Chapter 2
Literature Review

2.1 Solid lipid Nanoparticles

2.1.1 Introduction:

During the past decades, polymeric and lipid based submicron sized carrier systems have been widely investigated as drug delivery systems (Westesen K et al., 1996). Polymeric nanoparticles are solid colloidal particles consisting of nonbiodegradable synthetic polymers or biodegradable macromolecular materials of synthetic, semisynthetic or natural origin. A great deal of interest has been focused on lipid based carrier systems like liposomes, fat emulsions and solid lipid nanoparticles (SLNs). These vehicles are composed of naturally occurring physiological lipids such as phospholipids, cholesterol, cholesterol esters and triglycerides. Owing to the natural and biological origin of the carrier material, as resulting in much lower toxicological risk than that of the polymeric particles.

Amongst all lipid based carrier systems, attention has recently been focused on alternative nanoparticles made from solid lipids, the so called SLNs. These possess a solid lipid core matrix in the nanometer range, stabilized by a layer of surfactants. They have potential to carry lipophilic and hydrophilic drugs as well as diagnostic agents. SLNs combine the advantages of different colloidal carriers; for instance, they are physiologically acceptable like emulsions and liposomes and can provide controlled release of drug from the lipid matrix, like polymeric nanoparticles (Manjunath K et al., 2005). Other advantages of SLNs include use of biocompatible and biodegradable material, ease of preparation and scale up and high dispersibility in an aqueous medium. SLN formulations have been developed for various routes of application (parenteral, oral, dermal, ocular, pulmonary, and rectal) and are thoroughly characterized and evaluated for both *in vitro* and in *in vivo* criteria (Pinto JF & Muller RH., 1999, Demirel M et al., 2001, Cavalli R et al., 2002, Videira MA et al., 2002, Sznitowska M et al., 2001). Most recently, increasing research work is being carried out on delivery of macromolecules like proteins, oligonucleotides, DNA and RNA using SLNs as the carrier system. Nanostructured lipid carriers (NLC) are mixture of solid lipid and liquid lipid while Lipid Drug Conjugates (LDC) are water insoluble lipid carrier for loading of poorly lipid

soluble drugs. These new generation of lipid nanoparticles have been claimed to overcome the shortcomings of SLNs.

Solid lipid nanoparticles (SLNs) are emerging as alternative carriers to colloidal systems, for controlled and targeted delivery. These are in submicron size range (50–1000 nm) and are made up of biocompatible and biodegradable materials capable of incorporating lipophilic and hydrophilic drugs. The general structure of SLNs is shown in Figure 2.1.SLNs combine the advantages of different colloidal carriers, for instance like emulsions and liposomes, these are physiologically acceptable and like polymeric nanoparticles, controlled release of drug from lipid matrix can be anticipated. Additional advantages include lack of coalescence after reaching to room temperature (following their preparation or during their storage) and better physical stability. Since mobility of the incorporated drug molecule is drastically reduced in SLNs, there would not be any appreciable drug leakage from the particles. In recent years, much work has been focused in the development of SLNs as delivery systems for anticancer
drugs, peptides, genetic material, cosmetics, etc.

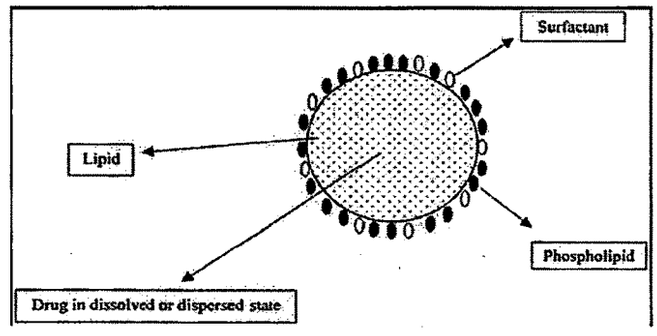


FIG. 2.1. General structure of SLN.

Advantages of Solid Lipid Nanoparticles:

- Possibility of controlled drug release and drug targeting
- Increased drug stability
- High drug payload
- Incorporation of lipophilic and hydrophilic drugs feasible
- No biotoxicity of the carrier
- Avoidance of organic solvents

- No problems with respect to large scale production and sterilization

Disadvantages:

- Low drug-loading capacity
- Drug expulsion after polymorphic transition during storage
- High water content of the dispersions (70–99.9%)
- Presence of alternative colloidal structures (micelles, liposomes, mixed micelles, drug nanocrystals)
- Complexity of the physical state of the lipid (transformation between different modifications, possibility of super- cooled melts) which cause stability problems during storage or administration (gelation, particle size increase, drug expulsion).

2.1.2 Formulation of SLNs

Common ingredients used in the formulation of SLNs are lipids (matrix materials), emulsifiers, co emulsifiers, and water. Charge modifiers, stealthing agents that improve long circulation time and targeting ability, are also used to meet the requirements of stability and targeting aspects. Various excipients used in the formulation of SLNs are listed in Table 2.1.

Preparation of Solid Lipid Nanoparticles:

The performance of SLNs greatly depends on the method of preparation which in turn influences the particle size, drug loading capacity, drug release, drug stability etc. Different approaches exist for the production of finely dispersed lipid nanoparticle dispersions. Few of the production processes such as high pressure homogenization and microemulsion dilution have demonstrated scaling up possibility, a prerequisite for introduction of a product to the market (Cavalli R et al., 1997, Cavalli R., 1998, Jening V et al., 2002).

2.1.2.1 High pressure homogenization (HPH)

High pressure homogenization (HPH) has emerged as a reliable and powerful technique for the preparation of SLNs. HPH is a suitable method for the preparation of SLN and can be performed at elevated temperature (hot HPH technique) or at or below room temperature (cold HPH technique) (Schwarz C et al., 1994, Cortesi R et al., 2002, Siekmann B et al., 2002). R.H. Muller was the first to report and patent this method. Skye

Pharma, London, currently holds the worldwide patent rights of this technology (Muller et al., 1996).

Table 2.1 Excipients used in solid lipid nanoparticle (SLN) drug delivery systems

Lipid matrices	Beeswax
	Behenic acid
	Caprylic/capric triglyceride (Miglyol 812)
	Cetylpalmitate
	Cholesterol
	Glyceryl trilaurate (Dynsan 112)
	Glyceryl trimyristate (Dynsan 114)
	Glyceryl monostearate
	Glyceryl tristearate (Dynsan 118)
	Glyceryl behenate (Compritol)
	Glyceryl monostearate (Imvitor 900)
	Glyceryl tripalmitate (Dynsan 116)
	Hardened fat (Witepsol E 85)
	Monostearate monocitrate glycerol (Acidan N12)
	Monosyeol (Propylene glycol palmitic stearate)
	Precirol ATO 5 (mono, di, triglycerides of C16-C18 fatty acids)
	Softisan 142/cetyl alcohol (75:25)
	Softisan 142
	Solid paraffin
	Stearic acid
	Superpolystate
	Synrowax HRSC (mixture of glycerol tribehenate and calcium behenate)
	Witepsol E 85/cetyl alcohol (75:25)
	Witepsol H5
	Witepsol W 35
Emulsifiers	Phosphatidyl choline 95% (Epikuron 200)
	Soy lecithin (Lipoid S 75, Lipoid S 100)
	Egg lecithin (Lipoid E 80)
	Poloxamer 188 (Pluronic F 68)
	Poloxamer 407
	Poloxamine 908
	Polysorbate 80
	Cremophor EL
	Solutol HS 15
Co-emulsifiers	Tyloxopol
	Taurocholate sodium salt
	Taurodeoxycholicacid sodium salt
	Sodium dodecyl sulphate
	Sodium glycocholate
	Sodium oleate
	Cholesteryl hemisuccinate
	Butanol
Cryoprotectants	Trehalose, Glucose, Mannose, Maltose, Lactose, Sorbitol, Mannitol, Glycine, Polyvinyl pyrolidone (PVP), Polyvinyl alcohol (PVA), Gelatin
Charge modifiers	Stearylamine
	Dicetylphosphate
	Dipalmitoyl phosphatidyl choline (DPPC).
	Dimyristoyl phophatidyl glycerol (DMPG).
Agents for improving circulation time	Polyethyleneglycol, poloxamer
Preservatives	Thiomersal

In the hot HPH technique, the lipids are melted by heating them to 5 -10 ^{0} C above their melting points and the drug is dispersed. The drug loaded lipids are dispersed in hot aqueous surfactant solution maintained at the same temperature. A hot preemulsion is formed by high speed stirring, generally by a high shear mixing device (Ultra – Turrax). The hot pre-emulsion is then processed in a high pressure homogenizer, at a temperature above the melting point of the lipids. The homogenization process is repeated till desired average particle size is obtained. The obtained nanoemulsion recrystallises upon cooling down to room temperature forming SLN.

The cold HPH technique is suitable for processing hydrophilic or temperature labile drugs. The drug is dispersed in the melted lipids followed by rapid cooling by means of liquid nitrogen or dry ice. This is then milled to microparticles by means of a ball mill or mortar. A pre-suspension is formed by high speed stirring of the particles in a cold aqueous surfactant solution. This pre-suspension is then homogenized at or below room temperature forming SLN. Thus cold homogenization is claimed to overcome the problems of temperature-induced drug degradation and drug distribution into aqueous phase during homogenization. However, the drug does get exposed to elevated temperature when dispersed in the molted lipid and when heat is generated during the homogenization process.

Artemisia arborescens L, a essential oil has been incorporated in Compritol 888 ATO as lipid matrix and sodium cocoamphoacetate as surfactant using hot high-pressure homogenization technique (Francesco Lai et al., 2006). The authors reported encapsulation efficiency was in the range of 87-92 % and particle size range of 199 – 207 nm. SLN formulation was stored for 60 days at different conditions (RT, 40 ^{0} C, 4 ^{0}C) and it was observed that particle size was less than 600 nm over the stability period. Oils are chemical instable in the presence of air, light, moisture, and high temperatures that can determine the rapid evaporation and degradation of some active components. But incorporation and stability of Artemisia arborescens L in SLNs shows that volatile compounds can remain stable in terms of Particle size, zeta potential and entrapment efficiency.

2.1.2.2 Microemulsion technique:

Microemulsions are clear, thermodynamically stable dispersions, composed of lipophilic phase (lipid), a surfactant, co-surfactant and water. Gasco & coworkers have developed and patented (Gasco, M.R., 1993) a suitable method for the preparation of SLN via microemulsions which has been adapted and/or modified by different labs (Cortesi R et al., 2002, Gasco MR., 1997, Igartua M et al., 2002, Bondi ML et al., 2007). First of all, the lipid (fatty acid/glyceride) is melted and the drug is dispersed in the molten lipid. A mixture of water, surfactant and co-surfactant is heated to a temperature at least equal to the melting temperature of the lipid. This aqueous surfactant solution is added to the lipid melt under mild stirring to obtain transparent microemulsion. This microemulsion is then dispersed in water at 2 - 10^0C under mild mechanical stirring. Typical ratio of hot microemulsion to cold water is in the range of 1:25 to 1:50. The excess water is removed either by ultra-filtration or by lyophilization in order to increase the particle concentration. The drawback of this technique is that high concentrations of surfactants and co - surfactants are necessary for formulation purposes, which is not desirable with respect to regulatory purposes and applications.

The microemulsion technique has been utilized for developing and stabilizing Curcuminoids loaded SLNs (Tiyaboonchai W et al., 2007). The workers optimized different processing parameters and found that the amount of lipid and emulsifier were critical factors for controlling particles size and entrapment efficiency. The curcumoids and curcuminoids loaded into SLNs were incorporated into a cream and its photostability was studied for 6 months. The percentages of the remaining curcumin, bisdemethoxycurcumin, and demethoxycurcumin were 91, 96, and 88, respectively in absence of sunlight while in the presence of sunlight, the values were 71, 83, and 62, respectively. It was concluded that the light and oxygen sensitivity of curcuminoids was strongly reduced by incorporating them into SLNs.

Large scale production of SLNs by the microemulsion technique also appears feasible and is at present under development at Vectorpharma (Trieste, Italy) (Boltri L et al.,1993). For the scale up operation, the microemulsion is prepared in a large, temperature-controlled tank and then pumped from this tank into a cold water tank for the precipitation step. Critical process parameters during the scaling up are the temperatures

of the microemulsion water, the temperature flow in the aqueous medium and the hydrodynamics of mixing. It is required that these should change as little as possible during scale upto maintain the same product characteristics (Muller RH et al., 2000).

2.1.2. 3 Solvent emulsification-evaporation or diffusion method

In the solvent emulsification-evaporation technique, the lipid is dissolved in a water-immiscible organic solvent (e.g. cyclohexane, chloroform) that is emulsified in an aqueous phase containing surfactants (Sjostrom B et al., 1995, Shahgaldian P et al., 2003, Dubes A et al., 2003).Upon continuous stirring, the solvent evaporates and results in precipitation of the lipid. This method is highly suitable for thermolabile drugs as it avoids use of heat for preparation of the SLNs. However, these dispersions are generally quite dilute, and need to be concentrated by means of ultra-filtration or evaporation.

In the solvent-diffusion technique, partially water miscible solvents (e.g. benzyl alcohol, ethyl formate) are employed (Dubes A et al., 2003, Trotta M et al., 2003). First of all, they are mutually saturated with water to ensure initial thermodynamic equilibrium of both liquids. Then a transient oil-in-water emulsion is passed into water and stirred continuously. This leads to solidification of dispersed phase as lipid nanoparticles due to diffusion of the organic solvent. Similar to the production of SLNs via microemulsions, the dispersion is fairly dilute and needs to be concentrated by means of ultra-filtration or lyophilization. Average particle sizes around 100 nm and very narrow particle size distributions can be achieved by both solvent evaporation based methods.

Trotta et al prepared SLNs by this technique using glyceryl monostearate and different pairs of surfactant mixtures. By using lecithin and taurodeoxycholic acid sodium salt and glyceryl monostearate (2%-5%), the mean diameters of the SLNs obtained were 205 and 320 nm with benzyl alcohol and butyl lactate, respectively (Hu FQ et al., 2002).

2.1.2. 4. Solvent Injection Method

The basic principle of the solvent diffusion method has been extended for the formation of SLNs by solvent injection method. Here, the lipids are dissolved in a water miscible solvent or water miscible solvent mixture and rapidly injected through an injection needle into an aqueous phase containing surfactants. Normally used solvents in this method are

acetone, isopropanol and methanol. Schubert and Muller-Goymann prepared SLNs by this method wherein the effect of process parameters such as volume of solvent injected, lipid concentration, emulsifier concentration in the aqueous phase, viscosity in the aqueous phase and ethyl acetate concentration in the organic phase on particle size was studied. Particle size was in the range of 80-300 nm, depending on the process parameters employed. About 96.5% of the employed lipid was transformed into SLNs and the formation of SLNs seemed to be diffusion controlled (Schubert MA & Muller-Goymann CC ., 2003).

This method offers clear advantages over the existing methods such as the easy handling and a fast production process without technically sophisticated equipment like high pressure homogenizer. A disadvantage of the 'solvent injection' method is the use of organic solvents. Although some of the solvents are pharmaceutically acceptable, they cannot be used for certain routes like parenteral, ocular etc. as any residual solvent may harm the patient.

2.1.2.5 w/o/w double emulsion method

The double emulsion method has been introduced as a novel method based on solvent emulsification - evaporation for the preparation of SLNs loaded with hydrophilic drugs (Cortesi R et al., 2002). Here, the hydrophilic drug along with a stabilizer is encapsulated in the internal water phase of a w/o/w double emulsion. The stabilizer is claimed to prevent drug partitioning to the external water phase during solvent evaporation. This technique has been used for the preparation of sodium cromoglycate-containing SLNs. However, the average size was in the micrometer range so that the term "lipospheres" rather than SLNs should be used for these 'SLNs'.

2.1.3 Characterization of SLNs:

A thorough characterization of the lipid nanoparticles is a necessity for the quality control of the formulation. However, like any colloidal system, the characterization of SLNs is a serious challenge.

2.1.3.1 Particle characterization:

Particle characterization of SLNs includes its Particle Size measurement, Surface Charge and Morphology. Different parameters and their relevant methods are enlisted in Table 1. Amongst all the methods used to measure particle size, Photon Correlation Spectroscopy (PCS) is the most widely employed. Particle size of the SLNs is influenced by different factors such as lipid matrix, drug to lipid ratio, surfactant blend, viscosity of lipid and aqueous phase and production parameters. SLNs prepared from different matrices were found to give mean particle sizes in the lower nanometric range (Westesen K et al., 1993).

Surface charge: Zeta potential is used as a measure of surface charge. This is valuable in preventing aggregation and imparts the physical stability to formulations. At higher zeta potential, particle aggregation is less likely to occur, due to electrical repulsions (Muller R.H., 1991). The uptake by the Reticuloedothelial system (RES) can be minimized by controlling Zeta Potential because the clearance behavior and tissue distribution of intravenously injected particulate drug carriers are greatly influenced by their size, surface characteristics and opsonization process. In general, particles with charged surface showed reduced uptake by organs of RES (Moghimi SM et al., 2001).Thus, Zeta potential measurement will help in designing dosage form with reduced RES uptake.

Morphology: Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM) are very useful in determining the shape and morphology of lipid nanoparticles and also allow determination of particle size and size distribution. SEM uses electrons transmitted from the specimen surface, while TEM uses electrons transmitted through the specimen. SEM has high resolution and the sample preparation is relatively easy. TEM allows visualization of nanoparticles after freeze-fracturing and freeze substitution. Another advanced microscopic technique used for characterization of nanoparticles is atomic force microscopy (AFM). AFM provides a three-dimensional surface profile unlike the electron microscopy which provides two-dimensional image of a sample. In this technique, the force acting between the surface and probing tip results in a spatial resolution up to 0.01 nm. It allows the analysis of non-conductive, hydrated and solvent containing samples (Manjunath K et al., 2005).

2.1.3.2 Crystallinity and polymorphic nature:

The Crystallinity as well as its polymorphic behavior of lipids strongly influences drug incorporation and release rates. Crystalline solids (lipids) have an orderly arrangement of units. The presence of emulsifiers, the preparative method, and the high dispersity as well as small particle size of the colloidal system influences crystallinity of lipids in the SLNs. Assessment of crystallization kinetics and polymorphism of the dispersed lipid in SLN suspensions has given insight into the complex stability aspects of the lipid nanoparticles. Moreover the crystallization process may also result in change of surface charge and cause stability problems such as gelling or expulsion of the incorporated drug (Freitas C, Muller RH., 1998, Bunjes H et al., 2000). Basic techniques used to investigate the states of the lipid are Differential Scanning Calorimetry (DSC) and X-Ray diffractometry (XRD) (Table 2.2).

Table 2.2. Summary of Characterization Techniques of Lipid Nanoparticles

Parameter	Method	Reference
Particle size	Photon Correlation Spectroscopy	Westesen K et al., 1993, Muller RH et al., 1993
	Laser Diffraction Spectroscopy	Schubert MA et al., 2006, Jores K et al., 2005
Surface charge	Zeta Potential	Freitas C et al., 1998, Schwarz C et al., 1999, Kuo YC et al., 2007, Radomska SA., 2007
Morphology	Scanning Electron Microscopy	Dubes A et al., 2003, Eldem T et al., 1991, Hatziantoniou S et al., 2007
	Transmission Electron Microscopy	Jores K et al., 2004 Schubert MA et al., 2006
	Atomic force microscopy	Dubes A et al., 2003, Shahgaldian P et al., 2003
Crystallinity & Polymorphic nature	Differential Scanning Calorimetry	Jenning A et al., 2000, Freitas C & Muller RH, 1999, Souto EB et al., 2006
	X Ray Diffractometry	Westesen K et al., 1993, Bunjes H et al., 2007
Structure & drug distribution	Nuclear Magnetic Resonance	Wissing S et al., 2004, Jenning V et al., 2000, Morel S et al., 1998
	Electron Spin Resonance	Jores K et al., 2003, Braem Braem C et al., 2007, Pegi A et al., 2003

	Parelectric Spectroscopy	Braem C et al., 2007, Lombardi Borgia S et al., 2005
	Raman Spectroscopy	Jores K et al., 2005, Saupe A et al., 2006
Encapsulation efficiency & drug loading capacity	Ultrafiltration	Venkateswarlu Vet al., 2004, Jian You et al., 2007
	Gel filtration	Li Y et al., 2006, Antonietta CM et al., 2006

2.1.3.3 Structure of SLNs and drug distribution:

The information on drug distribution and mobility in SLNs is very important for its design and evaluation as a potential drug carrier system. Drug incorporation implies the localization of the drug in the solid lipid matrix. However, several alternative incorporation sites (micelles, mixed micelles, liposomes, drug-nanosuspensions) exist in addition to the complex physicochemical status of the lipid (supercooled melt and several modifications). Formation of stacked lamella in triglyceride formulations have been reported by using small angle X-ray scattering (SAXS) and TEM. Nishikawa M et al. reported that particle shape and an increase in the concentration of the lipid affected particle self assembly. The formation of stacked lamella may accelerate formation instabilities such as gelation and particle growth and also has an impact on the rheological behavior of the formulations (Nishikawa M et al., 2001, Nishikawa M et al., 1993). Large number of hydrophilic drugs are postulated to be incorporated into SLNs. However, little data exists on the localization site and the physical state of the drug molecules. By electron diffraction study, Lukowski et al observed that the lipid and aciclovir nanoparticles were coexisting and aciclovir was not molecularly dissolved in the lipid matrix (Lukowski G et al., 1997).

Paraelectric spectroscopy (PS) is based on the frequency dependency of dipole density and dipole mobility when exposed to a changing electromagnetic field. This relatively novel method of PS has proven to be a versatile tool as it gives insight into the experimental details and function of the open-ended coaxial probes to be used when performing measurements on liquid dispersions or emulsions. This method has been

employed to determine the structure and dynamics of SLNs in topical formulations. (Sivaramakrishnan R et al., 2004, Craig D., 2001, Asami K., 2002, Schönhals A & Kremer F., 2003).

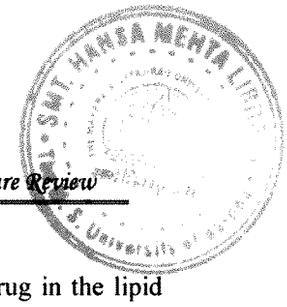
Proteins and antigens intended for therapeutic purposes may be incorporated or adsorbed onto SLNs for improved protein stability, to avoid proteolytic degradation, and to provide sustained release of the incorporated molecules. Important peptides such as cyclosporine A (Ugazio E et al., 2002), insulin (Zhang N et al., 2006), calcitonin (Garcia Fuentes M et al., 2005) and somatostatin (Reithmeier H et al., 2001) have been incorporated into SLNs.

2.1.3.4 Encapsulation efficiency and drug loading capacity:

The encapsulation efficiency of the SLNs is based on the separation of lipid and aqueous phases of the dispersion. To separate the dispersion medium, different methods like Ultrafiltration (Cheng SY, Zhu JB., 2002), Gel filtration (Sephadex column) (Jain SK et al., 2005) and dialysis (Heiati H et al., 1998) have been employed. Ultrafiltration consists of membrane filter (molecular weight cut-off 3 KD- 20 KD) at the base of the sample recovery chamber. The sample is placed in the outer chamber and subjected to centrifugation so that the aqueous phase moves into the sample recovery chamber through membrane filter. Analyzing drug concentration in an aqueous phase gives entrapment efficiency. The dialysis method is restricted, because the diffusion of the drug through the membrane might be the rate limiting step. It is also dubious whether this method can distinguish between adsorbed and incorporated drug.

Other parameter to be considered in the selection of a suitable lipid is loading capacity. Loading capacity is generally expressed in percent related to the lipid matrix. Tetracaine, etomidate and prednisolone have been used as model drugs to assess the drug loading capacity and entrapment efficiency of SLNs by Westsen et al (Westesen K et al., 1997). The drugs were incorporated in concentrations of 1%, 5% and 10% based on the lipid mass. The entrapment efficiency achieved with tetracaine and etomidate varied between 80% and 98% depending on SLN composition. With prednisolone, greater than 70% entrapment was achieved in tribehenin.

Drugs can usually be incorporated in the range of 5-10%. In case of ubedicarnone, the loading was found to be upto 40% (Bunjes H et al., 2001). Percentage drug loading



usually depends on the molecular weight of the drug, solubility of the drug in the lipid and the type of lipid used.

2.1.3.5 *In vitro* release:

Drug release from SLNs occurs by the diffusion of the drug molecules through the lipid matrix and in vivo degradation of lipid matrix. The main factors influencing release of drugs from SLNs are the method of preparation, solubility of the drug in the lipid, drug/lipid interactions, temperature employed during the preparation, surfactant used, composition of lipid matrix and particle size (Almeida AJ & Souto, 2007, Haynes CA & Norde W, 1994). Solubility of the drug in the lipid and drug/lipid interactions appear to influence the rate of release, perhaps through the partition coefficient. The temperature employed during the preparation of the SLN affects the position of the drug in SLNs and thereby influences the drug release. The aqueous solubility of the drug will be enhanced at elevated temperatures, leading to the drug localization at the surface.

The drug release can be immediate, prolonged or biphasic. Burst release from tetracaine and etomidate loaded glycerylmonobehenate SLNs has been observed (Annette zur et al.,1998). Prolonged /sustained release was observed with SLNs loaded with paclitaxel (Cavalli R et al., 2000), camptothecin (Yang SC et al., 1999), doxorubicin and idarubicin (Cavalli R et al., 1993). Biphasic release was observed with clobetasol propionate (Hu FQ et al., 2002) and 3-azido- 3-deoxythymidine palmitate (AZT-P) (Heiati H et al., 1997). Burst release is common in protein-containing SLNs, frequently followed by a well-defined slow release period (Garcia Fuentes M et al., 2005, dos Santos IR et al., 2002, Hu FQ et al., 2004, García-Fuentes M et al., 2002, Almeida AJ et al., 1997). Although the burst release provides an initial dose (Muller RH et al., 2000), it is not desirable for a controlled release formulation. After a decade of publications concerning protein incorporation in solid lipid particles, data on release mechanisms and full kinetic characterization are still scarce. In conclusion, Data so far available shows that prolonged *in vitro* release and subsequently sustained in vivo effects can be achieved for various pharmaceutical proteins incorporated into SLNs (Del Curto MD et al., 2003, Müller RH et al., 2006, Bekerman T et al., 2004).

2.1.4 Applications of SLNs:

2.1.4.1 Parenteral administration:

SLNs are generally injected either intravenously, intramuscularly or subcutaneously or to the target organ. The advantages the of SLNs for parenteral application are their excellent physical stability, protection of incorporated labile drugs from degradation, controlled drug release depending on the incorporation model, good tolerability and site-specific targeting. However they do have disadvantages like insufficient loading capacity, drug expulsion due to polymorphic transition during storage and dilute state of the dispersions (Shenoy VS et al., 2005, N. Scholar et al., 2005). SLNs have been reported to be accumulated in the Mononuclear Phagocytic Systems (MPS) or provide a sustained release depot of the drug when administered subcutaneously (N. Scholar et al., 2001). After intravenous administration, paclitaxel-loaded SLNs led to higher and prolonged plasma levels of paclitaxel. Both paclitaxel loaded non-pegylated and pegylated SLNs exhibited a low uptake by the liver and spleen macrophages but an increased uptake in the brain (Muller RH et al., 2000, Cavalli R, Brioschi A et al., 2007).

The safety and efficacy of the excipient plays important role in case of parenteral application. It should be kept in mind that GRAS (Generally Recognized as Safe) surfactants must be used for the preparation of SLNs to obtain approval and a good in vivo tolerability (Wissing SA et al., 2004). The comparison between polyester nanoparticles and SLN cytotoxicity showed that SLNs were ten-fold (Müller RH et al., 1996) or even twenty-fold (Müller RH et al., 1999) less toxic to human granulocytes compared to the polyester nanoparticles. Studying the pharmacokinetics of two anticancer agents, camptothecin and doxorubicin, drug accumulation into the brain was observed after both oral and I.V. administration when loaded into SLN (Yang SC et al., 1997, Zara GP et al., 1999). Although SLNs are of great clinical interest for parenteral administration, studies on the metabolic aspects haven't been explored as yet. In future, this area would be a matter of interest in understanding the in vivo metabolic activity of SLNs.

2.1.4.2 Oral administration

Oral delivery of drugs incorporated in SLNs is gaining interest as lipid provides protection of drug from chemical as well as enzymatic degradation, thereby delaying the in vivo metabolism. Due to small particle size, SLNs may exhibit bioadhesion to the GIT wall or enter the intervillar spaces thus increasing their residence time in the GIT that result in enhanced bioavailability (Lian Dong Hu et al., 2004, R.H. Muller et al., 2006). Reduction in particle size also leads to increase in area under plasma versus time curve, enhanced onset of action, peak drug level, reduced variability, and reduced fed/fasted effects. In vivo studies performed on orally administered lipid nanoparticles containing tobramycin (Cavalli R et al., 2000, Bargoni A et al., 2001), rifampicin, isoniazid and pyrazinamide (Pandey R et al., 2005) have been reported. In addition, orally administered SLNs containing proteins like calcitonin and insulin have shown promising results (Zhang N et al., 2006, García-Fuentes M et al., 2005).

Increased bioavailability and prolonged plasma levels have been described after peroral administration of cyclosporine containing lipid nanodispersions to animals (Penkler et al., 1999). However, the microclimate of the stomach favors particle aggregation due to its acidity and high ionic strength. The question concerning the influence of the stomach and pancreatic lipases on SLN degradation in vivo remains open, too.

2.1.4.3 Topical application:

Topical products based on SLN technology are having potential as short time - to market products. SLN is considered to be the next generation of delivery system after liposomes (Muller RH et al., 1998). Since the second half of the nineties, there has been an increasing interest in investigating the SLNs for dermal application, especially for cosmetic use. Cosmetic molecules incorporated include retinol and retinyl palmitate (Jenning V et al., 2001), vitamin E and vitamin E acetate (Dingler A et al., 1999) and coenzyme Q 10 (Wissing S et al., 2004). An example of pharmaceutical actives is the incorporation of corticoids, such as prednicarbate (Gasco MR et al., 1997).

SLNs possess a number of advantages for the topical route of administration. Their small particle size permits SLN to have close contact with the stratum corneum and thereby increase penetration of encapsulated drug into the viable skin. Sustained release of the drug from SLNs ensures that the drug is supplied to the skin over a prolonged period and thereby reduces systemic absorption. SLNs show occlusive properties as they form a film on the skin, which reduces transdermal water loss. Increase of water content in the skin reduces the symptoms of atopic eczema and also improves the appearance of healthy human skin. Occlusion also favors drug penetration into the skin (Jenning V et al., 2000). SLNs have also been shown to enhance the stability of active molecules compared to o/w emulsions. The stabilizing effect of the lipid nanoparticles was demonstrated for retinol. The aqueous lipid nanoparticles suspension contained 0.5% retinol, 4.5% Miglyol 812 and 10% Compritol (solid lipid) (total of lipid particle mass 15%) and 1.5% surfactant as stabilizer. After 3 months of storage at 40 °C, about 60% of retinol remained intact. In contrast to this, retinol degraded in o/w emulsions to remaining 40% within three months (Jenning V et al., 2001). SLNs ensure enhanced skin bioavailability and targeting of actives. Increased penetration of coenzyme Q 10 was described after application of a Q 10-loaded SLN suspension in comparison to solutions in isopropanol and liquid paraffin (Sivaramakrishnan R , 2004). Localization of corticoid prednicarbate loaded SLNs in the upper skin compared to an emulsion based formulation minimized side effects such as skin thinning.

2.1.5 Commercialization of SLNs:

Commercial feasibility is the most desired characteristic of any novel delivery system. A system that has great efficacy but poor commercial feasibility is merely futile. The commercial feasibility of any delivery system is governed by the availability of a large scale production method yielding a product of a quality that is acceptable by the regulatory authorities (e.g. FDA) and cost of the material. Considering these facts, the scenario is promising in case of SLNs. As SLNs require easily available reasonably priced triglyceride lipids, the material cost is much less than carriers like Poly Lactic Glycolic Acid (PLGA), Poly Lactic Acid (PLA), Polycaprolactone (PCL) or phospholipids. Moreover, as described earlier, SLNs are mainly manufactured by using

high-pressure homogenizers which are already used for the manufacturing of parenteral nutrition products since many years
(Muller et al., 1996). It may be used even without any modification (e.g. for production of SLNs by hot homogenization technique). Muller et al have successfully demonstrated the feasibility of scale up of SLN production with batch size varying from 2 kg to 150 kg. The production of SLNs on these lines can be performed in discontinuous or continuous modes (Müller RH et al., 2000, Gohla SH & Dingler A., 2001). The scale up and manufacturing is also possible when microemulsions are used as a template for SLN production (Muller RH et al., 2000). Gasco et al. have demonstrated the feasibility of using the microemulsion technique by conducting scale up studies (Marengo E et al., 2000).

2.1.6 Current and Future Developments:

Lipid nanoparticles have shown applicability in the encapsulation of various drugs as well as biopharmaceutical and biotechnological products including proteins, peptides and vaccines. Lipid nanoparticles have also shown promise as a delivery system in cancer therapeutics. The same may be extended in various ailments including osteoarthritis as well as gene delivery. The applicability is further fortified by the production methods that have commercial feasibility. Over the years, Solid lipid nanoparticles have metamorphosed into further improved delivery systems in the form of Nanostructured Lipid Carriers and Lipid Drug Conjugate that are anticipated to make inroads into the therapeutic and diagnostic roles of lipid nanoparticles in the near future. The potentiality of the lipid nanoparticles in the area of gene transfection is still being explored. One important aspect that these lipid based systems have given to researchers and industry is the opportunity for patentability. In recent years, numbers of research groups have been successful in receiving patent grants in this area. With the development of NLC and LDC, the feasibility of developing and patenting newer delivery systems will increase.
These lipid based nanoparticles may replace few of the problematic carriers in the near future due to their biocompatibility, production feasibility and scalability. Their application in the field of diagnostics and cosmetics also hold great potential. The various generations of SLNs have shown promise in drug targeting at all levels including organ,

tissue and cell targeting. They have the potential of being the ideal 'tailor made carrier' that can carry any drug molecule and deliver it to any part of the body in the required manner.

2.2 Nanosuspension:

2.2.1 Introduction

The formulation of poorly water-soluble drugs has always been a challenging problem faced by pharmaceutical scientists as at present 10% of the present drugs are poorly soluble and it is expected to increase because approximately 40 % or more of the new chemical entities being generated through drug discovery programmes are poorly water-soluble (Lipinski 2002) and even 60% of drugs coming directly from synthesis have a solubility below 0.1 mg/mL (Liversidge E.M.2002). The problem is even more pronounced than in the past because more and more new drugs are poorly soluble in aqueous media and simultaneously in organic media.

Receptor-based screening studies frequently lead to false acceptance of poorly soluble drugs. Many poorly water-soluble drugs show good activity in simple in vitro screening because of interaction with hydrophobic receptor domains. The recourse is to alter the drug environment, alter chemical structure of the drug, or modify the lattice structure of the solid. Modification of lattice structure, or crystallinity, may temporarily enhance solubility by affecting molecular cohesion within the solid. Altering chemical structure may drastically change pharmacological activity by modifying the affinity of drug for its receptor. Development of water-soluble derivatives is costly because of the need to demonstrate efficacy and safety of the new chemical species. In many cases formulation by any means may be viable. Limited membrane transport, despite strong receptor interaction in vitro, is an obvious roadblock that is difficult to overcome without synthetic modification of the drug.

In the case of drugs being poorly soluble in aqueous media, a range of formulation approaches are available (Muller R. H 1997) such as the use of solubilizing solutions, complexing agents such as cyclodexthns, and mixtures of water with organic media (e.g., water- ethanol, water - propylene glycol). However, for many drugs these approaches do not lead to a sufficiently high increase in solubility, dissolution velocity, and subsequent bioavailability. Especially for drugs that are simultaneously poorly soluble in water and organic media, these approaches are of limited success (e.g., solubilizing solutions) or cannot be used at all (e.g., mixture of water and organic media).

The use of solubilizing excipients in drug solubilization is primarily limited by their toxicity, their use at elevated concentration, or a combination of both factors. Alteration of the formulation vehicle is the most attractive option from the aspect of development cost time. However, complete solubilization of a drug with very low intrinsic solubility may be very difficult achievement.

The ability to form inclusion complexes with cyclodextrins is also limited for those compounds having very low intrinsic solubility. Solubility of the complex, viscosity of the resulting formulation limits the feasible concentration of cyclodextrins and only a fixed solubility enhancement can be realized at this critical level. Furthermore, a molar excess of cyclodextrins must usually be added to drive the equilibrium toward complexation. Because of their high molecular weight, large quantities of cyclodextrins may be required to reach the desired drug concentration. For example, the molecular weight of 2-hydroxypropyl- B-cyclodextrins (HPBCD), is approximately 1400 (degree of substitution is approximately 4). The commercial product SPORANOX TV3 contains 400 mg of HPBCD per 10mg of itraconazole (Janssen, SPORANOX package insert).

High excipient levels may also limit the route of administration. Oral delivery, for example, may be impractical because the tablet or capsule would be too large to swallow. Very low drug solubility in aqueous media may also hamper preparation of an emulsion. At fixed temperature and partitioning equilibrium, the drug concentration in the oil phase is limited by the intrinsic water solubility and the fractional oil volume. Furthermore, number of water-insoluble drugs also has limited solubility in oils. For example, the antimalarial drug artemisinin has poor solubility in both water and oil (Ashton et al., 1999). This dual insolubility often applies to high-melting solids for which a substantial fraction of the free energy of solution is attributable to high lattice energy. The same reasoning applies to liposomes other structured lipid dispersions. High drug loading may be limited by solubility in the aqueous voids, solubility within the hydrophobic lipid compartment, respective volumes of these compartments.

The principle limitation of all these approaches is that the drug needs to possess certain physicochemical properties (e.g. solubility in oils) or to fit' to the solubilizing principle (e.g. having the right molecular size to fit into the cyclodextrin ring). These formulation approaches were of limited success as clearly demonstrated by the relatively low number

of products on the market being based on such technologies. For example, there are only three main o/w emulsion products on the market with the drugs Diazepam, Etomidate and Propofol (Sznitowska M et al, 2001) would be much more elegant to have one universal formulation approach to process any poorly soluble drug. This is useful for drugs being poorly soluble in aqueous and organic media, excluding all formulation approaches involving any solvent mixture.

Much more attractive are the "generalized" formulation approaches; a very simple and effective one is micronization. Any drug can be micronized independent of its structure. In the past, this worked for quite a number of drugs; however, it is not effective anymore for the new drugs exhibiting even lower saturation solubilities. Consequently, the next step after "micronization" was "nanonization," which means that, instead of producing ultrafine drug micropartides (mean diameter approximately 2—10μm), one produces superfine drug nanocrystals (approximately 100 nm to just below 1000 nm) **(Muller et al 1999).** To be more precise, a drug showing poor solubility means that it has a low saturation solubility which is typically correlated with a low dissolution velocity dc/dt. The terms "solubility" and "saturation solubility" are used synonymously. In case a drug - despite having low saturation solubility - dissolves very fast and has a good permeability, this should not cause any bioavailability problems. However, this is normally not the case with poorly soluble drugs because, according to the law of Noyes—Whitney, the dissolution velocity 'dc/ dt' is directly correlated to the saturation solubility 'c'. The principle of micronization is to increase the surface area 'A' to which 'dc/dt' is also directly correlated. Nanonization is taking the increase in surface area 'A' one dimension further than micronization. There are also some additional features of drug nanocrystals improving the dissolution velocity (increase in dissolution pressure, reduction of diffusional distance 'h'), which further improve the enhancement of bioavailability.

Nanosuspensions of drugs are sub-micron colloidal dispersions of pure particles of drug, which are stabilized by surfactants (Na, G. C. et al., 1999). They are distinguished from nanoparticles, which are polymeric colloidal carriers of drugs (Horn, D. & Rieger, J., 2001), and from Solid Lipid Nanoparticles, which are lipidic carriers of drugs (Muller, R. H. et al.2000).

2.2.2 Formulation theory:

Nanoparticles can be formed by building particles up from the molecular state, as in precipitation, or by breaking larger micron-sized particles down, as in milling. In either case, a new surface area, ΔA, is formed, which necessitates a free-energy (ΔG) cost as defined by $\Delta G = \gamma s/l \cdot \Delta A$, in which $\gamma s/l$ is the interfacial tension. This arises because water molecules incur fewer attractive forces with other water molecules when located at a free surface. The system prefers to reduce this increase in surface area by either dissolving incipient crystalline nuclei, in the case of precipitation, or by agglomerating small particles, regardless of their formation mechanism. This tendency is resisted by the formulator through the addition of surface-active agents, which reduce the $\gamma s/l$ and therefore the free energy of the system (FIG. 2.2).

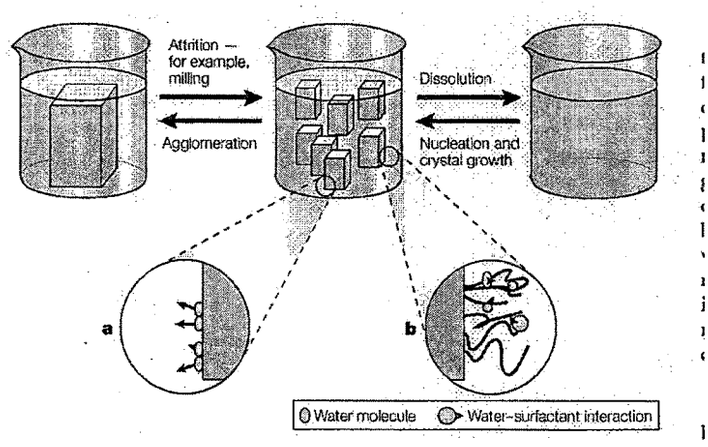


Figure 2.2 Creation and stabilization of nanoparticles, from the perspective of surface energetics.

a) In the case of unfavorable energetics, hydrophobic crystal surface directly contacting water molecules leads to crystal agglomeration, because water molecules are energetically driven to leave the surface, as shown by the arrows.

b) A surfactant stabilized crystal surface reduces interfacial tension by allowing attractive water surfactant interactions. The crystal surface is stabilized and shows reduced tendency to agglomerate.

These agents confer immediate protection and are more effective when present at the time of creation of the new, fresh surface than if added afterwards. By virtue of their complementary properties, surfactants of two classes are utilized: charged or ionic surfactants, which effect an electrostatic repulsion among the particles; and non-ionic polymers, which confer a steric repulsion that is, they resist compression. If the particles approach each other too closely, they will agglomerate. This must be prevented to ensure a stable system. Energetically, this requires the placement of a sufficiently high energy barrier at relatively long separation distances, to prevent the particles coming too close together. Therefore, a non-ionic polymeric surfactant is also used that coats the surface with a hydrophobic chain, and permits a hydrophilic tail to project into the water. Compression of the polymeric coating, as by the approach of a similarly coated particle, causes loss of entropy and is therefore unfavorable. This provides the necessary repulsive barrier between two neighbouring particles. The polymeric coating performs a dual role: inhibiting crystal growth and reducing particle size (Ziller, K. H. 1990)

Both electrostatic and steric mechanisms are enabled by combining polymers and ionic surfactants, which therefore complement each other. Entropic steric interactions are inherently more sensitive to temperature fluctuations than is electrostatic repulsion. Therefore, temperature cycling could disrupt a suspension stabilized only by polymer. To prevent this, ionic surfactants are used as well. There is a synergy between the two, because adding a neutral polymer to a surface stabilized with an ionic surfactant permits greater coverage by the ionic surfactant. This occurs because self-repulsion of the charged surfactant molecules is minimized, which therefore permits closer packing.

The repulsive energy of two similarly charged particles is given by the equation

$$VR = (\varepsilon a \psi 0/\kappa 2) \ln [1 + \exp(-\kappa Ho)]$$

Where,

a is the particle radius,

H_0 is the distance of separation between the two particles,

ε is the dielectric constant of the medium,

$\psi 0$ is the electrostatic surface potential, and

K is related to the thickness of the diffuse electric double layer .

The net repulsive energy decreases with separation of the particles (FIG. 2.3). At shorter distances of separation, there is an attractive force between the two particles due to van der Waals forces. The superposition of both these forces results in an attractive potential well provided the particles can overcome an energy barrier.

Therefore, regardless of the nature of the bulk particles, colloidal stability is determined primarily by the choice of surfactants, which affects the repulsive potentials. This does not mean that there is no effect of drug on the stability of suspensions; the drug affects the nature of the surface to which the surfactant must bind.

The design of a stable formulation for nanosuspensions can differ from that used for suspensions of larger particle size. The latter often strives for weak flocculation, which corresponds to the secondary minimum, Vs, (FIG. 2.3) and yields readily dispersible particles after agitation. This is designed to prevent slow settling, which promotes tight packing and consequent caking of the suspensions, which are then no longer dispersible (Tabibi, S. F & Rhodes, C. T.1996)

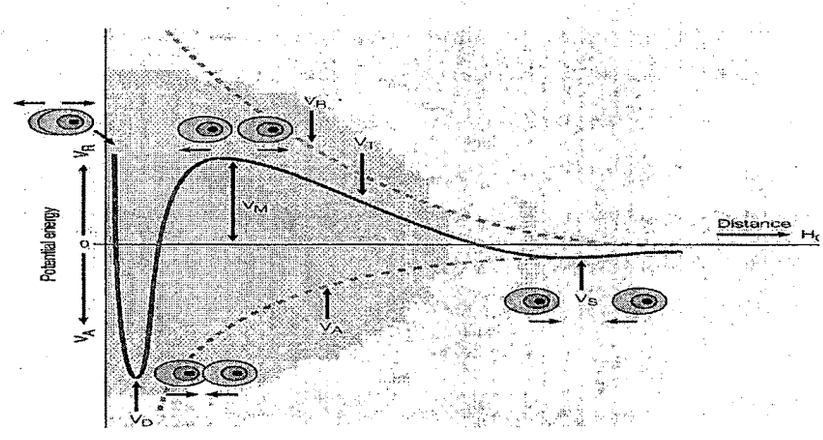


Figure 2.3 Potential energy curve for approach of two nanoparticles. The total potential energy curve V is a superposition of an attractive curve VA and a repulsive curve VR. As two particles approach, they can overcome the energy barrier VM, leading to attractive aggregation of the closely packed particles. To prevent this for microparticulate suspensions, one can formulate as a weak floc, making use of the secondary minimum (Vanderhoff, J. W.1996).

2.2.3 Manufacturing of nanosuspension:

The existing technologies can be divided into the so called 'bottom up' and the 'top down' technologies. The bottom up technologies starts from the molecules which are dissolved and precipitate them by adding the solvent to a non-solvent. The top down technologies are disintegration methods that mean various types of wet milling (List M. 1998, Gassmann P. et al 1994, Sucker H. 1994).

Examples for precipitation techniques are the hydrosols developed by Sucker (company Sandoz, nowadays Novartis), the product 'Nanomorph' by the company Soliqs/Abbott and a number of other precipitation techniques (Violante M.R. 1991, Thies J. 1998 , Kipp J.E. 2003) differing in precipitation details such as use of certain stabilizers (e.g. B.W. Muller/Kiel, Germany) (Rasenack N. 2002). Basically, the drug is dissolved in a solvent and this solution is added to a non-solvent. Addition of the solvent to the non-solvent is necessary to yield a very fine product. In the case of 'Nanomorph', amorphous drug nanocrystals are produced to further enhance dissolution velocity and solubility. The basic advantage of precipitation techniques is that they use relatively simple, low cost equipment. Scaling up is relatively easily possible by using static blenders (e.g. from the company Sulzer Chemtech) or micromixers (Institut fur Mikrotechnik Mainz/Germany) (Muller R. H. 2001). The use of a static blender maintains practically the precipitation conditions in a beaker on lab scale, stirring and mixing problems potentially occurring when moving from lab scale to a large product container.

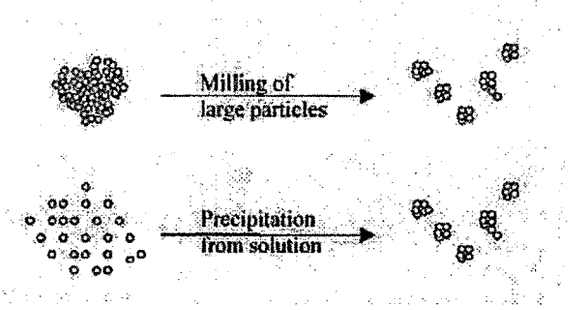


Figure 2.4: Schematic of the two general nanoparticles production techniques (Gupta Ram B. and Uday B. Kompella 2006)

However, there are basic problems associated with precipitation techniques. The particles produced need to retain their size after precipitation, particle growth to microcrystals needs to be avoided. In case a special crystalline state is given to the particle matrix (amorphous), this state needs to be maintained during the shelf life of the product to avoid a decrease in oral bioavailability. To sum up, the bottoms up techniques are not really widely used for drug nanocrystals production. Nowadays, the top down technologies of various milling techniques are more frequently used.

There are two basic disintegration technologies for drug nanocrystals:

Pearl/ball milling (wet milling)

High pressure homogenization with different homogenizer types/homogenization principles

2.2.3.1 Pearl milling (wet milling)

Wet milling is an attrition process in which large micron size drug crystals are wet milled in the presence of grinding media and a surface modifier (Liversidge G. G. 1992) In the method, the drug macrosuspension is filled into a milling container containing milling pearls from, e.g. glass, zirconium oxide, such as 95% ZrO stabilized with magnesia, zirconium silicate, and other media, such as glass, stainless steel, titania, alumina, and 95% ZrO stabilized with yttrium (Liversidge G. G. 1992) or special polymers such as hard polystyrene derivatives. The rigid grinding media is typically spherical in form, having an average size less than about 3 mm. The surface modifiers include various polymers, low molecular weight oligomers, natural products and surfactants, such as polyvinyl pyrrolidone, various grades of poloxamer, and lecithin.

Milling instruments to be used are;

1. Jet mill
2. Colloid mill (wet milling)
3. Pearl mill

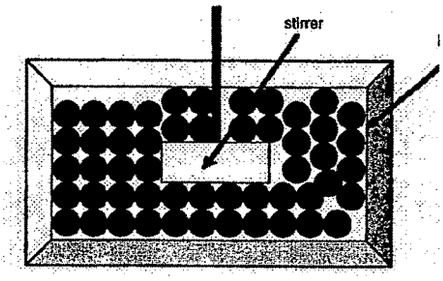


Figure 2.5. Principle of pearl mill type; pearls are moved by the stirrer.

The pearls are moved by a stirrer, the drug is ground to nanocrystals in between the pearls (Fig 2.5). The milling process is performed under controlled temperature. Processing temperatures commonly are less than 40 °C and processing pressures are up to about 20 psi. This is the basic technology developed by G. Liversidge and coworkers and nowadays used by the company Nanosystems (E. Merisko-Liversidge et al. 1996) (presently owned by Elan). First products on the market are Rapamune and Emend, launched in 2002 and 2003, respectively. The nanoparticles are typically less than 400 nm and are physically stabilized with a polymeric excipient. The registered trade name for their product is Nanocrystals. Intensive in vivo studies have been performed by NanoSystems, especially after peroral administration of the drug nanoparticles (Liversidge et al. 1996).

The milling technique clearly has some advantages:

1. Simple technology
2. low-cost process regarding the milling itself
3. Flexibility in handling the drug quantity, ranging from 1 to 400mg mL enabling formulation of very dilute as well as highly concentrated nanosuspensions.
4. Large-scale production possible to some extent (batch process).

Large-scale production is stated only to "some extent." This should be explained by a model calculation. A pearl mill of 1000 L volume containing milling pearls in hexagonal packaging has 76% pearls (760 L) and 24% void volume available for the drug suspension (240 L). The solid concentration in the suspension is limited; it should not be too pasty because such a highly viscous system is no longer processable. Assuming a

suspension with 20% solid would mean that 20% of the 240 L suspension is drug (48 L). This is equivalent to 96 kg of drug (assumed drug density: 2.0 g/mL). Depending on the density of the milling material used (glass: approximately 2—3 g/mL, zirconium oxide: 5.9 g/mL, and hard polystyrene: 1.05 g/mL), the weight of the milling material and suspension will be in the range of 1—2 tons plus the weight of the mill itself. The batch cannot be increased by just multiplying the dimensions of pearl mills (Muller R. H. et al 1997).

A basic problem when applying milling techniques is that the product contains not only nanoparticles but also a fraction of particles in the micrometer range. Jet milling-typically used for micronization - leads to relatively broad particle size distributions ranging from approximately a few hundred nanometers to about 25 μm, which means only a few percent of the product are nanoparticles. Erosion of materials from the balls has been reported when using a pearl mill (Buchmann S. Ct al. 1996). Ball mill materials used are zircon oxide and glass. Erosion of these low- toxicity materials is considered as not critical when preparing nanoparticles for oral and peroral administration (e.g., Nanocrystals in pellets or tablets). Depending on the mill type and batch volume, the milling process itself can last up to a few days or a week, which means that it can be time consuming. In addition, wet milling is a batch process. There is batch-to-batch variation detected in the quality of dispersion, processing time, crvstallinity degree of drugs, and particle size distribution. These variations will affect drug particle stability, powder flow properties, and efficiency of delivery system. Milling over a few days also brings the risk of microbiological problems, especially when performing the milling at 30°C or having dispersion media providing nutrition to bacteria.

Scaling up with pearl mills is possible; however, there is a certain limitation in the size of the mill due to its weight. Up to about 2/3 of the mill volume are the pearls leading to a heavy weight of the machinery thus limiting the maximum batch size. The batch size can be increased above the void volume (volume in between the hexagonal packaging of the pearls) using a mill with suspension circulation. The suspension is contained in a product container and continuously pumped through the mill in a circle. This increases the batch size but of course also the milling time because the required total exposure time of the drug particles per mass unit to the milling material remains unchanged.

2.2.3.2 High pressure homogenization:

Principle: During homogenization, the fracture of drug particles is brought about by cavitation, high-shear forces and the collision of the particles against each other. The drug suspension, contained in a cylinder of diameter about 3 mm, passes suddenly through a very narrow homogenization gap of 25 pm, which leads to a high streaming velocity.

In the homogenization gap, according to Bernoulli's equation, the dynamic pressure of the fluid increases with the simultaneous decrease in static pressure below the boiling point of water at room temperature. In consequence, water starts boiling at room temperature, leading to the formation of gas bubbles, which implode when the suspension leaves the gap (called cavitation) and normal air pressure is reached again. The implosion forces are sufficiently high to break down the drug microparticles into nanoparticles. Additionally, the collision of the particles at high speed helps to achieve the nano-sizing of the drug. To improve the efficiency of nano-sizing, the addition of viscosity enhancers is advantageous in certain cases as increasing the viscosity increases the powder density within the dispersion zone (homogenization gap).

In order to obtain an optimized formulation, the effect of the following process variables should be investigated.

Effect of homogenization pressure: As the homogenizer can handle varying pressures. ranging from 100 to 1500 bars, the effect of the homogenization pressure on the particle size should be investigated in each case in order to optimize the process parameters. It is expected that the higher the homogenization pressure, the lower the particle size obtained. The studies carried out on RMKP 22, 4-[N-(2-hydroxy-2-methyl-propyl)-ethanolaminoj-2,7-bis (cis-2,6-dimethylmorpholin-4-yl)-6-phenyl-pteridine, revealed that an inverse relationship exists between the homogenization pressure and the particle size (Muller & Böhm 1998; Muller & Peters 1998; Grau etal 2000).

Number of homogenization cycles: For many drugs it is not possible to obtain the desired particle size in a single homogenization cycle. Typically, multiple cycles are required. Hence, depending on the hardness of the drug, the desired mean particle size

and the required homogeneity of the product, homogenization can be carried out in three, five or 10 cycles. It is anticipated that the higher the number of homogenization cycles, the smaller the particle size obtained. The optimum number of homogenization cycles can be arrived at by analyzing the particle size and polydispersity index of the drug after each cycle.

The two homogenization principles/homogenizer types applied are:

1. Microfluidisation

2. Piston-gap homogenizers. Microfluidisation is a jet stream principle, the suspension is accelerated and passes with a high velocity an especially designed homogenisation chamber. In the 'Z' type chamber, the suspension changes a few times the direction of its flow 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. The microfluidisation technique for drug nanocrystals production (Parikh I., U. Selvaraj. 1999) has been pursued by the Canadian company Research Triangle Pharmaceuticals (RTP) (meanwhile acquired by SkyePharma PLC). A disadvantage of the technology is the sometimes high number of passes through the microfluidiser, examples in the various patents describe up to 75 passes. This is not very production friendly. In addition, from our experiences, the product obtained by microfluidisation can contain a relatively large fraction of micro- particles (especially in the case of hard drugs) thus losing the special benefits of a real homogeneous drug nanocrystal suspension.

In the knowledge of the potential problems associated with pearl/ball milling and the use of the microfluidisation principle, as an alternative a drug nanocrystal technology based on piston-gap homogenisers was developed in the middle of the 1990s. A first technology was based on homogenization of particles in pure water (Muller R.H. 1999), the trademark of the product is DissoCubes (trade name nowadays owned by SkyePharma). At the turn of the millennium, the second generation technology was developed, that means homogenisation of drug particles in non-aqueous media or in dispersion media with a reduced water content (i.e. mixtures of water with water-miscible liquids such as water—PEG or water—glycerol (e.g. isotonic suspensions for i.v. injections) (Muller R.H., Mader K., 2000). Registered trade name by the company PharmaSol GmbH/Berlin is Nanopure® (pure nanocrystals).

Advantages of High pressure homogenization method:

• Drugs that are poorly soluble in both aqueous and organic media can be easily formulated into nanosuspensions.

• Ease of scale-up and little batch-to-batch variation (Grau et al 2000).

• Narrow size distribution of the nanoparticulate drug present in the final product (Muller & Böhm 1998).

• Allows aseptic production of nanosuspensions for parenteral administration.

• Flexibility in handling the drug quantity, ranging from 1 to 400 mg mL thus enabling formulation of very dilute as well as highly concentrated nanosuspensions (Krause & Muller 2001).

Disadvantages High pressure homogenization method:

• Prerequisite of micronized drug particles.

• Prerequisite of suspension formation using high-speed mixers before subjecting it to homogenization.

2.2.4 Formulation considerations

2.2.4.1 Organic solvents: Organic solvents may be required in the formulation of nanosuspensions if they are to be prepared using an emulsion or microemulsion as a template. As these techniques are still in their infancy, elaborate information on formulation consideration is not available. The acceptability of the organic solvents in the pharmaceutical arena, their toxicity potential and the ease of their removal from the formulation need to be considered when formulating nanosuspensions using emulsions or microemulsions as templates. e.g. ethanol, isopropanol, ethyl acetate, ethyl formate, butyl lactate, triacetin, propylene carbonate and benzyl alcohol.

2.2.4.2 Stabilizers

Stabilizer plays an important role in the formulation of nanosuspensions. In the absence of an appropriate stabilizer, the high surface energy of nano-sized particles can induce agglomeration or aggregation of the drug crystals. The main functions of a stabilizer are to wet the drug particles thoroughly, and to prevent Ostwald's ripening (Muller & Bohm,

1998) and agglomeration of nanosuspensions in order to yield a physically stable formulation by providing steric or ionic barriers. The type and amount of stabilizer has a pronounced effect on the physical stability and in-vivo behavior of nanosuspensions. In some cases, a mixture of stabilizers is required to obtain a stable nanosuspension.

One concept that could prove useful in the selection of polymeric stabilizer is that of surface coverage. In principle, to fully provide steric stabilization, the polymeric stabilizers must fully adsorb onto the surfaces of the nanoparticles. While nanosuspensions can and have been formulated successfully, little attention is paid to the whereabouts of the stabilizer. Knowledge of the adsorption isotherm may help provide additional insights into the formulation efforts. Panmai and Deshpande described a convenient method for determining the adsorption isotherm of a nanosuspension, which involves the determination of the fractions of the stabilizer that are bound to the drug surface and unbound in solution for a given polymer concentration (Panmai S., Deshpande S., 2003).

The drug to stabilizer ratio in the formulation may vary from 1:20 to 20:1 and should be investigated for a specific case. Care should be taken not to add excessive surfactant as this can result in enhanced solubility and Ostwald ripening. e.g. cellulosics, poloxamers, polysorbates, lecithins and povidones, lecithin. SLNs stabilized with surfactant mixtures (Lipoid S 75/poloxamer 188 or tyloxapol/ lecithin) have lower particle size and higher storage stability (Siekmann, B.et al 1994) Uner et al. studied the influence of surfactants on the physical stability of SLNs formulation and found that 1.5% Tegocare 450 was the most effective stabilizer for the witepsol E 85 SLNs dispersion compared to Tween 80, tyloxapol and Pluronic F68 (Uner, M 2004).

2.2.4.3 Co-surfactants

The choice of co-surfactant is critical when using microemulsions to formulate nanosuspensions. Since co-surfactants can greatly influence phase behavior, the effect of cosurfactant on uptake of the internal phase for selected microemulsion composition and on drug loading should be investigated. e.g. bile salts, dipotassium glycerrhizinate, Transcutol, glycufurol, ethanol and isopropanol. Cavalli et al. used stearic acid as the lipid phase and compared an ionic surfactant cosurfactant system composed of Epikuron

100, taurodeoxycholate and monooctylphosphate with a nonionic system composed of Tween 80 and butanol [74]. The particle size of the SLN dispersion produced with the ionic surfactants was considerably smaller (70 ±2 nm) compared to the nonionic formulation (200±5 nm).

2.2.4.3 Other additives

Nanosuspensions may contain additives such as buffers, salts, polyols, osmogent and cryoprotectant, depending on either the route of administration or the properties of the drug moiety.

2.2.5 Characterization of Nanosuspensions:

The unique qualities and performance of nanoparticles as devices of drug delivery arise directly from their physicochemical properties. Hence, determining such characteristics is essential in achieving a mechanistic understanding of their behavior. A good understanding allows prediction of in vivo performance as well as allowing particle design, formulation development, and process troubleshooting to be carried out in a rational fashion. such determinations on nanoparticles. There are a large number of methods available to characterize nanoparticles. Some approaches, such as dynamic light scattering (DLS) for size, or nuclear magnetic resonance (NMR) for diffusivity, are unique to the analysis of nanoparticles compared to that of more macroscopic species. Other techniques, such as Differential Scanning Calorimetry (DSC) or X-ray diffraction, are not significantly affected by the submicrometer particle size. Rather, it is the interpretation of the results in the context of the problem at hand that renders the corresponding method relevant. Table 2.3 breaks the classification down orthogonally by summarizing the same information according to the likely properties of interest.

Table 2.3 Methods for assessing properties of nanoparticles

Property	Relevant analytical method(s)
Presence	Dark field optical microscopy
Size	Dynamic light scattering, Static light scattering, Ultrasonic spectroscopy, Turbidimetry, NMR, Single particle optical sensing, FFF Hydrodynamic fractionation, Filtration
Morphology	TEM, SEM, Atomic force microscopy
Surface charge	Electrophoretic light scattering, U-tube electrophoresis, Electrostatic-FFF
Surface hydrophobicity	Hydrophobic interaction chromatography
Surface adsorbates	Electrophoresis
Density	Isopycnic centrifugation, sedimentation-FFF
Interior structure	Freeze-fracture SEM, DSC, X-ray diffraction, NMR

Abbreviations: DSC, differential scanning Calorimetry; FFF, field fractionation; NMR, nuclear magnetic resonance; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

The essential characterization parameters for nanosuspension are:

2.2.5.1 Mean particle size and particle size distribution

The most basic property of nanoparticle is its size. The mean particle size and width of particle size distribution are important characterization parameters as they govern saturation solubility, dissolution velocity, physical stability and even biological performance of nanosuspension. Muller & Peters indicated that saturation solubility and dissolution velocity show considerable variation with the changing particle size of the drug (Muller R.H. & Peters K. 1998).

Photon correlation spectroscopy (PCS) *or* Dynamic light scattering (DLS) *or* quasi-elastic light scattering (QELS):

This technique can be used for rapid and accurate determination of the mean particle diameter of nanosuspensions (Muller & Muller 1984). It records the variation in the intensity of scattered light on the microsecond time scale (Pecora R. 2000, Chu B, Liu 2000). 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). Using standard assumptions of spherical size, low concentration, and known viscosity of the suspending medium, particle size is calculated from this coefficient.

The measuring range is approx. 3 nm to 3 pm, by using a short sample time the particles in the nanometer range are analyzed (Muller, R. H., Heinemann, S., 1992). In addition to the PCS mean diameter, a polydispersity index (PI) is obtained by characterizing the width of the size distribution. . The PI is an important parameter that governs the physical stability of nanosuspensions and should be as low as possible for the long-term stability of nanosuspensions. The PI of 0.100 still indicates a relatively narrow distribution, values of up to 0.250 are reported for parenteral fat emulsions. A polydispersity index of > 0.500 indicates a very broad distribution, no logarithmic normal distribution can definitely be attributed to such a high polydispersity index. The advantages of the method are the speed of analysis, lack of required calibration, and sensitivity to submicrometer particles. Drawbacks include the necessity of significant dilution to avoid artifacts, the need for cleanliness in sample preparation, the mathematical instability of the procedure used to extract decay constants, and the possible influence of interparticle interactions. DLS is a stand-by method for those working in the area of nanoparticles (Chu B, Liu 2000 , Ito T, 2004).

Laser Diffractometry (LD) :

Although PCS is a versatile technique, because of its low measuring range (3 nm to 3 μm) it becomes difficult to determine the possibility of contamination of the nanosuspension by microparticulate drugs (having particle size greater than 3 μm).

Hence, in addition to PCS analysis, Laser Diffractometry (LD) analysis of nanosuspensions should be carried out in order to detect as well as quantify the drug microparticles that might have been generated during the production process. Laser Diffractometry yields a volume size distribution and can be used to measure particles ranging from 0.05 - 80 μm and in certain instruments particle sizes up to 2000 μm can be measured. The typical LD characterization includes determination of diameter 50% LD (50) and diameter 99% LD (99) values, which indicate that either 50 or 99 % of the particles are below the indicated size. The LD analysis becomes critical for nanosuspensions that are meant for parenteral and pulmonary delivery. Even if the nanosuspension contains a small number of particles greater than 5 – 6 μm, there could be a possibility of capillary blockade or emboli formation, as the size of the smallest blood capillary is 5 -6 μm. It should be noted that the particle size data of a nanosuspension obtained by LD and PCS analysis are not identical as LD data are volume based and the PCS mean diameter is the light intensity weighted size. The PCS mean diameter and the 50 or 99 % diameter from the LD analysis are likely to differ, with LD data generally exhibiting higher values. The nanosuspensions can be suitably diluted with deionized water before carrying out PCS or LD analysis.

For nanosuspensions that are intended for intravenous administration, particle size analysis by the Coulter counter technique is essential in addition to PCS and LD analysis. Since the Coulter counter gives the absolute number of particles per volume unit for the different size classes, it is a more efficient and appropriate technique than LD analysis for quantifying the contamination of nanosuspensions by microparticulate drugs.

2.2.5.2 Particle charge (Zeta Potential):

The determination of the zeta potential of a nanosuspension is essential as it gives an idea about the physical stability of the nanosuspension. 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, though there are other approaches (Hunter J. 1981, Yang SC, Zhu JB. 2002). There are a number of instrumental configurations by which this is achieved, mostly using a Doppler shift, and the user should familiarize themselves with the particular approach implemented in their

equipment. Instrumentation concerns aside, the need for dilution begs the question of what is an appropriate diluent, because its choice can profoundly influence the surface chemistry and thus the results. One approach is to use a particle-free supernatant to dilute the sample. This will not account for concentration effects, however, and obtaining such a diluent is nontrivial as the particle size drops. Electroacoustic methods should in principal eliminate or reduce the need for dilution and its inevitable consequences (Dukhin AS, Goetz PJ. 1998). Nonpolar media and the combination of low mobility with high ionic strength are also problematic; however, phase analysis light scattering, a newer method in which a phase delay shift rather than a frequency shift is observed, addresses these issues (McNeil-Watson F., 1998). The zeta potential of a nanosuspension is governed by both the stabilizer and the drug itself In order to obtain a nanosuspension exhibiting good stability, for an electrostatically stabilized nanosuspension a minimum zeta potential of +30 mV is required whereas in the case of a combined electrostatic and steric stabilization, a minimum zeta potential of +20 mV is desirable (Muller & Jacobs 2002).

2.2.5.3 Crystallinity and polymorphism:

The crystalline structure of the nanosuspensions can be assessed by differential scanning calorimetry (DSC) (Jacobs C., 2000). This is especially important when a drug exists in different polymorphic forms. In addition, the high pressure homogenization technique for nanosuspensions can be used to particles with an amorphous fraction, thus leading to an enhancement of the saturation solubility. The extend of such a transform can be quantified and its stability during storage monitored by DSC preferentially in combination with X-ray analysis. Additionally, when nanosuspensions are prepared, drug particles in an amorphous state are likely to be generated. Hence, it is essential to investigate the extent of amorphous drug nanoparticles generated during the production of nanosuspensions. The changes in the physical state of the drug particles as well as the extent of the amorphous fraction can be determined by X-ray diffraction analysis (Muller & Böhm 1998; Muller & Grau 1998) and can be supplemented by differential scanning calorimetry (Shanthakumar etal 2004). In general, wide angle X-ray analysis (WAXS) is employed in contrast to Small angle X-ray analysis (SAXS) because it is easily accessible. The assessment of the crystalline state and particle morphology together helps

in understanding the polymorphic or morphological changes that a drug might undergo when subjected to nanosizing. The geometric scattering of radiation from crystal planes within a solid allow the presence or absence of the solid to be determined thus permitting the degree of crystallinity to be assessed (Calvo P. 1996).

2.2.5.4 Saturation solubility and dissolution velocity:

The determination of the saturation solubility and dissolution velocity is very important as these two parameters together help to anticipate any change in the in-vivo performance (blood profiles, plasma peaks and bioavailability) of the drug. As nanosuspensions are known to improve the saturation solubility of the drug, the determination of the saturation solubility rather than an increase in saturation solubility remains an important investigational parameter. The saturation solubility of the drug in different physiological buffers as well as at different temperatures should be assessed using methods described in the literature. The investigation of the dissolution velocity of nanosuspensions reflects the advantages that can be achieved over conventional formulations, especially when designing the sustained-release dosage forms based on nanoparticulate drugs. The dissolution velocity of drug nanosuspensions in various physiological buffers should be determined according to methods reported in the pharmacopoeia.

According to the Kelvin equation (Sinsonelli A. P. 1970), the vapor pressure above curved surface is increased compared to a flat surface. The vapor pressure increases further with increasing curvature of the surface, which means decreasing particle size. The Kelvin principle is exploited in the spray- drying process, in which increase in surface area and increase in vapor pressure lead to a rapid evaporation of the sprayed liquid. The transition of a molecule from the liquid phase (droplet) to the surrounding gas phase is comparable to the transition of molecules from a solid phase (particle) to the surrounding liquid phase. Analogously to the vapor pressure, the dissolution pressure of a substance increases with decreasing particle size. The saturation solubility around a particle depends on the tendency of the molecule to move from the solid to the liquid phase (vapor pressure) and the tendency to recrystallize, i.e., the saturation solubility increases with reduction in particle size (Fig. 2.6, top). .According to the Prandtl equation (Mosharraf M. et al 1995), for small particles the diffusional distance 'h' decreases with

decreasing particle size (Fig. 2.6, bottom). The decrease in h and the simultaneous increase in Cs leads to an increase of the gradient (cs— c)/dh and, according to Noyes-Whitney, to an increase in the dissolution velocity. Bearing these theoretical considerations in mind, particle size analysis of nanosuspensions by Coulter Counter was performed in drug saturated 0.9% sodium chloride-solutions to avoid dissolution effects (Fig. 2.6).

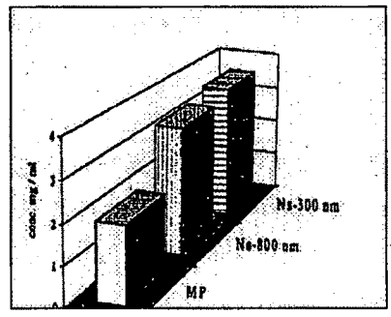


Figure 2.6 Solubility Cs of RMKP 22 microparticles (MP) with a mean diameter of 2400 nm and of two RMKP 22 nanosuspensions with diameters of 800 and 300 nm (Ns-800, Ns-300). (Modified from Ref. Muller R. H. 1996).

Saturation was performed using jet-milled drug with a mean particle diameter of 2.4 μm (laser diffractometer, volume distribution). This should avoid or minimize dissolution of the nanosuspension due to the size effect on the saturation solubility. Surprisingly, the nanosuspensions dissolved very quickly within the subsequent measurements in the Coulter counter Multisizer II (Fig. 7). Determination of the saturation solubility revealed an approximately two fold increase for the nanosuspensions compared to the 2.4 μm drug particles

2.2.5.5 Electron Microscopy

Scanning and transmission electron microscopy, SEM and TEM respectively, provide a way to directly observe nanoparticles, with the former method being better for morphological examination (Jores K et al 2004, Molpeceres J. 2000). TEM has a smaller size limit of detection, is a good validation for other methods, and affords 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 (Mosqueira VCF 2001). Sample corruption resulting from the extensive sample preparation is always a possibility, though lower vacuum (environmental- or E-SEM) instrumentation reduces this manipulation, albeit at the loss of some resolution (Nizri G et al 2004)

Electron microscopic graphs show that most of the nanosuspensions are cube-like (Fig. 2.7) or possess cuboid shape. The special dissolution properties and the cuboid shape lead to the trade name 'DissoCubes' for this delivery system. In some cases, the drug nanoparticles are long-shaped and rod-like. Nanosuspensions were produced using identical drugs and varying surfactant compositions and concentrations. These studies showed that the shape of the particles is primarily determined by the crystalline structure of the drug and not by the type and concentration of the surfactant used (Muller R.H.1996).

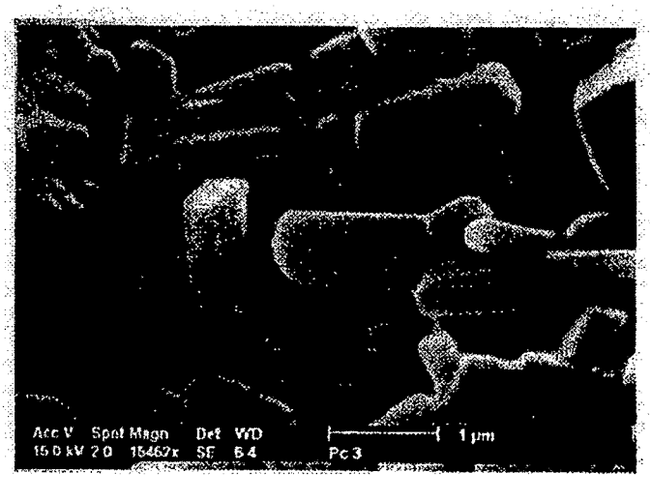


Figure 2.7 Electron micrograph of prednisolone nanosuspension (Muller R.H.1996).

2.2.5.6 In vitro release

The solubilization of active components from the individual nanoparticles is of obvious interest. This process can involve release of compound from a polymer or lipid matrix, or dissolution of the entire particle. In either case, separation of the ultra small particles from the release media is critical so that the nanoparticles are not mistaken for solubilized drug. In the latter case, the high rate of dissolution is frequently an additional complicating factor. In typical experiments, it is the appearance of solubilized material that signifies that dissolution is taking place. Using conventional filtration to remove undissolved material for in situ experiments presents serious challenges. The nanoparticles can easily pass through most filter membranes typically used for this purpose, if not at the beginning of the experiment, then at the end, when the particle size may have dropped sufficiently. Small filter pore sizes - as low as 0.02 μm - are commercially available, but can be plugged easily. The separation issue can be avoided by using a method, such as polarography, where only solubilized material is detectable (Kontoyannis C. G., Douroumis D 2001). In this way, the need for separation is obviated. Use of dialysis membranes and diafiltration is an option because they are less prone to blockage and the pore size is very small. The nanoparticles can be placed within a dialysis sac and samples taken from the large receiving medium (Leo E. et al 2004). Alternatively, the reverse approach can be used with the nanoparticles dispersed throughout the larger volume and the receiving media located within the sac (Calvo P et all 1996). Diffusion cells have also been used. Separation of particles can also be effected by centrifugation, or avoided implicitly by using two immiscible phases with one containing the nanoparticles and the other serving as the receiving medium (Tobio M. 1998, Chorny M. 2002).

When nanoparticles are used to increase the dissolution rate, a significant drawback to these approaches is the time it takes for the dissolved material to diffuse across a membrane or boundary. While this transfer function can be determined experimentally, the associated time constant can be on the scale of tens of minutes, if not hours. Such a long lag precludes the deconvolution of the drug release rate from the experimental data when the dissolution occurs within a few minutes or less. Rather than detecting drug as it appears in a solubilized form, dissolution information can also be derived by observing

the disappearance of the undissolved form, i.e., loss of the nanoparticles themselves. Spectroscopic methods such as light scattering or turbidity are good means of making such observations, and are useful because the corresponding measurements are essentially instantaneous in time, thus eliminating the deconvolution problem. Indeed, the limitation on measurable dissolution rate then arises from issues such as mixing times. Deliberately using nonsink conditions is a way of slowing down the process to avoid these problems.

2.2.6 Applications of Nanosuspension:

Nanosuspensions are used to advantage in diverse dosage forms. In some cases, their small size and increased surface area leads to an increased dissolution rate and increased bioavailability. In other cases, their particulate nature dictates targeting of the Monocyte Phagocytic System (MPS), with unusual pharmacokinetic consequences

Table 2.4: Potential benefits of Nanosuspensions

Physicochemical characteristic	Potential benefits
Increased drug amount in dosage form without harsh vehicles (extreme pH, co-solvents)	Intravenous: reduced toxicity, increased efficacy
Solid state : increased drug loading	Reduced administration volumes: essential for intramuscular, subcutaneous, ophthalmic use
Reduced particle size: increased drug dissolution	Oral: increased rate and extent of absorption, increased bioavailability of drug, area under plasma versus time curve, onset time, peak drug level, reduced variability, reduced fed/fasted effects. Pulmonary: increased delivery to deep lung
Particulate dosage form	Intravenous: potential for intravenous sustained release via monocyte phagocytic system targeting, reduced toxicity, increased efficacy. Oral: potential for reduced first-pass hepatic metabolism
Solid state: increased stability	Increased resistance to hydrolysis and oxidation, increased physical stability to settling

2.2.6.1 Oral drug delivery:

The oral route is the preferred route for drug delivery because of its numerous well-known advantages. The efficacy or performance of the orally administered drug generally depends on its solubility and absorption through the gastrointestinal tract. Hence, a drug candidate that exhibits poor aqueous solubility and or dissolution-rate limited absorption is believed to possess low and or highly variable oral bioavailability. Owing to low oral bioavailability, such a drug candidate would have to be administered in a larger excess than actually required in order to achieve a therapeutically active concentration, thus making the therapy costly. Orally administered antibiotics such as atovaquone and bupravaquone reflect this problem very well. Nanosizing of such drugs can lead to a dramatic increase in their oral absorption and subsequently bioavailability. The amelioration in oral bioavailability can be attributed to the adhesiveness of the drug nanosuspension, increased surface area (due to reduction in particle size by 10 to 50-fold), and increased saturation solubility, leading to an increased concentration gradient between the gastrointestinal tract lumen and blood, and increased dissolution velocity. This enhancement in bioavailability will lead to a subsequent reduction in drug dose, rendering the therapy cost-effective and obliterating any undue drug dumping in the body.

Surface charge and surfactant type of the nanoparticles determine the particles' adsorption to, and penetration through, the mucus layer to reach the underlying epithelial cells before the particles are shed from the mucus. Perturbed absorption resulting from an initial coating of salivary proteins on nanoparticles might be prevented by enteric coating. Targeting to macrophages in inflamed colon mucosa might also be possible. By reducing the size of particles to the sub-micron level, the uptake of intact gastrointestinal polymeric particles has been shown to occur preclinically, by mechanisms involving M-cells in Peyer's patches of the gastrointestinal lymphoid tissue. This uptake pathway communicates with the mesenteric lymph ducts, and empties via the thoracic duct into the systemic blood circulation. This approach therefore provides a route for avoiding first-pass metabolism, as well as for targeting sanctuaries of lymphatic-mediated diseases. The low drug uptake by this pathway might be enhanced, as shown in cell-based studies, by coating drug particles with agents, such as vitamin B12 , that dock into transporter

receptors on the intestinal epithelium. The use of surfactants has also been found to have an impact on decoupling the intestinal Pglycoprotein drug-efflux pump as well as interfering with lipidic chylomicron transport systems.

Atovaquone, an antibiotic indicated for treating opportunistic Pneumocystis carinii infections in HIV patients, non-complicated P. falciparum malaria and leishmanial infections (Looareesuwan et al 1999), shows poor bioavailability (10 –15%) because of its dissolution-rate limited absorption and has to be administered in high doses (750mg twice daily). Administration of atovaquone as a nanosuspension resulted in a 2.5-fold increase in oral bioavailability as compared to target drugs to the desired site of action, even when they show physical problems like poor solubility.

Danazol, a poorly bioavailable gonadotropin inhibitor, showed a drastic improvement in bioavailability when administered as a nanosuspension as compared to the commercial danazol macrosuspension 'Danocrine' (Liversidge & Cundy 1995). Danazol nanosuspension led to an absolute bioavailability of 82.3 %, whereas the marketed danazol suspension was 5.2% bioavailable. In addition, danazol nanosuspension resulted in a reduction in the inter- subject variability and fed fasted ratio of danazol.

Possible formulations are drink suspensions made from lyophilized or spray- dried nanosuspensions (sachet) or from an effervescent tablet. Other possibilities are the production of pellets using the aqueous nanosuspension for pellet production, or production of tablets using the nanosuspension in the granulation process. It is also possible to spray aqueous nanosuspensions on the surface of nonpareilles. Hard gelatin capsules can be filled with freeze- dried or spray-dried nanosuspensions, soft gelatin capsules with drug nanoparticles dispersed in PEG-400 or PEG-600. A very straightforward approach would be the production of nanosuspensions using liquid PEG as external phase instead of water. Apart from improving oral absorption, nanosuspensions offer the following advantages:

- improved dose proportionality
- reduced fed fasted state variability
- reduced inter-subject variability.

Numerous drug candidates that are poorly water-soluble are required to be taken over a prolonged period of time for effective medication. However, many of them cannot be

formulated into sustained-release dosage forms because of the risk of dose dumping and poor in-vivo performance. Although approaches such as a change in microenvironment and complexation with cyclodextrins have resulted in the successful incorporation of some poorly water-soluble drugs in sustained-release dosage forms (Chowdhary et al 2003), these solutions are not applicable to all poorly water-soluble drugs. Nanosuspensions,

the other hand, enable incorporation of all hydrophobic drugs in well-established sustained-release technologies. However, while doing so, the effect and the interaction of dosage form excipients with the nanocrystalline drug must be critically investigated. Drug nanosuspensions can also be incorporated into dosage forms such as tablets, capsules and fast melts by means of standard manufacturing techniques. Ketoprofen nanosuspension has been successfully incorporated into pellets to release the drug over a period of 24h (Remon et al 2001).

2.2.6.2 Parenteral drug delivery:

Nanosuspensions can be used to transform poorly soluble, noninjectable drugs into a formulation suitable for intravenous injection. This opens the perspective to utilize many drugs for therapy, which have a too low bioavailability after oral administration or parenteral administration as microparticles (e.g., IM or P) In addition, conventional formulations with undesirable side effects due to problematic excipients can be replaced by a new well-tolerated nanosuspension formulation. One drug candidate is paclitaxel, being presently marketed in Taxol (Bristol Myers Squibb). Taxol contains Cremophor EL, which can cause anaphylactic reactions. Paclitaxel can be transformed to drug nanoparticles by pearl milling (E. Merisko-Liversidge et al. 1996) or by high-pressure homogenization (Bohm et al 1997). Paclitaxel nanosuspensions were prepared by high-pressure homogenization at 1500 bar and 10 cycles. The nanosuspension was lyophilized and showed little difference in size distribution after reconstitution (Bohm et al 1997).

Several pharmacokinetic profiles can result following the injection of nanosuspensions. If the particles dissolve in the blood readily, both the pharmacokinetics, and therefore tissue distribution, will be equivalent to those for the solution formulations, affording a relatively fast onset of action. Alternatively, depot delivery via subcutaneous,

intramuscular or intradermal routes offers prolonged drug release, because of the ability to load more drugs safely into a small injectable volume. The greater loading capacity (up to 30%) distinguishes nanosuspensions from polymeric nanoparticulate vehicles. Following iv administration to mice of either the nanocrystalline or a control liposomal formulation at a dose of 20 mg clofazimine/kg bodyweight, drug concentrations in livers, spleens and lungs reached comparably high concentrations, well in excess of the MIC for most Mycobacterium avium strains. When C57BL/6 mice were experimentally infected with M. avium strain TMC 724, nanocrystalline clofazimine was as effective as liposomal clofazimine in reducing bacterial loads in the liver, spleen and lungs of infected mice (K. Peters et al.; 2000).

2.2.6.3 Ocular drug delivery

Nanosuspensions can prove to be a boon for drugs that exhibit poor solubility in lachrymal fluids. For delivery of such drugs, approaches such as suspensions and ointments have been recommended. Although suspensions offer advantages such as prolonged residence time in a cul-de-sac (which is desirable for most ocular diseases for effective treatment) and avoidance of the high tonicity created by water-soluble drugs, their actual performance depends on the intrinsic solubility of the drug in lachrymal fluids. Hence suspension may fail to give consistent performance. However, nanosuspensions, by their inherent ability to improve the saturation solubility of the drug, represent an ideal approach for ocular delivery of hydrophobic drugs. Moreover, the Nanoparticulate nature of the drug allows its prolonged residence in the cul-de-sac, giving sustained release of the drug. To achieve sustained release of the drug for a stipulated time period, nanosuspension can be incorporated in a suitable hydrogel base or mucoadhesive base or even in ocular inserts. An approach that has recently been investigated to achieve the desired duration of action of the drug is the formulation of polymeric nanosuspension loaded with the drug. The bioerodible as well as water soluble/permeable polymers possessing ocular tolerability (Pignatello et al, 2002a) could be used to sustain the release of the medication. The nanosuspensions can be formulated using the quasi-emulsion and solvent diffusion method. The polymeric nanosuspensions of flurbiprofen and ibuprofen have been successfully formulated using acrylate polymers

such as Eudragit RS-100 and Eudragit RL-100 (Bucolo et al 2002; Pignatello et a!, 2002b). the polymeric nanosuspension have been characterized for the drug loading, particle size, zeta potential, in vitro drug release, ocular tolerability and in vivo biological performance. The designed polymeric nanosuspensions revealed superior in vivo performance over the existing marketed formulations and could sustain drug release for 24 hr. The scope of this strategy could be extended by using various polymers with ocular tolerability.

2.2.6.4 Pulmonary drug delivery

Nanosuspensions may prove to be an ideal approach for delivering drugs that exhibit poor solubility in pulmonary secretions. Currently such drugs are delivered as suspension aerosols or as dry powders by means of dry powder inhalers. The drugs used in suspension aerosols and dry powder inhalers are often jet milled and have particle sizes of microns. Because of the microparticulate nature and wide particle size distribution of the drug moiety present in suspension aerosols and dry powder inhalers, the following disadvantages are encountered:

• limited diffusion and dissolution of the drug at the site of action because of its poor solubility and microparticulate nature, which may affect the bioavailability of the drug. • rapid clearance of the drug from the lungs because of ciliary movements (Muller & Jacobs 2002)

• less residence time for the drugs, leading to absence of prolonged effect

• unwanted deposition of the drug particles in pharynx and mouth.

Nanosuspensions can solve the problems associated with conventional systems because of their versatile nature. The nanoparticulate nature of the drug allows the rapid diffusion and dissolution of the drug at the site of action. At the same time, the increased adhesiveness of the drug to mucosal surfaces (Ponchel et al 1997) offers a prolonged residence time for the drug at the absorption site. This ability of nanosuspensions to offer quick onset of action initially and then controlled release of the active moiety is highly beneficial and is required by most pulmonary diseases. Moreover, as nanosuspensions generally contain a very low fraction of micro- particulate drug, they present unwanted

deposition of particles in the mouth and pharynx, leading to decreased local and systemic side-effects of the drug.

Additionally, because of the nanoparticulate nature and uniform size distribution of nanosuspensions, it is very likely that in each aerosol droplet at least one drug nanoparticle is contained, leading to even distribution of the drug in the lungs as compared to the microparticulate form of the drug. In conventional suspension aerosols many droplets are drug free and others are highly loaded with the drug, leading to uneven delivery and distribution of the drug in the lungs. Nanosuspensions could be used in all available types of nebulizer. However, the extent of influence exerted by the nebulizer type as well as the nebulization process on the particle size of nanosuspensions should be ascertained.

Budesonide, a poorly water-soluble corticosteroid, has been successfully formulated as a nanosuspension for pulmonary delivery (Muller & Jacobs 2002). A good relationship was obtained between increasing the drug concentration in the formulation and the number of micrograms of drug delivered per 2 s actuation.

2.2.7 Products on the market/in clinical phases

Looking at the time between invention of a technology and the first products on the market, this time period is very short for the drug nanocrystals. The liposomes were invented in 1968 by Bingham, the first pharmaceutical products appeared on the market at the beginning of the 1990s that means approximately 20 years in between. The first drug nanocrystal patents were filled at the beginning of the nineties by the company Nanosystems (nowadays élan), the first product Rapamune was placed on the market in the year 2000 by the company Wyeth. The product is a tablet containing 1 or 2 mg of sirolimus. To achieve this, the drug nanocrystal concentration in a tablet has a certain upper limit. In case this limit is exceeded, particles are getting in contact and might fuse under the compression. For Rapamune it was beneficial that a very low drug nanocrystal amount had to be incorporated into the tablet, just 1—2 mg, the total tablet weight is approximately 360 mg. That means the drug nanocrystal content is below 1% causing no formulation difficulties. The second product Emend was already introduced in the following year 2001 by the company Merck. It is a capsule containing 125 mg of the drug

aprepitant. The filling materials of a capsule are pellets. A single dose of 125 mg is in relation to typical weights of oral dosage forms between 400 and 500 mg relatively high. It corresponds to approximately 30 and 25% of drug nanocrystals in the total dosage form. In case of such a higher content it might be beneficial to apply less force during the production process that means replacing compression by an extrusion procedure (i.e. producing pellets). In the middle of the 1990s there was reluctance of pharmaceutical companies to employ the drug nanocrystal technology. At this stage it was a relatively hearing technology. A prerequisite for companies to use a technology is the availability of large scale production facilities. At the very beginning these facilities were unavailable. In addition, before moving to a novel technology one tries to employ technologies already existing in the company. In case of a successful formulation, large scale production technologies are available for these formulations approaches. The situation changed with the establishment of large scale production facilities and the pressure' created by an increasing number of poorly soluble compounds, i.e. the very low solubility, in both aqueous and organic media excluding the use of many traditional formulation approaches. Large scale production units for pearl milling were realized by circulating the suspension through the pearl mill (élan), the company Baxter established an aseptic production line based on piston-gap homogenizers for the product platform NANOEDGE. This led to the acceleration of the development of formulations based on drug nanocrystals. Quite a number is meanwhile in clinical phases. Table 2.5 gives an overview:

Drug	*Indication*	*Drug delivery company*	*Pharma company*	*Route*	*Status*
Paclitaxel	Anticancer	American BioScience	American Pharmaceutical partners	*iv*	Phase III
Undisclosed multiple	Anti-infective	Baxter NANOEDGE	Undisclosed	Oral / *iv*	Preclinical to phase II
Undisclosed	Anticancer	Baxter NANOEDGE	Undisclosed	Oral / *iv*	Preclinical to phase I
Rapamune	Immuno-suppressant	élan Nanosystems	Wyeth	Oral	marketed
Emend	Antiemetic	élan Nanosystems	Merck	Oral	marketed
Cytokine inhibitor	Crohn's disease	élan Nanosystems	Cytokine PharmaSciences	Oral	Phase II
Diagnostic agent	Imaging agent	élan Nanosystems	Photogen	*iv*	Phase I/II
Thymectatin	Anticancer	élan Nanosystems	NewBiotics/Ilex Oncology	*iv*	Phase I/II
Fenofibrate	Lipid lowering	SkyePharma	Undisclosed	Oral	Phase I
Busulfan	Anticancer	SkyePharma	Supergen	Intrathecal	Phase I
Budesonide	Asthma	élan Nanosystems	Sheffield Pharma	Pulmonary	Phase I

Table 2.5: Solid-particulate-nanosuspension-based formulations in development and in the market.

Perspectives: Nanosuspensions are an attractive and promising alternative for the formulation of poorly soluble drugs. They can be used for the optimized design of topical, ophthalmological, oral, peroral and parenteral formulations. Drug nanocrystals independent of their way of production represent a technology to overcome solubility problems and bioavailability problems of drugs which can be generally applied to all poorly soluble drugs. Any drug can be transformed to drug nanoparticles leading to an increase in saturation solubility, dissolution velocity, and providing the general feature of an increased adhesiveness to surfaces. Surface modification of the drug nanocrystals can

further increase the benefits, e.g. by producing mucoadhesive nanosuspensions for oral application or surface-modified site-specific nanoparticles for intravenous injection (e. g. targeting to the brain, bone marrow etc.). A further advantage is that this innovative nanosuspension technology can be combined with traditional dosage forms e.g. incorporating drug nanoparticles into pellets or tablets for oral delivery. An outstanding feature of the technology is its simplicity. The more simple the system is, the higher are the chances to launch products on the market. The success of drug nanoparticles, especially the third generation product nanosuspensions, will be demonstrated by the number of products on the market in the near future.

There is a definite need for a novel formulation of poorly soluble drugs. In the laboratories, the drug nanocrystals— independent of which top-down technology is employed for their production—proved to be such a formulation; many convincing data are available. The final criterion for a technology is its feasibility, proven by the appearance of products on the market, Polymeric nanoparticles have been investigated for almost 30 years; despite this huge input, to our knowledge, no product is on the market (only polymeric microparticles). In contrast to this, the first nanoparticle/ nanocrystal product Rapamune® has recently been launched. Due to the huge number of drug candidates in the pipeline, a high number of products should follow.

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DRUG PROFILES:

2.3 Saquinavir

Saquinavir was the first protease inhibitor licenced for the treatment of human immunodeciency virus (HIV) infection. It is highly active and selective against HIV-1 and HIV-2 *in vitro*, exerting antiretroviral activity at low nanomolar concentrations. Saquinavir is commercially available for oral administration as liquid-filled (soft gelatin) capsules containing saquinavir (Fortovase®) or as hard gelatin capsules or film-coated tablets containing saquinavir mesylate (Invirase®).

2.3.1 Description

Category: Anti-HIV Agent.

Generic Name: Saquinavir

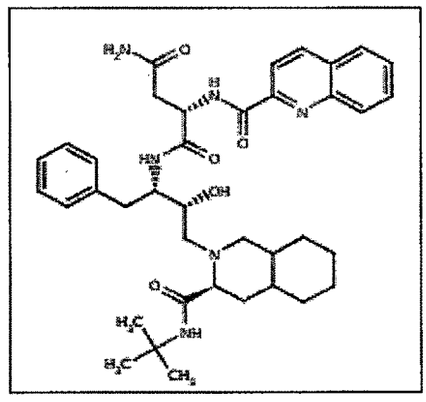
Chemical name: (2S)-N-[(2S,3R)-4-[(3S)-3-(tert-butylcarbamoyl)- 3,4,4a,5,6,7,8,8a-octahydro-1H-isoquinolin-2-yl]-3-hydroxy-1-phenylbutan-2-yl]-2-(quinoline-2-carbonylamino)butanediamide

CAS Number: CAS Number: Saquinavir: 127779-20-8 / Saquinavir mesylate: 149845-06-7

Empirical formula: $C_{38}H_{50}N_6O_5$

Molecular weight: 670.8408.

Structure:



Physical properties:

Appearance: A white to off white powder

Solubility: It is practically insoluble in water; slightly soluble in methanol, ethanol, and isopropanol; soluble in acetone and chloroform; and freely soluble in tetrahydrofuran. Its log p value is 4.1.

Melting point: 249.84 ^{0}C

2.3.2 Analytical method:

UV method : Determine the amount of Saquinavir ($C_{38}H_{50}N_6O_5$) dissolved by employing UV absorption at the wavelength of maximum absorbance at about 240 nm on filtered portions of the solution under test, suitably diluted with Dissolution Medium, in comparison with a Standard solution having a known concentration of USP Saquinavir Mesylate RS in the same medium (USP/NF 25, 2003).

HPLC method: High performance liquid chromatography (HPLC) analysis of saquinavir is reported in IP 2007. Stationary phase is a porous silica (5 μm) -C18 column (25 cm x 4.6 mm) and the mobile phase consists of acetonitrile, methanol, and 0.1-M sodium dihydrogen phosphate buffer (pH 4.0) in a 32:10:58 volume ratio. Detection of saquinavir is performed at 210 nm using a UV detector.

2.3.3 Clinical Pharmacology

Mechanism of Action

Saquinavir is a protease inhibitor with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Protease inhibitors block the part of HIV called protease. HIV-1 protease is an enzyme required for the proteolytic cleavage of the viral polyprotein precursors into the individual functional proteins found in infectious HIV-1. Saquinavir binds to the protease active site and inhibits the activity of the enzyme. This inhibition prevents cleavage of the viral polyproteins resulting in the formation of immature non-infectious viral particles. Protease inhibitors are almost always used in combination with at least two other anti-HIV drugs.

Saquinavir inhibits the HIV viral proteinase enzyme which prevents cleavage of the gag-pol polyprotein, resulting in noninfectious, immature viral particles.

Antiviral activity:

The antiviral activity of saquinavir is directly related to its concentration in the plasma, (Gieschke R Et al 1997, Beattie D 1996, Fischer L. 1998)

In vitro antiviral activity of saquinavir was assessed in lymphoblastoid and monocytic cell lines and in peripheral blood lymphocytes. Saquinavir inhibited HIV activity in both acutely and chronically infected cells. IC_{50} and IC_{90} values (50% and 90% inhibitory concentrations) were in the range of 1 to 30 nM and 5 to 80 nM, respectively. In cell culture, saquinavir demonstrated additive to synergistic effects against HIV-1 in combination with reverse transcriptase inhibitors (didanosine, lamivudine, nevirapine, stavudine, zalcitabine and zidovudine). Saquinavir in combination with the protease inhibitors amprenavir, atazanavir, or lopinavir resulted in synergistic antiviral activity.

Pharmacokinetics:

The pharmacokinetic properties of saquinavir when administered as Fortovase (Saquinavir soft gelatin capsule formulation) have been evaluated in healthy volunteers (n=207) and HIV-infected patients (n=91) after single-oral doses (range: 300 mg to 1200 mg) and multiple-oral doses (range: 400 mg to 1200 mg tid). The disposition properties of saquinavir have been studied in healthy volunteers after intravenous doses of 6, 12, 36 or 72 mg (n = 21).

Absorption and Bioavailability in Adults

The absolute bioavailability of saquinavir administered as Fortovase has not been assessed. Bioavailability of saquinavir mesylate from hard gelatin capsules is low, averaging 4% (CV 73%, range:1% to 9%) (www.aidsinfo.nih.gov). The relative bioavailability of saquinavir in Fortovase is estimated to average 331% (95% CI: 207% to 530%) that of INVIRASE (saquinavir mesylate hard gelatin capsules). This represents a calculated average oral bioavailability from the soft gelatin capsules of 13%. Peak plasma concentrations and area under the concentration-time curve (AUC) of the drug in soft gelatin capsules are about two times higher in HIV-infected patients than in healthy volunteers (AHFS Drug Information – 2003) . HIV-infected patients receiving multiple doses of Fortovase (400 mg to 1200 mg tid) and healthy volunteers receiving single doses

of Fortovase (300 mg to 1200 mg) , a greater than dose-proportional increase in saquinavir plasma concentrations has been observed. Although the soft gelatin formulation (Fortovase) provides roughly a 3-fold higher extent of systemic exposure (as measured by the AUC) compared with Invirase, a wide interindividual variation in oral pharmacokinetics remains (Kilby et al., 2000).

The mean 12-hour AUC after a single 800-mg oral dose of saquinavir in healthy volunteers (n=12) was increased from 167 ng.h/mL (CV 45%), under fasting conditions, to 1120 ng.h/mL (CV 54%) when Fortovase was given following a high-fat breakfast (45 g protein, 76 g carbohydrate, 55 g fat; 961 kcal).

After administration of Invirase (600 mg tid) in healthy individuals following meal, following values were obtained (AHFS drug information, 2004):

F (oral bioavailability):		4 %
Cmax	:	0.09 μg/ml
t_{max}	:	3.3 hr
$t_{1/2}$	:	13.2 hr
Apparent clearance	:	1650 ml/min
Vd	:	10 L/kg
Protein binding	:	approx. 98 %

Distribution

The mean steady-state volume of distribution following intravenous administration of a 12-mg dose of saquinavir (n=8) was 700 L (CV 39%), suggesting saquinavir partitions into tissues. It has been shown that saquinavir, up to 30 μg/mL, is approximately 97% bound to plasma proteins. Distribution of the drug into body tissues and fluids (such as cerebrospinal fluid) has not been fully characterized. Saquinavir is about 97% bound to plasma proteins in concentrations up to 30 mcg/ml.

Metabolism and elimination

The drug is metabolized in the liver to several monohydroxylated and dihydroxylated inactive metabolites. Metabolism is mediated by cytochrome P450; the isoenzyme CYP3A4 is involved in more than 90% of this metabolism. Low oral bioavailability is often attributed to extensive hepatic metabolism, but the human intestinal mucosa also

expresses a variety of drug-metabolizing enzymes, with CYP3A4 being identified as the predominant P450 isoform in human small intestinal enterocytes (Kolars et al. 1992, Prueksaritanont et al. 1996). In addition, the P-glycoprotein efflux pump can reduce absorption and increase gut wall metabolism by causing drugs such as the HIV-protease inhibitors to cycle through the enterocytes, so raising their chance of presystemic biotransformation (Kim et al. 1998). The Fortovase formulation is thought to be well absorbed and the resultant higher concentrations, compared with those of *Invirase*, partially saturate the processes which reduce the oral bioavailability of saquinavir (Steimer et al. 1998). Metabolism of saquinavir has been studied and shown that significant amount of its metabolism occurs at the small intestine (Fitzsimmons M.E. 1997).

Systemic clearance is rapid. Saquinavir is excreted primarily in the feces, both as unchanged drug and as metabolites 9AHFS Drug Information – 2003). Saquinavir is excreted principally in the feces, both as unabsorbed drug and metabolites. Following oral administration of 600 mg of radiolabeled saquinavir or IV administration of 10.5 mg of radiolabeled drug, 88 or 81% of the dose, respectively, is recovered in feces and 1 or 3%, respectively, is recovered in urine within 5 days. While about 13% of an oral dose of radiolabeled saquinavir reaches systemic circulation unchanged, 66% of an IV dose of radiolabeled saquinavir is present in systemic circulation as unchanged drug. These findings indicate that saquinavir undergoes substantial first-pass metabolism.

2.3.4 Indications and Usage

Fortovase is indicated for use in combination with other antiretroviral agents for the treatment of HIV infection. This indication is based on studies that showed increased saquinavir concentrations and improved antiviral activity for Fortovase 1200 mg tid compared to Invirase 600 mg tid. Invirase may be used only if it is combined with ritonavir, which significantly inhibits saquinavir's metabolism to provide plasma saquinavir levels at least equal to those achieved with Fortovase. When using saquinavir as the sole protease inhibitor in an antiviral regimen, Fortovase is the recommended formulation

2.3.5 Adverse events / Toxicity:

Saquinavir and saquinavir mesylate appear to be well tolerated. In clinical studies, the most frequently reported adverse effects included abdominal discomfort, diarrhea, and nausea. Other reactions include abdominal pain, anxiety, asthenia, buccal mucosa ulceration, constipation, depression, dizziness, dyspepsia, eczema, fatigue, flatulence, headache, insomnia, libido disorder, musculoskeletal pain, numbness in extremities, paresthesia, peripheral neuropathy, rash, taste alteration, verruca, and vomiting (Hoffmann- La Roche, Inc. - - Fortovase Prescribing Information; p. 18 , Hoffmann-LaRoche, Inc. - Invirase Prescribing Information; p. 15).

In clinical studies there have been rare reports of serious adverse effects that may be related to treatment with saquinavir or saquinavir mesylate. These rare effects included confusion, ataxia, and weakness; seizures; headache; acute myeloblastic leukemia; hemolytic anemia; thrombocytopenia; thrombocytopenia and intracranial hemorrhage resulting in death; attempted suicide; Stevens-Johnson syndrome; bullous skin eruptions and polyarthritis; severe cutaneous reaction associated with increased liver function test results; isolated elevation of transaminase values; exacerbation of chronic liver disease with elevated liver function tests, jaundice, ascites, and upper left and right quadrant abdominal pain; fatal pancreatitis; intestinal obstruction; portal hypertension; thrombophlebitis; peripheral vasoconstriction; drug fever; nephrolithiasis; and acute renal insufficiency (Hoffmann-La Roche, Inc. - Fortovase Prescribing Information; p. 17).

2.3.6 Contraindications

Saquinavir and saquinavir mesylate are contraindicated in patients with clinically significant hypersensitivity to the drugs or any components in the formulations. Caution should be used when administering saquinavir or saquinavir mesylate to patients with impaired hepatic function or hemophilia (USP DI – 2003).

2.3.7 Dosage and Administration

Mode of Delivery: Oral.(USP DI - 2003; p. 2439)

Dosage Form: Saquinavir: Soft gelatin capsules containing saquinavir 200 mg; this formulation was discontinued by USFDA because of decreased clinical demand.(FDA -

Fortovase discontinuation [Dear Health Care Professional Letter]. New Jersey: Roche Pharmaceuticals; May 27, 2005 at http://www.invirase.com)

Saquinavir mesylate: Tablets containing saquinavir 500 mg; hard gelatin capsules containing saquinavir 200 mg. Saquinavir and saquinavir mesylate are not bioequivalent and cannot be used interchangeably. The recommended dose of saquinavir is 1,200 mg (taken as six 200 mg capsules) three times a day or 1,000 mg coadministered with 100 mg of ritonavir two times a day. The recommended dose of saquinavir mesylate is 1,000 mg (taken as either two 500 mg tablets or five 200 mg capsules) coadministered with 100 mg of ritonavir two times a day. Both saquinavir and saquinavir mesylate should be taken within 2 hours after a full meal. (Hoffmann-La Roche, Inc. - Fortovase PI, December 2003 , Hoffmann-La Roche, Inc. - Invirase PI , December 2004), Saquinavir mesylate is now the preferred formulation.

2.3.8 Adults (Over the Age of 16 Years)

Fortovase Administered Without Ritonavir:

- Fortovase 1200-mg tid (6 x 200-mg capsules)
- Fortovase should be taken with a meal or up to 2 hours after a meal

Fortovase Administered With Ritonavir:

- Fortovase 1000-mg bid (5 x 200-mg capsules) in combination with Ritonavir 100-mg bid
- Ritonavir should be taken at the same time as Fortovase.
- Fortovase and ritonavir should be taken within 2 hours after a meal

2.3.9 Storage:

Saquinavir: Store at 2 to 8 °C (36 to 46 °F) until dispensed. Patients can keep refrigerated capsules until expiration date. Once brought to room temperature (at or above 25 °C [77 °F]), capsules should be used within 3 months (Hoffmann-La Roche, Inc. - Fortovase Prescribing Information; p. 22).

2.3.10 Prior work done:

Using biodegradable poly(ethylene oxide)- modified poly(epsilon-caprolactone)-based nanoparticles of less than 200 nm in diameter, Amiji et. al. showed enhanced delivery and prolonged residence of SQV in THP-1 monocytes/macrophage cells (Shah and Amiji, 2006).

To examine oral bioavailability and distribution to vital organs including the brain, SQV was incorporated in the nanoemulsions and administered orally to conscious Balb/c mice. Intravenous administration was also carried out to determine the relative bioavailability values of SQV following oral administration in different formulations. Control preparation of SQV was made as aqueous suspension containing all of the other ingredients (e.g., surfactants) except the oils (Amiji et al 2008). the results of this study show tremendous

promise of nanoemulsions, made with PUFA-rich oils, for enhancing oral bioavailability and efficient brain delivery of anti-HIV drugs.

Permeability of SQV across BBB under exposure to Electromagnetic field (EMF) was investigated *in vitro*. Here, BBB model was based on human brain-microvascular endothelial cells SQV was incorporated with Polybutylcyanoacrylate, methylmethacrylate-sulfopropylmethacrylate , and SLN. Effects of systematic parameters of EMF, including power, wave types, frequency, modulation and depth of amplitude modulation (AM) wave, and modulation and deviation of frequency modulation (FM) wave, were especially examined. Experimental results revealed that a high power of EMF caused apoptosis of HBMEC. For penetration across HBMEC monolayer, permeability of SQV was significantly enhanced by incorporation with the three carriers. Also, an apparent increase in permeability coefficient of SQV incorporated with the three carriers was resulted from an increase in continuous electromagnetic frequency, modulation or depth of AM wave, or modulation or deviation of FM wave.

M. Martin-Facklam et al. evaluated influence of increasing oral doses of cremophor EL on the pharmacokinetics of saquinavir in healthy individuals (M. Martin-Facklam et al. 2001). They have shown that a single oral administration of the pharmaceutical aid cremophor EL dose-dependently increases the bioavailability of single doses of oral saquinavir (Invirase), a PGP and CYP3A substrate with extremely low bioavailability. Cremophor EL probably acts by inhibiting intestinal PGP. Because the increase in bioavailability is likely to occur with oral cremophor doses used in clinical settings, cremophor has to be considered as a pharmacologically active pharmaceutical aid. Ponchel G. et al investigated interactions between HPBCD and saquinavir leading to an HPBCD–saquinavir inclusion complex and formulation of saquinavir loaded combined

hydroxypropyl-cyclodextrin and poly(alkylcyanoacrylate) nanospheres (Ponchel G. et al 2001). Author concluded that the present formulation may be considered as a valuable tool for improving the delivery of saquinavir and it may have a potential for improving saquinavir bioavailability and simultaneously reducing the oral dosing of saquinavir in HIV-infected patients.

2.4 Adefovir Dipivoxil

Adefovir dipivoxil, previously called bis-POM PMEA, with trade names Preveon and Hepsera, is an orally-administered acyclic nucleotide analog reverse transcriptase inhibitor (RTI) used for treatment of hepatitis B.

2.4.1 Description

Category: Antiviral (Reverse Transcriptase Inhibitors).

Generic Name: Adefovir Dipivoxil.

Chemical name: 9-[2-[[bis[(pivaloyloxy)methoxy]-phosphinyl]- methoxy]ethyl]adenine

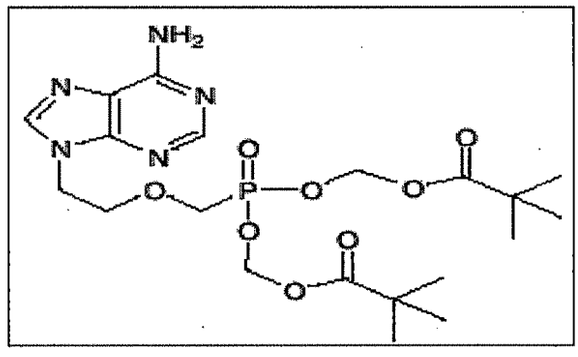
CAS Number: 142340-99-6

Marketed preparations available: Tablet for oral administration.

Empirical formula: $C_{20}H_{32}N_5O_8P$.

Molecular weight: 501.48

Structure:



Appearance: Adefovir dipivoxil is a white to off-white crystalline powder. Adefovir dipivoxil has an octanol/aqueous phosphate buffer (pH 7) partition coefficient (log p) of 1.91.

Solubility: It has an aqueous solubility of 19 mg/mL at pH 2.0 and 0.4 mg/mL at pH 7.2. Intrinsic aqueous solubility is 0.34 mg/ml.

Melting point: 99-100 ^{0}C

2.4.2 Mechanism of action: Adefovir is a synthetic nucleotide analog of adenosine 5-monophosphate. In vivo, adefovir dipivoxil is converted to the parent compound, adefovir, and through two phosphorylation reactions to adefovir diphosphate. Adefovir

diphosphate inhibits HBV DNA polymerase (reverse transcriptase) by competing with the natural substrate deoxyadenosine triphosphate and by causing DNA chain termination after its incorporation into viral DNA. The inhibition constant (Ki) for HBV DNA polymerase was 0.1 mM. Adefovir diphosphate is a weak inhibitor of human DNA polymerases A and G with Ki values of 1.18 mM and 0.97 mM, respectively.

2.4.3 Antiviral Activity: The concentration of adefovir that inhibited 50% of viral DNA synthesis (EC50) in HBV transfected human hepatoma cell lines ranged from 0.2 to 2.5 M. The combination of adefovir with lamivudine showed additive anti-HBV activity.

Pharmacokinetics:

Adefovir dipivoxil is a diester prodrug of the active moiety adefovir. Based on a cross study comparison, the approximate oral bioavailability of adefovir from HEPSERA is 59%. In preclinical studies, oral administration of adefovir dipivoxil led to enhanced delivery of adefovir to the systemic circulation. The oral bioavailability of adefovir from the prodrug in cynomolgus monkeys (_25%) was shown to be independent of the formulation used (Cundy, K. C et al 1994) In rats, the oral bioavailability of adefovir from a solution of prodrug in PEG 400 was 38.2% (Shaw, J.P 1994).

Following oral administration of a 10 mg single dose of HEPSERA to chronic hepatitis B patients, the peak adefovir plasma concentration (Cmax) was 18.4 ± 6.26 ng/mL (mean ± SD) and occurred between 0.58 and 4.00 hours (median=1.75 hours) post dose. The adefovir area under the plasma concentration-time curve (AUC0-∞) was 220 ± 70.0 ng·h/mL. Plasma adefovir concentrations declined in a biexponential manner with a terminal elimination half-life of 7.48 ± 1.65 hours. Adefovir dipivoxil diffuses passively into cells, unlike adefovir, which appears to require an endocytosis like transport process (Palu G. 1991, Cihlar T. 1995).

The pharmacokinetics of adefovir in subjects with adequate renal function was not affected by once daily dosing of 10 mg HEPSERA over seven days. The impact of long-term once daily administration of 10 mg HEPSERA on adefovir pharmacokinetics has not been evaluated. Adefovir exposure was unaffected when a 10 mg single dose of HEPSERA was administered with food (an approximately 1000 kcal high-fat meal). HEPSERA may be taken without regard to food.

The oral bioavailability of adefovir dipivoxil from a tablet formulation is limited by the relatively low intestinal permeability of the prodrug and its biological conversion to adefovir in the intestine, rather than by disintegration or dissolution of the dosage form. The Caco-2 cell permeability of adefovir dipivoxil appears to be an acceptable indicator of the extent of absorption of the prodrug. No correlation was observed between in vitro dissolution of adefovir dipivoxil tablets and oral bioavailability in dogs. It is therefore unlikely that such a correlation would exist between the in vitro dissolution of adefovir dipivoxil tablets and the corresponding in vivo human oral bioavailability (Cundy Kenneth et al 1997).

In vitro binding of adefovir to human plasma or human serum proteins is ≤4% over the adefovir concentration range of 0.1 to 25 mg/mL. The volume of distribution at steady-state following intravenous administration of 1.0 or 3.0 mg/kg/day is 392 75 and 352 9 mL/kg, respectively. Following oral administration, adefovir dipivoxil is rapidly converted to adefovir. Forty-five percent of the dose is recovered as adefovir in the urine over 24 hours at steady state following 10 mg oral doses of HEPSERA. Adefovir is renally excreted by a combination of glomerular filtration and active tubular secretion.

2.4.4 Contraindications: HIV infection, kidney problems, liver transplant, allergies. This medication should be used only when clearly needed during pregnancy.

2.4.5 Adverse effects: Gastrointestinal disturbances, hepatic effects and delayed renal abnormalities are the principal adverse events seen with adefovir dipivoxil. Reductions in serum free carnitine levels may occur and co administration of L-carnitine is recommended. Chronic administration of HEPSERA (10 mg once daily) may result in nephrotoxicity, although the overall risk of nephrotoxicity in patients with adequate renal function appear low.

2.4.6 Indications: For the treatment of chronic hepatitis B in adults with evidence of active viral replication and either evidence of persistent elevations in serum aminotransferases (ALT or AST) or histologically active disease.

2.4.7 Dosage and administration: The recommended dose of HEPSERA in chronic hepatitis B patients with adequate renal function is 10 mg, once daily, taken orally, without regard to food. The optimal duration of treatment is unknown.

Dose Adjustment in Renal Impairment: Significantly increased drug exposures were seen when HEPSERA was administered to patients with renal impairment. Therefore, the dosing interval of HEPSERA should be adjusted in patients with baseline creatinine clearance <50 mL/min using the following suggested guidelines (Table 2.6). The safety and effectiveness of these dosing interval adjustment guidelines have not been clinically evaluated.

Table 2.6 Dosing interval adjustment of HEPSERA in patients with renal impairment

	Creatinine Clearance (mL/min)			
	≥50	20-49	10-19	Hemodialysis Patients
Recommended dose and dosing interval	10 mg every 24 hours	10 mg every 48 hours	10 mg every 72 hours	10 mg every 7 days following dialysis

2.4.8 Prior work done:

Cundy K. attempted to correlate the in vitro performance of adefovir dipivoxil tablets with in vivo oral bioavailability in beagle dogs (Cundy K.1997). The effect of pH on oral bioavailability of a tablet formulation of adefovir dipivoxil was assessed using fasted dogs with or without pentagastrin pretreatment. Finally, the effect of food on the oral bioavailability of the clinical tablet formulation was evaluated. the oral bioavailability of adefovir dipivoxil from a tablet formulation is limited by the relatively low intestinal permeability of the prodrug and its biological conversion to adefovir in the intestine, rather than by disintegration or dissolution of the dosage form. The Caco-2 cell permeability of adefovir dipivoxil appears to be an acceptable indicator of the extent of absorption of the prodrug. No correlation was observed between in vitro dissolution of adefovir dipivoxil tablets and oral bioavailability in dogs. It is therefore unlikely that such a correlation would exist between the in vitro dissolution of adefovir dipivoxil tablets and the corresponding in vivo human oral bioavailability.

Augustijns, P.et al have examined whether the lipophilic modification of Adefovir and the subsequent incorporation of the prodrug into LacNeoHDL increases the uptake of the

drug by parenchymal liver cells and reduces its renal uptake in rats.A lipophilic prodrug of Adefovir (PMEA-LO) was prepared by conjugating the drug with lithocholic acid-3aoleate using ethylenediamine as a spacer. The linkage between PMEA and the spacer is acid labile, which ensures the release of Adefovir once the prodrug is delivered to the acidic lysosomes in the target cell. PMEA-LO readily incorporates into LacNeoHDL without appreciably affecting the physicochemical properties of the carrier. Furthermore, Augustijns, P.et al have determined the intracellular routing and metabolic fate of PMEA-LO in the liver. Lipophilic modification of Adefovir and its subsequent incorporation into LacNeoHDL results in a dramatically increased uptake of the drug by parenchymal liver cells. The kidney association of the drug was substantially reduced. After transport to the lysosomes, PMEA was rapidly released from the carrier and readily enters the cytosol, where the drug is phosphorylated to the active metabolite PMEApp. The dramatically improved biological fate of PMEA holds great promise that the present carrier-mediated approach may lead to a more effective therapy for chronic hepatitis B.

Using Caco-2 monolayers, Augustijns et al previously demonstrated that adefovir dipivoxil undergoes significant metabolism to the intermediate mono (pivaloyloxymethyl) - ester [mono (POM)-PMEA] and adefovir (Augustijns, P. 1997). Transport of adefovir dipivoxil and its metabolites across Caco-2 monolayers was modulated by at least two different efflux carrier mechanisms (Annaert P 19998).The results obtained with the Caco-2 model were studied using two models of intestinal absorption - they are *ex vivo* model (rat excised intestinal sheets mounted in using chambers) and an *in situ* intestinal perfusion model Augustijns P.et al,2000). Compared with the *in vitro* Caco-2 model, specific characteristics featured by the *ex vivo* and *in situ* models include: (1) adequate paracellular permeability as in small intestinal epithelium, (2) presence of a mucus layer, and (3) relevant expression of transport proteins and drug metabolizing enzymes. Hence, these models could provide additional information regarding the relative contribution of metabolism and active efflux mechanisms that were shown to play a role during *in vitro* transport of adefovir dipivoxil across Caco-2 monolayers.

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