but requires more cleanliness than DLS.

Acoustic Spectroscopy

Acoustic spectroscopy measures the attenuation of sound waves as a means of determining size through the fitting of physically relevant equations[63].

Turbidimetry

For nonabsorbing particles, turbidity is the complement to light scattering because it represents the amount of incident radiation not reaching a detector, that is, light lost to scattering[64].

Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) can be used to determine both the size and the qualitative nature of nanoparticles. The selectivity afforded by chemical shift complements the sensitivity to molecular mobility to provide information on the physicochemical status of components within the nanoparticle [65].

Single-Particle optical Sensing

A particle counting method, SPOS, which is also known as optical particle counting, involves recording the obscuration or scattering of a beam of light that results from the passage of individual particles through a sensor. Signal magnitude is translated to the size of the particle via use of a previously determined calibration curve using standards approximating the sample in terms of shape and optical properties.

Electron Microscopy

Scanning and transmission electron microscopy, SEM and TEM, respectively, provide a way to directly observe nanoparticles. SEM is better for morphological examination[67,68,77]. TEM has a smaller size limit of detection, is a good validation for other methods,andaffords structural information via electron diffraction, but staining is usually required, and one must be cognizant of the statistically small sample size and the effect that vacuum can have on the particles. Very detailed images data can result from freeze-fracture approaches in which a cast is made of the original sample[69,70].

Atomic Force Microscopy

In this technique, a probe tip with atomic scale sharpness is rastered across a sample to produce a topological map based on the forces at play between the tip and the surface. The probe can be dragged across the sample (contact mode), or allowed to hover just above (noncontact mode), with the exact nature of the particular force employed serving to distinguish among the subtechniques[72,73].

Filtration

A simple, yet effective, approach of determining particle size is filtration, in which the concentration of a suspension is determined before and after passage through filter membranes of various sizes.

Field-Flow Fractionation

The nature of the perpendicular force defines the type of field-flow fractionation (FFF) and thus the particle property on which separation occurs: sedimentation (buoyancy, size), flow (hydrodynamic size), electrostatic (charge), or thermal (diffusion)[67].

Hydrodynamic Chromatography

In a sufficiently narrow channel of parabolic flow, particles of different size will on average experience different flow lines because of their differential ability to approach the channel wall. The particles will separate based on that property, with those that are smaller eluting later just as they would in flow-FFF.

Hydrophobic Interaction Chromatography

In this method the analyte is first adsorbed onto a chromatographic stationary phase using a high concentration of an antichaotropic salt[82].

Electrophoresis

This process will determine the clearance and biodistribution of the colloid, so evaluating the exact nature of the surface coverage is required to achieve a useful understanding. The small size of nanoparticles allows their electrophoretic behavior to be observed using bioanalytical tools such as isoelectric focusing and 2-D polyacrylamide gel electrophoresis[83].

Isopycnic Centrifugation

This self-focusing separation allows nanoparticle density to be determined, which along with particle size and bulk substituent concentration can in turn be used to calculate a number concentration[70].

Zeta Potential

Zeta potential is used as a surrogate for surface change, and is often measured by observing the oscillations in signal that result from light scattered by particles located in an electric field. Doppler shift is generally used for this purpose.

Scanning Probe Technique

This technique uses the interaction between a sharp tip and a surface to obtain the image. The sharp tip is held very close to the surface to be examined and is scanned back and forth. As the tip is scanned across the sample, the displacement of the end of the cantilever is measured, using a laser beam. This can image insulating materials simply because the signal corresponds to the force between the tip and the sample, which reflects the topography being scanned[2].

Optical tweezers

Optical tweezers use a single laser beam (focused by a high-quality microscope objective) to a spot on the specimen plane. The radiation pressure and gradient forces from the spot create an optical trap, which holds a particle at its center. Small interatomic forces and displacements can be measured by this technique. Samples that can be analyzed range from single atoms to micrometer-sized spheres to strands of DNA and living cells[2].

Challenges to Nanoparticulate drug delivery systems:

The development of nanoparticulate drug delivery system requires understanding of both surface chemistry of nanomaterials as well as the interaction chemistry of these materials with the biological systems. The challenges can be due to biological, safety, manufacturing and financial issues[89]

- Biological challenges- Problems such as rapid clearance by immune system, low targetting efficiency and difficulty in crossing barriers may be encountered. Knowledge of mechanism underlying the intracellular uptake, processing and fate of the nanoparticulate in the complex biological systems is needed.
- Safety challenges- the negative impact of interaction between nanomaterials and biological systems is dealt in Nanotoxicology. Investigations have shown that toxicity has lead to several harmful effects on the biological systems[90] have reported many evidences for nanotoxicology. Gold and polystyrene nanoparticles have caused hemolysis and blood clotting. Carbon nanotubes showed platelet aggregation[91], oxidative stress, mitochondrial dysfunction[92]. Quantum dots have showed cytotoxicity by induction of reactive oxygen species causing damage to nucleus and mitochondria[93,89] suggested the establishment of proper standards and testing protocols for nanoparticulates.
- Manufacturing challenges- Due to complexity in methods and high cost of materials employed, they may not be compatible with large scale production. Proper statistical approaches can be followed to improve the scale up for increased production and commercialization of nanoparticles.

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- Economical challenges- Despite the number of patents, commercialization is a problem still due to high costs of development[94].

Emerging Trends in Nanoparticulate drug delivery systems:

Nanotechnology offers great potential benefits for drug delivery and therapy of respiratory and systemic diseases. Nanoparticles have been of significant interest for some time because they can be designed to simultaneously carry a drug payload, specifically target features of diseased tissues, and carry an imaging molecule to track drug accumulation and clearance in tissues. Moreover, they can be engineered to tailor drug delivery and improve pharmacokinetics. A variety of Nanoparticles have been investigated in experimental animal models as tools to improve the delivery and therapeutic efficacy of drugs or genes delivered to the lung or other organ systems. The emerging applications of nanoparticles shown in below table 4.

Table 4: Emerging applications of Nanoparticles

Emerging trend	Application	Ref
Biological Analysis and Discovery	Segregation of proteins and nucleic acids based on size and shape, sequencing of a genome and sum total of genes in an organism.	95
Nanoparticulate Tagging	Attachment of nanoparticles such as quantum dots and nanowires to molecules of interest are being used in detection technologies	96
Nanostructured materials	Used as portable biodetectors that can be used for detection of chemicals based on colour change in hazardous environments	96
Single-Molecule Detection	Nanostructures such as quantum dots that can emit a photon in presence of a particular molecule can be used	97
Protective Nanoparticles against pathogens	By virtue of their physical nature, cause disruption of bacteria and viruses hence used as cream for lacing fabrics in hospitals	96
Nanotubes and Cellular Manipulations	Removal and insertion of nucleus from one cell to another, cloning and making probes	98
Nanoengineered Prosthetics	Replacement and implantation of organs	96
Thiomer Nanoparticles	Possess mucoadhesive, enzyme inhibitory and permenation enhancing properties, used in insulin and calcitonin systems	99
Nanostructured Monoliths	Protein and peptide analysis based on nanocoupling technique	2
Antibody coated Nanospheres	Targeted delivery of drugs	2
Nanocrystallites	Increased solubility and bioavailability using pluronics	2
Nanohybrids	Poorly soluble non ionic drug delivery, non viral vector for gene delivery	100,101
Nanocontainer technology	Versatile carriers used to evade immune system and for drug delivery	102
Electrospun Nanofibers as Drug-Delivery Systems	Complete release of poorly soluble drugs, for buccal and topical uses	103

CONCLUSION

Developments in the field of nanotechnology are very promising. Many of them are therapeutically in use and others are in various stages on preclinical and clinical development. Thus they are expected to have a tremendous impact on medicines for decades to come. The understanding of properties of nanoparticles shows unique characteristics such as increased surface area, ease of suspension, magnetic and optical properties and high drug loading. Using recent technologies of production they can be used for any types of drugs. Their characterization uses various techniques to determine physiologically relevant parameters. The value of nanotechnology enabled compounds is expected to reach $ 220 billion by 2015. Therefore the use of nanoparticles will transform medical treatment by targeting the drugs to specific areas of the body without side effects or toxicity concerns since lower doses can be used. Therefore further advances in this field would turn it into the next generation of drug delivery system.

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