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Nanoparticles for Improved Delivery of Poorly Soluble Drugs

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Abstract

Nanoparticle based drug products are of growing interest in the field of pharmaceutics. Enhancing saturation solubility and dissolution velocity by preparing drug nanoparticles correlates with faster absorption rates. The faster absorption rates can correlate into better bioavailability, reduction in fed and fast effects and inter-subject variability with improved therapeutic response. Drug nanoparticles have shown potential applications in developing several oral and parenteral dosage forms with improved therapeutics. Suitable formulations for the commonly used routes of administration can be identified employing nanoparticle technology. Drug nanoparticles provide the discovery scientist an alternate avenue for screening and identifying a superior drug delivery system. For toxicologist, the approach provides a means for dose escalation with minimum amount of drug substance. In the recent years, formulating poorly water-soluble compounds using a nanoparticulate approach has evolved from a conception to a realization whose versatility and applicability are being vastly recognized. In the present review, industrially relevant production technologies are critically reviewed. The nanoparticle characterization techniques and potential challenges involved in the development of drug nanoparticles were discussed in detail.

Introduction

With the advent of new technologies in drug discovery, combinatorial chemistry, and computer aided drug design, there was an exponential increase in the development of new chemical entities with good therapeutic potential. However because of the complex chemistry, nearly 40% of the drug candidates in the development pipeline and about 60% of new drugs produced by chemical synthesis are associated with poor aqueous solubility resulting in low and variable bioavailability [1,2]. The poor solubility of drug may result in sub-optimal dosing and concomitantly poor therapeutic response. Parenteral administration of poorly soluble drugs as microsuspensions (e.g. i.m. or i.p.) often fails to achieve required drug concentration levels due to limited volume of solute at the site of injection. They are instances wherein the solubilizing agents used to improve the solubility of drug have resulted in allergic and toxic reactions. For example, Cremphor EL used as solubilizing agent in Taxol® formulation have shown some adverse effects such as allergic shock [3].

The commonly used approach to overcome poor aqueous solubility is by preparation of salts that had limited success. From a formulation stand point, a crystalline salt is preferred foreseeing the potential physical and chemical stability issues associated with an amorphous form of drug substance. Identification of a crystalline salt with adequate aqueous solubility requires screening of various counter-ions and crystallization conditions and at times isolation of a crystalline material itself is a tough task. In some instances the salt formed may be highly hygroscopic posing formulation challenges during drug product development [1].

An alternate approach is to identify analogs or prodrugs with enhanced solubility. This approach was not successful since the chemically modified drug molecule is often abandoned in its early phase of development or the drug product is launched with suboptimal properties including poor bioavailability, fed and fast variability, lack of optimal dosing, presence of excipients at high concentrations that pose limitations with respect to dose escalation, and ultimately, poor therapeutic outcome. Generally, chemical modification methods are expensive compared to manipulation of drug with formulation strategies because once the chemical structure is modified, the associated pharmacological activity may not be same and it is expensive to re-demonstrate the efficacy and safety of the chemically altered drug. Therefore, screening of suitable formulation technology for improving the therapeutic performance of drug is a preferred approach to develop a viable product for poorly soluble drug [4].

At present, they are limited formulation approaches available to address the problems associated with drug's poor aqueous solubility and bioavailability. The most commonly used approaches are incorporation of drug into complexing agents (cyclodextrins), using lipid carriers (liposomes, self-emulsifying systems), micronization, and solid dispersions of the drug in water-soluble carriers, etc. However the success of these techniques is mostly dependent on specific properties of drug molecule therefore, have limited scope for general application. For example, ability to ionization, solubility in oils, lipids, molecular size, structure and shape to fit into the hydrophobic cavities, etc. Liposomes have demonstrated reasonable success in formulating poorly soluble drugs however because of the poor stability issues and expensive product costs, these approaches are not suitable for all the drugs, particularly of those which are not

soluble in either aqueous or organic solvents[5].

For many years, micronization was successfully applied in the formulation of poorly soluble drugs. Micronization often results in colloidal drug particles with a particle size>1 μm with less fraction in the sub-micron range. Micronization of drug shall result in a moderate increase in surface area that may not be significant in terms of improving the dissolution rate or saturation solubility to impact the bioavailability[6].

Solid dispersions comprise of dispersion of the drug in a solid matrix, where the matrix can be a polymer or lipid based surface active carrier that can rapidly emulsify, upon contact with the dissolution media. Formation of molecular dispersions (solid solution) provides a means of reducing the particle size of the drug to nearly molecular level. As the carrier dissolves, the drug is exposed to the dissolution media as fine colloidal particles in amorphous form. The reduced particle size and increased surface area, results in improved dissolution rate and oral absorption. There are several formulations in the market, e.g., Sandimmune®/Neoral® (cyclosporine microemulsion), Norvir® (Ritnovir) and Fortovase® (Saquinavir). This approach is suitable for highly potent compounds with low dose requirement and thus not applicable for drugs with low potency where the dose requirements are relatively high[7]. Thus there is a need for a versatile technology that can address formulation issues associated with poorly soluble drugs.

In recent years nanoparticle formulation technology is gaining considerable interest in the formulation scientists. For example, when the particle size of the drug is reduced from micro scale to nano scale (8 μm to 200 nm), a significant increase in surface area (40 fold) was observed[8]. The nanoparticle formulation approach is proven to be very useful and promising in all stages of the drug product development and has opened opportunities for revitalizing marketed products with sub-optimal delivery.

For pharmaceutical industries, nanoparticle formulations haveprovided new opportunities for addressing the issues associated with poorly soluble compounds. In new chemical entities (NCE's) development, the technology has been of great value when it is used as a screening tool during preclinical efficacy and / or safety studies in the early development phase. During later drug product development, nanoparticle formulations can be post processed into various types of patient friendly dosage forms that provide maximal drug exposure. For commercial products requiring lifecycle extension, nanoparticle formulation strategies provide a means to develop a new drug-delivery platform incorporating the marketed drug, thus creating new opportunities for addressing the unmet medical needs. The present research indicate that nanoparticle solutions in drug delivery will capture significant percentage of the total market based on their ability to reduce the product development time to reach the market, extend product life cycles and provide patent fencing. The advantages of nanotechnology based drug delivery include lower drug toxicity, improved bioavailability and reduced cost of treatment. The potential benefits of nanoparticle technology in various stages of drug product development are shown in Figure 1.

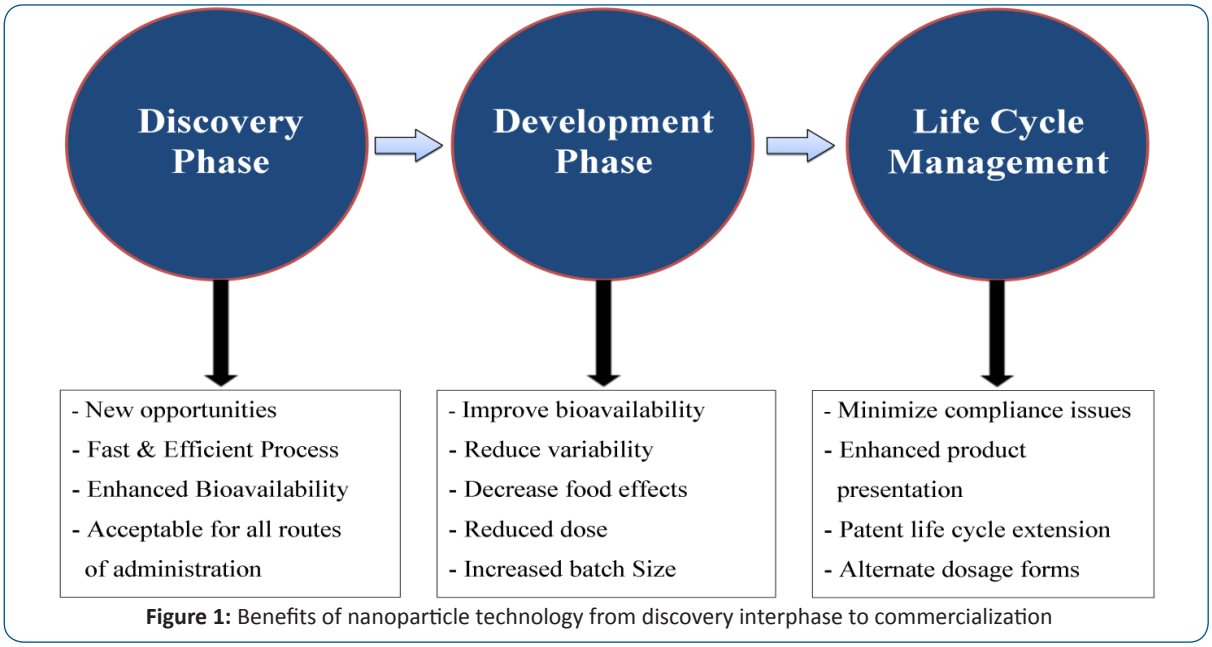

Figure 1: Benefits of nanoparticle technology from discovery interphase to commercialization

History

Nanoparticle technology has a long development and application history. In the early 19th century, heterogeneous catalysts were among the first nanoparticles reported[1,9]. The first example of pharmaceutical drug product was danazol which was formulated using a bead milling process. The resultant nanosuspension with a median particle size of 169 nm, showed significant enhancement in the oral bioavailability (82.3 ± 10.1%) compared to the conventional drug suspension (5.1 ± 1.9%). Micro fluidization was used in the production of atovaquone nanoparticles (100–3000 nm). Rapamune® (Sirolimus), an immunosuppressant developed by Wyeth's pharmaceuticals using nanoparticle technology was the first product approved by FDA. An anti-emetic drug, Emend® was second product approved for commercialization. The subsequent product developed by Abbott Laboratories was Tricor®, which was successor for fenofibrate following patent expiry. Triglide® was another product containing fenofibrate nanoparticles developed by Skyepharma with improved product performance. Par Pharmaceutical company developed Megace ES® (ES stands for enhanced solubility) using nanoparticle technology. Elan nanosystems developed the megestrol acetate nanosuspension which has demonstrated reduced the fed and

fast variability. Megestrol acetate nanosuspension has shown long term physical stability indicating its potential over the product shelf life. A list of approved products developed using nanoparticle technology is summarized in Table 1 [9,10].

Fabrication of existing drugs with maximal drug exposure, less toxicity, expanded intellectual property by drug life cycle management and minimized competition during the drug's life time can be achieved through nanoparticle based drug delivery systems. In fact, viable formulations for poorly soluble drugs with maximum drug exposure can be developed potentially by nanoparticle technology, which has opened the stage gates for reviving currently marketed products, leading to better clinical and commercial benefits. Some of the key nanotechnology based approaches for the enhancement of drug solubility and oral

Table 1: Overview of nanoparticle technology based marketed products [58]

Trade Name	Drug	Indication	Drug Delivery Company	Innovator Company	Status
Rapamune®	Rapamycin, Sirolimus	Immunosuppressant	Elan Nanosystems	Wyeth	Marketed
Emend®	Aprepitant	Anti-emetic	Elan Nanosystems	Merck & Co.	Marketed
Tricor®	Fenofibrate	Hypercholesterolemia	Abbott Laboratories	Abbott Laboratories	Marketed
Megace ES®	Megestrol	Anti anorexic	Elan Nanosystems	Par Pharmaceuticals	Marketed
Triglide®	Fenofibrate	Hypercholesterolemia	IDD-P Skyepharma	Schiele Pharma Inc.	Marketed
Avinza®	Morphine sulphate	Phychostimulant drug	Elan Nanosystems	King Pharmaceuticals	Marketed
Focalin	Dexmethyl-Phenidate HCl	Attention Deficit Hyperactivity Disorder (ADHD).	Elan Nanosystems	Novartis	Marketed
Ritalin	Methyl Phenidate HCl	CNS Stimulant	Elan Nanosystems	Novartis	Marketed
Zanaflex Capusules™	Tizanidine HCl	Muscle relaxant	Elan Nanosystems	Acorda	Marketed

Table 2: Key nanotechnology-based approaches for the enhancement of drug solubility and oral bioavailability

Company	Nanotechnology-based formulation Approach	Description and Reference
American Biosciences (Blauvelt, NY, USA)	Nanoparticle albumin-bound technology. e.g. paclitaxel-albumin nanoparticles	Paclitaxel albumin nanoparticles [59]
Baxter Pharmaceuticals (Deerfield, Illinois, USA)	Nanoedge technology: Particle size reduction was achieved by homogenization, micro precipitation, lipid emulsion and other dispersed systems.	Nano lipid emulsion [59]
BioSante Pharmaceuticals (Lincolnshire, Illinois, USA)	Calcium phosphate based nanoparticles were produced for improved oral bio-availability of hormones/proteins and vaccine adjuvants	Calcium phosphate nanoparticles [60]
ElanPharma International (Dublin, Ireland)	Nanoparticles (< 1μ) were produced by wet milling technique using surfactants and stabilizers. The technology was applied successfully in developing of ap-prepitant and reformulation of siroliums.	Nanocrystal drug particle [60]
Eurand Pharmaceuticals (Vandalia, Ohio USA)	Nanocrystal or amorphous drug is produced by breakdown of crystal lattice and stabilized by using biocompatible carriers (swellable microparticles or cyclodex-trins)	Cyclodextrin nanoparticle [58]
iMedd Inc. (Burlingame, CA, USA)	Implantable drug delivery system using silicon membrane with nano pores (10–100 nm)	Stretchable silicon nanomembrane [58]
pSivida Ltd (Watertown, MA, USA)	The solubility and bioavailability of hydrophobic drugs was achieved by incor-porating drug particles within the nano-width pores of biocompatible silicon membranes or fibers.	Silicon nanoparticles [61]
PharmaSol GmbH (Berlin, Germany)	High pressure hominization was used to produce nanostructured lipid particles dispersions with solid contents that provide high-loading capacity for hydrophilic drugs	Drug encapsulated in lipid nanoparticles [62]
SkyePharma Plc, (Piccadilly, London, UK)	Nanoparticulate systems of water insoluble drugs were produced by applying high shear or impaction and stabilization was achieved by using phospholipids.	A polymer stabilizing nano reactor with the encapsulated drug core [62]

bioavailability according to Saffie- Siebert and co-workers[11] are highlighted in Table 2.

Formulation Theory

The principle of nanonization is based on the increase in surface area of drug particles. According to Noyes-Whitney equation, the dissolution rate of poorly water soluble drug can be increased by reducing the drug particle size to nano scale and increasing its surface area[12]. Increasing the surface area by reducing the particle size generally correlates with improved dissolution and drug absorption.

Development of nanoparticle formulation for poorly water soluble drugs results in enhanced dissolution rate which is the driving force for its improved pharmacokinetic properties. Particle size and intrinsic solubility are the important parameters influencing the dissolution rate of a drug. As described by the Nernst-Brunner and Levich modification of Noyes-Whitney model the rate of drug dissolution is directly proportional to surface area;

$dx/dt = (A \times D/\delta) \times (C-X/V)$

Where X is the amount of drug in solution, t is time, A is the effective surface area, D is the diffusion coefficient of the drug, δ is the effective diffusion boundary layer, C is the saturation solubility of the drug, and V is the volume of dissolution medium.

Saturation solubility of a drug depends on the dissolution pressure and temperature. The dissolution pressure is a function of the curvature of the nanoparticle surface. Greater the curved surface of the particles, stronger will be the dissolution pressure. For particles below a size of 1 μm, the dissolution pressure increases significantly leading to an increase in the saturation solubility. In addition the concentration gradient is increased due to decreased diffusional distance on the surface of the drug nanoparticle. This increase in surface area and increase in concentration gradient results in enhanced dissolution velocity and saturation solubility compared to the products containing micronized particles[13]. Saturation solubility and dissolution velocity are important parameters affecting the bioavailability of poorly soluble drugs administered orally.

Fabrication of Drug Nanoparticles

There are various techniques reported for the production of drug nanoparticles. The existing technologies are classified as 'bottom up' and 'top down' technologies. The bottom-up technologies involves controlled precipitation by adding a suitable non-solvent. The top down technologies are milling or homogenization methods. However the combination techniques, a pre-treatment step followed by subsequent size reduction are also being employed for the production of drug nanoparticles. In some instances solvent evaporation and supercritical fluid technologies are also used but they are less industrially relevant at present.

Bottom-Up technologies

These technologies are also known as precipitation methods. Precipitation has been applied for many years in the preparation of small particles, particularly in the development of photographic films,[14] and from the last decade, precipitation methods have been successfully used in the preparation of sub-micron particles for drug delivery. Examples for precipitation techniques are the hydrosols[15], developed by Sucker (Sandoz, presently Novartis) and the product Nanomorph by Soliqs/Abbott (previously Knoll/BASF).

In this process, the drug is dissolved in a solvent and the resulting solution is subsequently added to a non-solvent. This results in high super saturation, rapid nucleation and formation of many small nuclei[16]. Upon solvent removal, the suspension may be sterile filtered and lyophilized. Addition of the solvent to non-solvent is necessary to yield a very fine product by passing the Ostwald Mier area fast[17]. The mixing processes may vary considerably. Therefore, through careful control of this addition process, a particle with a narrow size distribution can be obtained. In the case of Nanomorph, amorphous drug nanocrystals are produced to further enhance solubility and dissolution velocity[18].

Another precipitation method was reported for the preparation of amorphous drug nanoparticles, for example, carotene nanoparticles in the food industry[19], (Lucarotin® or Lucantin®). In this process, solutions of the carotenoid together with a surfactant in digestible oil, are mixed with an appropriate solvent at a specific temperature. To obtain the solution, a protective colloid was incorporated in the formulation. This resulted in an O/W two phase system. The carotenoid stabilized by the colloid localizes in the oily phase and after lyophilization, the X-ray analysis showed that approximately 90% of the carotenoid was in an amorphous state. This technology is used for pharmaceuticals by Soliqs (Ludwigshafen, Germany) and is advertised by the trade name, NanoMorph®.

The precipitation technique in comparison to other technologies is relatively simple and requires no expensive equipments. The method does not necessitate high energy process like disintegration which prevents denaturation of drug. However, precipitation methods have numerous limitations; it is very difficult to control nucleation and crystal growth to obtain a narrow particle size distribution. Often a metastable solid, usually amorphous, is formed which is converted to more stable crystalline form[20]. Furthermore, non-aqueous solvents utilized in the precipitation process must be reduced to toxicologically acceptable levels in the end product and due to the fact that many poorly soluble drugs are sparingly soluble not only in aqueous, but also in organic media. To sum, the bottom up techniques are not extensively used for production of drug nanocrystals.

Top-Down technologies

Top-down technologies refer to the mechanical breakdown of larger particles in to nanoscale. The two top down technology frequently used for producing drug nanoparticles include;

i. High pressure homogenization

ii. Milling methods

High pressure homogenization technique

High-pressure homogenization is one of the disintegration method used for size reduction. The two-homogenization principles/homogenizer types used are;

i. Microfluidization (Microfluidics, Inc.)

ii. Piston-gap homogenizers (e.g. APV Gaulin, Avestin, etc.)

Micro-fluidization for production of drug nanoparticles

Micro-fluidization uses the high shear forces and impaction to produce the drug nanoparticles. It works on a jet stream principle, where the suspension is passed at a high velocity in a specially designed 'Y' and 'Z' type homogenization chambers. In the 'Z' type chamber, the drug suspension changes the direction of its flow at a high velocity leading to particle collision and shear forces. In the second type of chamber, the 'Y'-type, the suspension stream is divided into two streams, which then collide frontally resulting in high shear and particle size reduction.

A disadvantage of this technology is increased process time which is due to the requirement of more number of passes through the microfluidizer to obtain particles in the sub-micron range. In addition, the product obtained by microfluidization may contain a relatively large fraction of microparticles, especially in the case of hard drugs.

Piston-gap technologies

Utilizing microfluidization principle, the second generation nanoparticle technology based on piston-gap homogenizers was developed in 1990's. Homogenization can be performed in water (DISSO CUBES) or alternatively in nonaqueous media or hydro alcoholic media (NANOPURE). There is also a combination process of precipitation followed by a second high-energy step, e.g. homogenization to avoid the particle growth (NANOEDGE)[21].

A high energy input and impact forces are required for effective size reduction. Piston gap homogenization generally produces greater turbulent energy by cavitation. In piston gap homogenization process, the suspension formulation is pumped through a narrow gap at high pressure (15,000–30,000 psi). In this homogenizer type, the dispersion (emulsion or suspension) passes through a very thin gap with an extremely high velocity. Prior to entering the gap, the suspension is contained in a cylinder with a relatively large diameter compared to the width of the following gap. In APV LAB 40, the diameter of the cylinder is about 3 cm, it narrows to about roughly 25 mm (varies with applied pressure). The resultant particle size is a function of the pressure applied and number of homogenization cycles. Increase in temperature during the homogenization process is one of the important parameter which can be controlled by placing a heat exchanger before the homogenizer valve. The major limitation of high pressure homogenization process is that it is difficult to handle dispersions with high solid content (usually > 10% w/w).

Milling Techniques

In 1990, the first generation mill based on disintegration technique was developed by Liversidge which lead to the production of sub-micron particles[22]. Conventional milling methods generally produce larger particles (>1μm). These milling techniques were later refined to produce solid drug particles of sub-micron range. In 20th century bead mills are employed for the production of fine suspensions. In this method, the suspension comprising of drug, surfactant and stabilizers along with milling media are charged into the recirculation chamber (grinding chamber). The particle size reduction mainly occurs due to the high shear forces of impact, generated by the agitation of the milling media. In contrast to high pressure homogenization, it is a low energy milling technique. The pearls or beads consist of ceramics (cerium or yttrium stabilized zirconium dioxide), stainless steel, glass or highly cross linked polystyrene resin-coated beads. Bead milling technique is relatively simple and applicable to drugs that are insoluble in both aqueous and non-aqueous media. However, metallic contamination due to erosion from the milling media during the milling process is a common problem with this technology. To overcome this issue, the milling beads are often coated[23]. Another problem with milling process is the adherence of product to the inner surface area of the mill (consisting mainly of the surface of the milling beads and the inner surface of the milling chamber). The milling time depends on several factors such as drug concentration, surfactant concentration, hardness of the drug, viscosity of the suspension, temperature, energy input, milling media etc. The milling time to produce the desired particle size may vary from 30 minutes to several hours/days depending on the properties of the drug[24,25].

In wet bead milling process, the drug suspension is passed through a grinding chamber containing beads of sizes ranging from 0.2 to 3 mm. To achieve the desired particle size; drug concentration in the suspension can be varied from 5 – 40% w/v. To prevent aggregation / agglomeration of the dispersed particles, stabilizers and surfactants are used in the formulation. An ideal stabilizer must be capable of wetting the drug particles providing steric and ionic barrier. In absence of appropriate stabilizers, the high surface energy of the nanoparticles would lead to aggregation of dispersed particles. The concentration of polymeric stabilizers can range from 0.5 – 10% w/v and the concentration of surfactants is generally < 1 % w/v. If necessary, other excipients such as sugars, lactose or mannitol can be added to the dispersion to enhance stability and further processing of the wet product [26].

Following the addition of grinding/milling media in to the milling chamber, the suspension comprising of surfactant and stabilizer is charged in to the mill. The milling chamber has a rotor fitted with disks that can be accelerated at a desired speed. The milling media is agitated by the rotation of the rotor disk radially in the chamber. When the product flows axially through the chamber, due to high shear forces generated during impaction of the milling media with the drug provides the energy input to fracture the drug into nanoparticles. The milling chamber temperature is controlled by circulating coolant through the outer jacket and the process can be performed either in a batch mode or in a continuous mode (re-circulation). The milled product is subsequently separated from the milling media using a separation system.

Scaling up the process using bead mills is relatively simple and convenient[27]. The batch size can be increased above the void volume in a recirculation mode. The suspension is continuously pumped through the mill in a circular motion. This increases the batch size with corresponding increase in the milling time because the required exposure time of the drug particles per unit mass to the milling material remains unchanged.

Surfactants and stabilizers are incorporated in the formulation to ensure physical stability of the nanosuspensions. The stabilizers act as an energy barrier across the dispersed particles to prevent aggregation or agglomeration. During the manufacturing process the drug substance is dispersed by high speed stirring or homogenizer in a surfactant and/or stabilizer solution to yield a uniform suspension. The choice of surfactants and stabilizers largely depends on the physical stabilization (steric versus electrostatic

stabilization) and the route of administration. In general, steric stabilization is less susceptible to physiological electrolytes therefore, it is recommended choice. Electrolytes in the GI reduce the surface charge of the drug particles leading to physical instability, especially of ionic surfactants. In some instances combination of a steric stabilizer with an ionic surfactant, i.e, the combination of steric and electrostatic stabilization is preferred for stabilization of the drug product. There are various charged surfactants generally regarded as safe (GRAS) available in case of drug nanoparticles for oral administration. There are wide choices of bead mills available in the market, ranging from laboratory to commercial scale. The ability of the technology for production at large scale is a prerequisite for the launching of the product into market. To sum up, among the drug nanoparticle technologies available, bead milling offers a convenient process for production of nanoparticle dispersions at high concentrations for solid dosage form that offers ease of scale-up to enable commercial manufacturing. The pros and cons of nanoparticle technologies are summarized in Table 3.

Translation of nanosuspension into solid intermediate

The conversion of nanosuspension into solid intermediate can be achieved by removing the solvent from the suspension by using drying operations such as fluid bed coating, spray granulation, spray drying and lyophilization (freeze drying). Lyophilization is considered as a more complex and cost intensive process predominantly applicable to highly sensitive drug products. The challenge in this process is to retain the dispersibility of the nanoparticles up on reconstitution with water or gastric fluids. If aggregation or agglomeration of drug particles occurs then the potential benefits obtained from the drug nanoparticles due to increased surface area may be completely lost or compromised. The re-dispersants must be added to the nanosuspensions prior or during the drying process. Commonly used re-dispersants are sugars such as lactose, sucrose and mannitol etc.[28]. The objectives of drug nanoparticle system is to release the drug nanoparticles in the gastrointestinal tract (GIT) as fine non-aggregated suspension following oral administration and increase the physical stability for long term storage of the drug product[29]. Spray drying is especially suitable for drugs that can withstand high temperatures. Depending on the formula composition and spray conditions, the resulting dry powders can easily be filled into capsules or blended with extra granular excipients and compressed in to tablets[30]. In case of drugs

Table 3: The pros and cons of nanoparticle technologies[63]

Technology	Registerd Name	Key parameters	Pros	Cons
Precipitation	• NanoMorph®	• Stirring rate • Antisolvent to solvent ratio • Drug Content • Temperature	• Simple • No expensive equipments required • Avoids high energy process • Generates amorphous drug	• Not applicable to all drugs • Drug should be soluble in one solvent • Residual solvent • The solvent should be miscible with anitisolvent
Piston-gap High pressure homogenizer	• Dissocubes®, SkyePharma • Nanopure®, Abbott Laboratories	• Homogenizer pressure • Homogenization cycles • Temperature • Hardness of the drug	• Universally applicable • Simple technology • High productivity • Less metallic contamination	• Not applicable to temperature sensitive drugs • Poor homogenity in particle size distribution
Media Milling	NanoCrystal ™, Elan Pharma Nanomil ™, Elan Pharma Dyno®-Mill, Glen Mills, inc	• Drug concentration • Milling speed • Milling time • Temperature	• Simple process • Circumventing harsh chemicals or co-solvents • High drug loading • High productivity	• Time consuming • Metallic contamination • Batch size limitations
Combinative techniques (Combination of precipitation and high energy shear forces)	• Nanoedge™	• Temperature • Hardness of the drug • Homogenization cycles • Homogenizer pressure	• Reduced crystal growth • Less concernes about physical stability of amorphous materials	• Applicable to drugs soluble in at least one solvent • Residual solvent • Expensive process

which are susceptible to gastric fluids, the capsule or tablet can be coated with enteric polymers to protect the drug from gastric environment.

An alternative way to convert nanosuspension into solid intermediate is by layering the nanosuspension onto an inert carrier such as sugar beads, lactose, cellulose derivate, etc.[31]. The suspensions can be layered onto a water soluble or insoluble carrier's at a predetermined spray rate using a top spray fluid bed process. The granules are suspended in air stream as they move up and suspension shall be sprayed from the top of the system onto the fluidized bed, resulting in granules with a uniform size distribution.

Characterization of Drug Nanoparticles

Characterization of drug nanoparticles is mainly performed to understand the behavior of the drug product during manufacturing processes and to have better control of the product quality. There are various techniques reported for detecting, measuring and characterizing the drug nanoparticles. There is no single method that can be considered as the "best" for analysis. Most often the method is chosen to balance the restriction of the nature of sample, data required, time constraints and the cost of analysis. Following methods are used for characterization of drug nanoparticles[32].

Particle Size Distribution

Measuring the particle size and understanding its effects on the product and processes can be critical to the success of manufacturing businesses. The particle size characterization is primarily performed to obtain information about the changes in average particle size and particle size distribution during the manufacturing process and storage (e.g. aggregation or agglomeration). Particle size distribution of drug nanoparticles can be measured by following techniques

Photon Correlation Spectroscopy (PCS)

Photon correlation spectroscopy is one of the most widely used light scattering techniques for measuring the particle size and particle size distribution. PCS is based on dynamic light scattering in which the movement of sub-micron particles in random direction (Brownian motion) is measured as a function of time. The principle involved in this technique is that smaller particles move with higher velocity than the larger particles. When a laser beam is diffracted by sub-micron particles in the suspension, the diffusion of particles causes rapid fluctuations in scattering intensity of the laser around a mean value at a certain angle (between 10 to 90˚) which is dependent on the particle size. The measured correlation function results in a diffusion coefficient for a given temperature and viscosity which can be converted into particle size. The extent of increase in the particle size is a measure for the extent of instability of the suspension. Therefore, PCS is considered as a reliable instrument to detect the instabilities during storage[33]. The technique is used for determination of the average particle size in a range between 3 nm and 3000 nm. In addition a polydispersity index (PI) is obtained as a measure for the width of the distribution. If the value of PI is 0, it indicates a monodisperse particle population. In case of narrow distribution the PI values are around 0.10 – 0.20, the values of 0.5 and higher indicate a very broad distributions. From the values of mean particle size (z- average) and PI, the changes in nanoparticles size with time can be measured.

Laser Diffraction

Laser Diffractometry (LD) was developed around 1980 and is used as a routine method in many research laboratories. When a laser beam passes thought a sample containing colloidal particles in sub-micron range, light diffraction occurs and LD measures the angular variation in intensities of the light scattered and the angular scattering intensity data is then analyzed to calculate the size of the particles responsible for creating the scattering pattern, using the Mie theory of light scattering. Smaller particles scatter light at larger angles relative to the laser beam and larger particles scatter light at smaller angles. Mie theory requires knowledge of the optical properties (refractive index and imaginary component) of both the sample being measured, along with the refractive index of the dispersant. Unfortunately for most of the pharmaceutical solids the refractive indices are unknown. However, because of its simplicity, laser diffractometry is frequently used as the second characterization method for drug nanoparticles[25].

Morphology

Microscopy based techniques are considered as most direct measurements of particle size and morphology. These techniques can be used to study a wide range of materials with a broad distribution of particle sizes, ranging from the nanometer to the millimeter scale. Instruments used in this technique include optical light microscopes, transmission electron microscopes (TEM), scanning electron microscopes (SEM) and atomic force microscopes (AFM). The choice of instrument for evaluation is determined by the size range of the particles being studied, magnification, and resolution. However, the cost of analysis is increased exponentially as the size of the particles decreases due to requirements of higher magnification, improved resolution, greater reliability and reproducibility. The analysis cost also depends upon the instrument being studied, as it dictates the techniques of sample preparation and image analysis[34].

Surface potential

The interactions occurring between nanoparticles are measured by the surface charge density (zeta potential). The particle charge is one of the important factors in determining the physical stability of nanosuspensions. The higher the particles are equally charged, the greater is the electrostatic repulsion between the particles and longer is the physical stability. Typically the particle charge is quantified as zeta potential, which is measured e.g. via the electrophoretic mobility of the particles in an electrical field. A zeta potential of -30 mV for electrostatic and -20 mV for sterically stabilized systems is desired to obtain a stable nanocrystal suspension formulation[35].

Solid State Properties

Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) is used to determine the nature of crystallinity within nanoparticles by measuring the glass transition temperature, melting point and their associated enthalpies. This method along with XRPD is used in determination of the extent of which multiple phases exists in the interior and their interaction with the drug.

X-ray powder diffraction (XRPD)

XRPD is used to study single crystal or polycrystalline materials. A beam of x-rays is passed through a sample and the way

the beam is scattered by the atoms in the path of the x-ray is studied. The scattered x-rays constructively interfere with each other. This interference can be looked at using Bragg's Law to determine various characteristics of the crystal or polycrystalline material[35].

Saturation Solubility

Determination of saturation solubility is very important not only in assessing the benefits compared to the microparticulate formulation but also in predicting the *in vivo* performance such as plasma levels and bioavailability following dosage administration. Saturation solubility evaluations are carried out in buffer media at different pH conditions using a shake flask method. The saturation solubility is a function of crystalline structure (lattice energy) and particle size [18].

In vitro Dissolution

In vitro dissolution is an important step in the characterization of drug nanoparticle formulations. Drug nanoparticles have been used as a drug delivery tool to improve the solubility and/or dissolution of a poorly soluble drug. Hence, it is important to know the dissolution profile of the prepared formulation in a physiologically discriminating dissolution media. This will help in understanding to rate and extent of drug release and absorption characteristics from the administered dosage form *in vivo.* The *in vitro* dissolution of nanoparticle formulation can be carried out using United States Pharmacopoeia type-I or II apparatus (or as described in other Pharmacopoeia) upon compressing into a tablet or filling them into a suitable capsule.

In-vivo Pharmacokinetics

The *in vivo* pharmacokinetic studies on rate and extent of release of drug from the administered dosage form, absorption, distribution and elimination are evaluated by dosing the nanoparticle formulation in suitable animal model or human subjects. The *in vivo* pharmacokinetic studies provide a mathematical platform to estimate the time course of drug and its effects *in vivo.*

Future Trends

In the future, active targeting of drug nanoparticles by altering the functional surface will be the next important aspect in the development of nanoparticles. The surface modification of nanoparticles may significantly impact the drug adsorption pattern and regulates the cellular uptake.

Nanoscale drug loaded polymeric micelles have been widely reported for intracellular delivery of potent drugs[36-39]. pH-responsive polyion complex micelles[40,41], cholesterol-enhanced, amphiphilic di, tri block copolymer[42-44], thermo-responsive polypeptide[45], galactopeptide micelles[37] have demonstrated enhanced cellular internalization and receptor-mediated tumor cellular proliferation inhibition in malignancy chemotherapy. PEG–PLGA copolymers have shown potential applications as drug delivery platforms *in vivo* with enhanced stabilities during circulation and accelerated drug release at targeting lesion sites[46,47]. Reduction-responsive polymeric nanogels for tumor-specific targeting have shown potential for targeted intra-cellular delivery of chemotherapeutic drugs in cancer therapy[48-54]. Thermo-responsive and pH-responsive polypeptide vesicles revealed a promising approach for smart drug delivery with enhanced efficacy and security[54-57] demonstrating a prospective future of nanoparticle drug delivery in the disease management.

Conclusion

Conceptually nanoparticle technology based products are expected to revolutionize the field of modern medicine. It is believed that nanoparticles will provide potential solutions to encounter the problems emerging from pharmaceutical industry's drug discovery pipeline. In combination with conventional approaches drug nanoparticles can be incorporated into solid dosage forms such as tablets and capsules. Further advancements in nanoparticle technology will spur the complete evolvement of drug nanoparticles as potential drug delivery system.

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