2. LITERATURE REVIEW

2.1. Drug Review

PRAZIQUANTEL

Praziquantel is an acylated isoquinoline-pyrazine (Figure. 1) that has been originally developed for veterinary applications, and is now one of the most important drugs that has been added to the armamentarium of anti-parasitic drugs for human and veterinary use since the 1970s.

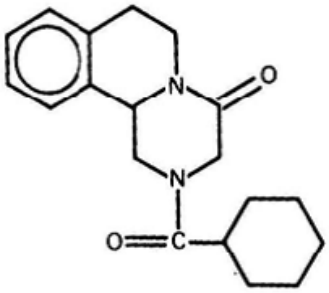

Figure.1.Praziquantel

2.1.1. Physico-Chemical Properties:

It is present as a white or practically white crystalline powder; odorless or with a faint characteristic odour. It is very slightly soluble in water (0.4mg /ml), freely soluble in alcohol (97 mg/ml) and chloroform (567mg/ml) (Analytical profiles of drug substances and excipients, vol. 25). It is hygroscopic, bitter tasting and exhibits polymorphism.

Chemically, it is 2-cyclohexylcarbonyl-1, 2, 3, 6, 7, 11b – hexahydropyrazino (2,1-a) iso-quinolin - 4- one. It has mol. wt. 312.4 dalton, Log P: 2.42 and shows M.P. 136 – 140 ºC. Commercial

preparations contain equal mixtures of levo (-) and dextro (+) optical isomers. The levo (-) enantiomer is the active form and is responsible for interaction with the parasite and therefore the pharmacological effect in vivo and in vitro (Andrews et al., 1983; Liu et al., 1986; Tanaka et al., 1989; Wu et al.,1991; Xiao and Catto, 1985;Staudt et al., 1992; Xiao and Catto, 1989).

2.1.2. Mode of action

Praziquantel has activity against a wide spectrum of trematodes and cestodes, but is generally ineffective against other organisms such as nematodes. Although the mode of action is not exactly known at present, there is experimental evidence that in case of cestodes, at low concentrations *in vitro*, the drug appears to impair the function of the worms' suckers, while at higher concentrations *in vitro*, praziquantel increases the contraction (irreversibly at very high concentrations) of the worm's strobilla (chain of proglottids) (Cioli and Pica-Mattoccia, 2003). Moreover, Praziquantel also causes irreversible focal vacuolization with subsequent cestodal disintegration at specific sites of the cestodal integument (Martin, 1997). In schistosomes and other trematodes, praziquantel directly kills the parasite, possibly increasing calcium ion (Ca^{++}) flux into the worm by increasing the permeability of the membranes of parasite cells for Ca^{++}. (Greenberg, 2005 a, b). The drug thereby induces contraction of the parasites resulting in paralysis in the contracted state (Martin, 1997; Greenberg, 2005 a, b). The dying parasites are dislodged from their site of action in the host organism and may enter

systemic circulation or may be destroyed by host immune reaction (phagocytosis). Additional mechanisms such as focal disintegrations and disturbances of oviposition (laying of eggs) are seen in other types of sensitive parasites (Dollery, 1999). Focal vacuolization of the integument follows and the parasite is phagocytized.

Another hypothesis on the mechanism of action of praziquantel has also been recently reported. The drug seems to interfere with adenosine uptake in cultured worms. This effect may have therapeutical relevance given that the schistosome, as the taenia and the echinococcus (other praziquantel sensitive parasites), is unable to synthesize purines de novo. Praziquantel may adversely affect the parasite's glutathione and intracellular calcium concentrations, with secondary effects on the metabolism and antigenicity (Ribeiro et al., 1998; Dollery, 1999).

2.1.3. Pharmacokinetics

The pharmacokinetics and metabolism of PZQ have been quite extensively studied and reported in the literature. There is considerable inter-individual variation for unknown reasons in the rate of absorption and clearance, so C_{max}, T_{max} and AUC show wide ranges (Dollery, 1999, Dayan, 2003; Mandour et al, 1990; Castro et al., 2003).

2.1.3.1. Absorption:

In helminths, Praziquantel is rapidly taken up by schistosomes, other flukes, and adult tapeworms. In humans it is rapidly absorbed after oral doses (approximately 80%) from the gastrointestinal tract. Absorption

has been shown to be by passive diffusion (Gonzalez- Esquivel et al., 2005). Peak plasma concentrations occur 1 to 3 hours after a dose. In normal liver function plasma concentrations occurs 0.2 to 2.0 μg per ml after therapeutic doses.

2.1.3.2. Distribution:

In helminths, Praziquantel is evenly distributed throughout. In humans, Praziquantel is distributed throughout the body, across the intestinal wall and crosses the blood-brain barrier into the CNS thus explaining its effectiveness on parenchymal brain cysticercosis (Sotelo and Jung, 1998). CSF concentrations are approximately 15 to 20% of the total amount of free and bound praziquantel in serum. Praziquantel levels in the bile, feces, and breast milk range from less than 10% to 20% of plasma concentrations. The volume of distribution for praziquantel has not been determined, and about 80% of the drug is bound to plasma proteins in man and animals (Mandour et al., 1990).

2.1.3.3. Biotransformation:

In helminths, Praziquantel does not appear to be metabolized by cestodes or schistosomes. Praziquantel is stereoselectively metabolized in the liver, yielding preferentially monohydroxylated metabolites (Meier and Blaschke, 2001). First pass metabolism in the liver is rapid; 15 min after an oral dose of ^{14}C[PZQ], the serum radioactivity consisted of 99% metabolites in the rat, 84% in the dog and a similar proportion in humans. Due to extensive first-pass metabolism, only a small amount of active

drug likely to reach the systemic circulation after ingestion. PZQ is rapidly and completely metabolized to inactive mono-,di- and polyhydroxylated derivatives, many of which are conjugated and thought to be inactive (Dollery, 1999). A few of the metabolites possess some but weaker antiparasite activity; however PZQ itself is the important therapeutic agent (Andrews et al., 1983). The main metabolites are cis-and trans-4-hydroxylpraziquantel. Different enantimoers of praziquantel are metabolized to qualitatively and quantatively different degrees in isolated rat hepatocytes (Meier and Blaschke, 2001; Godawska-Matysik et al., 2004). Praziquantel has a serum half-life of 0.8 to 1.5 hours in adults with normal renal and liver function. Metabolites have a half-life of 4 to 5 hours.

Praziquantel is metabolized through the cytochrome P450 pathway via CYP3A4, judged by selective inhibition experiments (Masimirembwa and Hasler, 1994). It has been reported that agents that induce or inhibit CYP3A4 such as phenytoin, rifampin, and azole antifungals will affect the metabolism of praziquantel and may displace praziquantel from protein binding sites thus may reduce the bioavailability of praziquantel (Bittencourt et al., 1992; Masimirembwa and Hasler, 1994; Sotelo and Jung, 1998).

Grapefruit juice, which is known to inhibit the first-pass metabolism of many drugs mainly by suppression of the cytochrome P450 enzyme (CYP3A4) in the small intestine (Lown et al., 1997) has been shown to significantly alter several kinetic parameters of praziquantel in healthy

men (Castro et al., 2002) and beagle dogs (Giorgi et al., 2003). It is likely that the reported effect of co-administration of ALB in increasing the plasma level of PZQ is due to inhibition of P450 enzymes (Homeida et al., 1994).

2.1.3.4. Elimination:

Praziquantel and its metabolites are mainly excreted renally. The metabolites in the urine account for about 38, 66 and 80% of an oral dose in rats, dogs and humans, respectively, within 8 h and about 15% in bile (EMEA, 1996). In man about 80% of an oral dose is excreted within 4 days; small amounts also excreted in feces but less than 0.1% eliminated as the unchanged drug. The elimination half-life is approximately 1-2 h in most species and 3 hours in the dog (Dayan, 2003). Overall clearance is rapid, with a $t_{1/2el}$ of unchanged PZQ of 1–2 h in most species and of metabolites of 3–8 h (EMEA, 1996 and Dollery, 1999). Clearance of metabolites, which is limited by the rate of metabolism, is slower but even in man metabolites are completely cleared by 4 days after an oral dose.

Praziquantel and its major metabolites are excreted in human milk, at a quarter of their levels in plasma and following the same rapid time course (Putter and Held, 1979).

2.1.4. Analytical methods

There are several high performance liquid chromatographic (HPLC) methods for the determination of praziquantel in serum, urine or tissue homogenates has been reported. These include, for example, the methods

of Xiao *et al.* (1983), Liu and Stewart (1997) and Dollery (1999). Newer HPLC/mass spectrometry methods can detect praziquantel itself and its different phase I metabolites, in addition to their glucuronidated and sulfated conjugates (e.g. Meier and Blaschke, 2000; Meier and Blaschke, 2001). There is a good HPLC technique for the racemate, suitable for application to plasma and other samples from animals and man (Dollery, 1999, Hanpitakpong et al., 2004). However, for resolving the isomers and suitable for application to clinical samples, there is need of a chirally sensitive technique.

2.1.5. Indications and therapeutic properties

Praziquantel is used against many helminthic and protozoan infections in humans (Adachi et al., 2005; Kjetland et al., 2006; Liu and Weller, 1996; Markoski et al., 2006), domestic animals (Bushara et al., 1983; Jenkins, 2005) and pet rodents and reptiles (Jenkins, 2005, Mehlhorn et al., 2005). The drug is generally used in the treatment of several varieties of trematodal infections such as schistosomiasis, fascioliasis, and cestodal infections. Common human cestodes treatable by praziquantel include *Taenia solium* and *Diphyllobothrium latum* and common human trematodes, or flukes, which are treatable, are *Clonorchis sinensis* and *Opisthorchis viverrini*. Although praziquantel can treat human neurocysticercosis (Oral, 16.7 to 33 mg per kg of body weight three times a day for fourteen to thirty days and may be repeated in two to six months if required). Recently, a case report indicated the success of praziquantel (at a single oral dose of 600 mg) in completely treating the intestinal

cestodes *Diphyllobotrium latum* in Brazil (Santos and de Faro, 2005). The treatment was complete and not accompanied by any adverse effects.

Praziquantel has a particularly dramatic effect on patients with schistosomiasis. Studies of have shown that within six months of receiving a dose of praziquantel, up to 90% of the damage done to internal organs due to schistosomiasis infection can be reversed. It has been shown that a carbohydrate – rich meal enhances absorption in man, probably by increasing the dissolution of the drug (Mandour et al., 1990; Castro et al., 2002), and that schistosomiasis decrease the $t_{1/2}$ of the drug, as it was 11.9 ± 5.4 h in patients with schistosomiasis and 2.3 ± 0.4 h in the controls (Mandour et al., 1990), hence the lower plasma levels and longer duration of action of praziquantel may be advantageous in reducing side effects and prolonging exposure of the schistosomes to the drug.

Surveys of long-term effect of single-dose praziquantel on morbidity and mortality from *Schistosoma mansoni* was investigated in Central Sudan and in children under six years of age living in an endemic area for *Schistosoma haematobium* in Zimbabwe. In Sudan, it has been shown that praziquantel treatment was effective in reducing the prevalence of infection from 53% to 34%, and the intensity of infection from 31% to 18% (Kheir et al., 2000). In Zimbabwe, praziquantel therapy reduced infection prevalence and mean intensity from 51.8% and 110 eggs per 10 ml urine, respectively, before starting re-treatment programme to very low levels thereafter. Praziquantel was not accumulated after periodic

administration (once every eight weeks for two years) in children (Mduluza *et al*., 2001). PZQ is effective in Hymenolepiasis, 25 mg per kg of body weight as a single Oral dose. Heavy infection may require repeated therapy after ten days.

2.1.6. Resistance to praziquantel

With the ever increasing use of praziquantel there is a possibility of the development of resistance by schistosomes (and other susceptible parasites) to the drug that may occasionally result in low cure rates in hyperendemic areas (Cioli and Pica- Mattoccia, 2003), hence the necessity to explore the activities of other compounds (Bennett *et al*., 1997; Southgate *et al*., 2005). The first alarming reports of possible praziquantel resistance came from an intensive focus in northern Senegal, where the drug had produced very low cure rate (Ciolo and Pica- Mattoccia, 2003). A decade ago in Egypt, it has been shown that a number of schiztosome isolates were established in the laboratory from the eggs excreted by patients who had been treated unsuccessfully (3 times) with praziquantel (Ismail *et al*., 1999). The same group of workers in Egypt suggested that there is no rapid emergence of resisitance to praziquantel, as there has not been an increase in the drug failure, despite more than a decade of therapueutic pressure in those villages where there had been resistant infections and worms with decreased response to praziquantel (Botros *et al*., 2005). However, investigators continue to find, for various ill-defined reasons, field isolates showing decreased responsiveness to praziquantel.

2.1.7. Adverse effects

The adverse effects of praziquantel in humans at the therapeutic dose ranges are not very serious. A pre- and post-treatment symptom questionnaire to Ugandans patients given single and repeated (6 weeks apart) praziquantel treatment (40 mg/kg) in a *Schistosoma mansoni*-endemic focus revealed a broad range of side effects, including abdominal pain and diarrhoea. However, no serious or long lasting complications affecting compliance were observed (Kabatereine *et al*., 2003).

When used orally, praziquantel can cause anorexia, vomiting, lethargy or diarrhea in dogs, but the incidence of these effects is less than 5%. In cats, adverse effects were quite rare (<2%). An increased incidence of adverse effects has been reported after using the injectable product. In dogs, pain at the injection site, vomiting, drowsiness and/or a staggering gait were reported from field trials with the drug. Some cats (9.4%) showed symptoms of diarrhea, weakness, vomiting, salivation, sleepiness, transient anorexia and/or pain at the injection site (Zajac, 1993).

2.1.8. Toxicity studies

Praziquantel has a wide margin of safety. In rats and mice, the oral LD_{50} is at least 2 g/kg. An oral LD_{50} could not be determined in dogs, as at doses greater than 200 mg/kg, the drug induced vomiting. Parenteral doses of 50 - 100 mg/kg in cats caused transient ataxia and depression. Injected doses at 200 mg/kg were lethal in cats. No particular toxic effects were noted in rats treated with praziquantel up to 1000 mg/kg/day for 4

weeks, and in dogs with up to 180 mg/kg/day for 13 weeks (Frohberg, 1984; Frohberg 1989). The no-observed-effect levels (NOELS) for those experiments were 60 mg/kg/day for dog. Rabbits given praziquantel at a dose rate of 2000 mg/kg died 10-20 hours following the treatment (Kheir *et al*., 1995). Recently, Omar *et al* (2005) reported that praziquantel (1500 mg/kg, weekly for 6 weeks) induced a significant increase in the mean values of some liver function tests (AST, ALT and bilirubin) with areas of hyaline degeneration, fatty changes, dysplasia and necrosis in the liver sections.

2.1.9. Genetic toxicity

A very large number of studies described in the initial reports and subsequently by many independent investigators have convincingly concluded that PZQ lacks mutagenic potential for man. (EMEA ,1996). An earlier report by International Commission for Protection against Environmental Mutagens and Carcinogens (ICPEMC) also concluded that if PZQ had any mutagenic effect in man, which was very improbable, it would be too small to be detected in the treated population (Kramers et al., 1991).

Although initial reports on genotoxicity of praziquantel gave mostly negative results in tests in bacteria, yeasts, mammalian (mice) reproductive cells and Drosophila, more recent reports in humans and pigs have suggested that praziquantel induces a greater frequency of hyperploid lymphocytes as well as structural chromosomal aberrations, but not in all the individuals treated. It was concluded that at the dose and

duration used, praziquantel has hepatotoxic, genotoxic and carcinogenic effects in albino rats. Recently, however, concerns over its genotoxicity have mounted. Due to its extensive use in multiple reinfections, infected and non-infected as well as non-diagnosed individuals, there are fears that the dug may not only be mutagenic per se, but also contribute to the development of neoplasm.

2.1.10. Reproduction toxicity

Fetal death and fetal resorption were found when praziquantel was administered in high doses to pregnant rats between the 6th and 10th day of gestation (Frohberg, 1989). However, clinically the drug is reported safe to use during pregnancy (Adam *et al*., 2004; Adam *et al*., 2005). The safety of a single dose of praziquantel (40 mg/kg) orally administered during different stages of pregnancy was confirmed in 25 Sudanese women afflicted with schistosomiasis mansoni (Adam *et al*., 2005). Neither the treated mothers, nor their babies (who were followed for up to one year of age) were adversely affected by the treatment. This confirms earlier work which concluded that praziquantel is safe in pregnant and lactating mothers (Olds, 2003). PZQ has not shown harmful effects in Segment I (fertility) and Segment II (fetal and maternal toxicity and teratogenicity) experiments at up to 300 mg/kg/day. A limited Segment III test (peri/post-natal toxicity) also showed no harmful effect of PZQ 300 mg/kg/day in the dams or their pups. PZQ is approved for use in cats, dogs and in sheep, potentially including pregnant animals (Zajac, 1993; Dayan, 2003,EMEA, 1996).

2.1.11. Carcinogenicity

The drug was not carcinogenic in the rat or hamster treated for 104 and 80 weeks, respectively (Frohberg, 1984 and Frohberg, 1989). It also did not accelerate tumorigenesis in a putative short-term test in which Syrian hamsters pre-treated with dimethylnitrosamine and infected with *O. viverrini* were subsequently given PZQ on 55 occasions and followed for 80 weeks ; the regime was intended to explore the potential influence of PZQ on tumorigenesis in the damaged liver.

2.1.12. Veterinary practice

Praziquantel is used in veterinary practice for the treatment of immature and mature tapeworms in dogs and cats. It is indicated for the treatment of *Echinococcus granulosus*, *Echinococcus multilocularis*, *Taenia multiceps*, *Taenia hydatigena*, *Taenia ovis*, *Taenia pisiformis*, *Taenia taeniaeformis* and *Dipylidium caninum*. The tablet formulation is administered at a dose of 5 mg/kg orally. An injectable preparation is available at a variable dose of 3.5–7.5 mg/kg, with smaller animals receiving a relatively larger dose because of their higher metabolic rate. The injectable formulation can be given either subcutaneously or intramuscularly and a brief pain response may be elicited if given subcutaneously. It is contraindicated in hound breeds by injection. There is also a spot-on preparation for transcutaneous delivery to cats at a dose of 8 mg/kg. Several combination preparations containing praziquantel with pyrantel embonate and

febantel are also available for combined tapeworm and round worm infestations.

2.2. Nanosponges

Nanosponges are a new class of materials and made up of polymers having particles with few nanometers wide cavities, in which a large variety of substances can be encapsulated. These particles are capable of carrying both lipophilic and hydrophilic substances and of improving the solubility of poorly water soluble molecules. Nanosponges can be used as a vessel for pharmaceutical principles to improve aqueous solubility of lipophilic drugs, to protect degradable molecules and to formulate drug delivery systems for various administration routes besides the oral one. The main disadvantage of these nanosponges is their ability to include only small molecules. The nanosponges could be either paracrystalline or in crystalline form. The loading capacity of nanosponges depends mainly on degree of crystallisation. Paracrystalline nanosponges can show different loading capacities. The nanosponges can be synthesized to be of specific size and to release drugs over time by varying the proportion of cross linker to β-CD.

Because of their nanoporous structure, nanosponges can advantageously carry water-insoluble drugs. These complexes can be used to increase the dissolution rate, solubility and stability of drugs, to mask unpleasant flavors and to convert liquid substances to solids.

NS are solid particles with spherical morphology that have been reported to have a very high solubilizing power for poorly soluble molecules, to protect degradable molecules, to mask unpleasant flavors and to formulate drug delivery systems for various administration routes such as Oral, Parenteral, Topical or Inhalation routes (Trotta *et al*., 2009). For the oral administration, the complexes may be dispersed in a matrix of excipients, diluents, lubricants and anticaking agents suitable for the preparation of capsules or tablets (Jenny *et al*., 2011). The nanosponges has been used in the formulation of drugs such as Tamoxifen (Jenny *et al*., 2011), Paclitaxel (Torne *et al*., 2010; Ansari *et al*., 2011), Camptothecin (Swaminathan *et al*., 2009; Rosalba *et al*., 2011), Resveratrol (Khalid *et al*., 2011), Itraconazole (Swaminathan *et al*., 2007), Dexamethasone (Lala *et al*., 2011), Curcumin (Darandale and Vavia, 2012), Telmisartan (Rao *et al*, 2012).

2.3. Solid lipid Nanoparticles

Solid lipid nanoparticles (SLN) have been used as an alternative drug delivery system to colloidal drug delivery systems namely oil-in -water emulsions, liposomes, microparticles and polymeric nanoparticles. They consist of spherical lipid particles in nanometer size range. SLN are used for the controlled and targeted delivery of drugs and for the incorporation of hydrophilic and lipophilic drugs. SLNs are made up of solid lipids, emulsifier and/or coemulsifier and water.

SLN are prepared from lipids which are solid at room temperature as well as at body temperature. Different solid lipids are exploited to produce

SLN, such as, tripalmitin/Dynasan® 116, trimyristin/Dynasan® 114,tristearin/Dynasan® 118, cetyl alcohol, cetyl palmitate, Compritol® 888 ATO, Glyceryl monostearate, Precirol® ATO5, stearic acid, Imwitor® 900. (Mehnert and Madar, 2001)

All classes of emulsifiers, either by itself or in combination have been utilized to stabilize the lipid dispersion. Examples of some of the emulsifiers that have been investigated are lecithin, bile salts such as sodium taurocholate, nonionic emulsifiers such as ethylene oxide/propylene oxide copolymers, sorbitan esters, fatty acid ethoxylates, and their combinations (Muller *et al*., 1995). Deionized water is used as a dispersion medium.

SLN have been claimed to combine the advantages of some colloidal carriers and simultaneously avoid their disadvantages (Muller et al.,2000).

2.3. 1. Advantages of SLNs over microparticles

- Smallest blood capillaries in body are approximately 5-6 μm and hence particles should be less than 5 μm in the blood stream without forming aggregates to minimize embolism. Therefore SLNs are better suited for I.V. delivery.
- Size of the microparticles is a limitation to cross the intestinal lumen into lymphatic system following oral delivery of vaccines, peptides, and other biomacromolecules. Microparticles remain in Peyer's patches while SLNs are disseminated systematically [Porter and charman,2001].

2.3. 2. Advantages of SLNs over liposomes

- Avoidance of organic solvents when desired
- Excellent reproducibility and feasible large scale production
- Unique ability to create controlled release and drug targeting Increased product stability of about 1 year

2.3.3. Advantages of SLN over polymeric nanoparticles

- Lipids are biodegradable and hence have better biocompatibility
- Avoidance of organic solvents when desired
- Feasibility of large scale production and sterilization
- Excellent reproducibility with cost effective
- Increased stability of the actives

2.3. 4. Solid Lipid Nanoparticles production methods:

High-Pressure Homogenization

High-Pressure Homogenization is a reliable and suitable technique for the preparation of lipid nanoparticles. There are following two types of high-pressure homogenization:

Hot high-pressure homogenization.

In this technique, first the lipids are melted at 5–10°C above their melting points and the drug is homogeneously dispersed in the melted lipids. A hot aqueous surfactant solution (preheated at the same temperature) is then added to the melted drug–lipid mixture and homogeneously dispersed (pre-emulsion) by a high shear homogenizer. Subsequently,

this hot pre-emulsion is subjected to a high-pressure homogenizer at the same temperature. This homogenization process is repeated till the nanoemulsion of desired average particle size is obtained. The obtained nanoemulsion is then cooled down to room temperature. During this cooling down, lipid droplets of the nanoemulsion re-crystallize and form lipid nanoparticles with solid matrix.

Cold high-pressure homogenization.

In cold high-pressure homogenization, the lipids are melted at 5–10°C above their melting points and the drug is dissolved or homogeneously dispersed in the melted lipids in the cold HPH technique. Then the drug-lipid melt is rapidly cooled down by means of liquid nitrogen or dry ice and subsequently milled to microparticles by means of a ball mill or mortar. These microparticles are suspended in a cold aqueous surfactant solution and then homogenized at or below room temperature forming lipid nanoparticles. This technique is suitable for hydrophilic or thermolabile drugs as this method is expected to avoid temperature-induced drug degradation and drug distribution into aqueous phase during homogenization. However, complete avoidance of drug exposure to high temperature is impossible as the drug needs to dissolve or disperse in the molten lipid and some heat is generated during the homogenization process.

Generally, scaling up of a process encounters several problems. Nevertheless, usage of the larger scale machines during HPH leads to an even better quality of the product with regard to a smaller particle size

and its homogeneity (Mulleret al., 2011). Additionally, HPH technique is widely used and well-established technique in pharmaceutical and food industry. SLN prepared by HPH can also be produced in non-aqueous dispersion media as long as the dispersion medium does not dissolve the lipid, e.g., liquid polyethylene glycol or oils (e.g., mineral oil).

Microemulsion

Microemulsion method for the preparation of SLNs was developed by Gasco *et al*., 1993. In this method, first the solid lipids are melted and the drug is dispersed in the molten lipids. After that, hot aqueous surfactant solution is added to the lipid melt with mild agitation to obtain transparent micro-emulsion. Subsequently, the micro-emulsion is dispersed in cold water (2–10°C) with mild agitation, where the micro-emulsion breaks into ultrafine nano-emulsion droplets which immediately crystallize to form SLNs.

Strong dilution of the particle suspension due to usage of large volume of water (ratio of hot microemulsion to cold water=1:25–1:50) is the main concern of this technique. Thus, the excess water needs to remove either by ultrafiltration or by lyophilization to obtain a concentrated dispersion. Another disadvantage of this method is the necessity of high concentrations of surfactants and cosurfactants, which is not desirable. Industrial scale production of lipid nanoparticles by the microemulsion technique is possible. In the large-scale production, a large temperature controlled tank is used to prepare the microemulsion. Subsequently, the microemulsion is pumped into a cold water tank for the precipitation

step. The temperature of the microemulsion and water, temperature flow in the aqueous medium, and hydrodynamics of mixing are the critical process parameters in the large-scale production.

Solvent Emulsification-Evaporation

In this technique, first the lipids are dissolved in a water-immiscible organic solvent (e.g., cyclohexane, chloroform) and then emulsified in an aqueous phase containing surfactants under continuous stirring (Sjostrom *et al*., 1992),). The organic solvent evaporates during emulsification, which results in lipid precipitation. As the whole formulation procedure can be conducted in room temperature, this technique is highly suitable for thermo-labile drugs. However, the major concern is the production of very dilute dispersion that needs to be concentrated by means of ultra-filtration or evaporation. Another concern is the use of organic solvent, some of which may remain in the final preparation.

Solvent Injection

The basic principle of the solvent injection method is similar to the solvent diffusion method. In case of solvent injection method, lipids are dissolved in a water-miscible solvent (e.g., acetone, isopropanol, and methanol) or water-miscible solvent mixture and quickly injected into an aqueous solution of surfactants through an injection needle (Rawat et al., 2010). The advantages of this method are the easy handling and fast production process without technically sophisticated equipment (e.g.,

high-pressure homogenizer). However, the main disadvantage is the use of organic solvents (Rawat et al., 2011).

Emulsification-Sonification

Briefly, in this method the lipids are melted at a temperature of 5–10°C above their melting points and the drug is dispersed in the melted lipids. Then a hot aqueous surfactant solution is added to the drug-lipid melt and homogeneously dispersed by a high shear homogenisation. Coarse hot o/w emulsion obtained is ultrasonicated using probe sonicator till the desired sized nanoemulsion is formed. Finally, lipid nanoparticles are obtained by allowing hot nanoemulsion to cool to room temperature. However, metallic contamination of the product may happen during sonication by probe sonicator (Venkateshwarlu and Manjunath,2004).

2.3. 5. Characterization

Characterization of the lipid nanoparticles is critical due to complexity of the system and colloidal size of the particles. Nevertheless, proper characterization of the formulations is necessary to control the product quality, stability, and release kinetics. Hence, accurate and sensitive characterization methods should be used. There are several important characterization techniques as follows.

Particle Size

Particle size plays a crucial role in the gastrointestinal uptake and their clearance by the reticuloendothelial system. Hence, the precise determination of the particle size is very important. Particle size less than

100 nm are advisable for the intestinal transport (Porter and Charman, 2001). Photon correlation spectroscopy (PCS) and laser diffraction (LD) are the most powerful and widely used techniques for the particle size measurement of lipid nanoparticles. PCS is also known as dynamic light scattering. The fluctuation of the intensity of the scattered light, caused by particles movement, is measured by this technique. PCS is relatively accurate and sensitive method. However, only size range from few nanometers to about 3 μ can be measured by PCS. This size range is enough to characterize lipid nanoparticles. On the other hand, LD can measure bigger particle sizes (>3 μ). LD covers a broad size range from the nanometer to the lower millimeter range. This method is based on the dependence of the diffraction angle on the particle radius. Smaller particles lead to more intense scattering at high angles than the larger particles. However, it is always recommended to use both PCS and LD method simultaneously as both methods do not directly measure particle sizes, rather particle sizes are calculated from their light scattering effects. This is because particles are non-spherical in many instances

Polydispersity Index

As SLNs/NLCs are usually polydisperse in nature, measurement of polydispersity index (PI) is important to know the size distribution of the nanoparticles. The lower the PI value, the more monodispered the nanoparticle dispersion is. Most of the researchers accept PI value less than 0.3 as optimum value. PI can be measured by PCS.

Zeta Potential

The zeta potential (ZP) indicates the overall charge a particle acquires in a specific medium. Stability of the nanodispersion during storage can be predicted form the ZP value. The ZP indicates the degree of repulsion between close and similarly charged particles in the dispersion. High ZP indicates highly charged particles. Generally, high ZP (negative or positive) prevents aggregation of the particles due to electric repulsion and electrically stabilizes the nanoparticle dispersion. On the other hand, in case of low ZP, attraction exceeds repulsion and the dispersion coagulates or flocculates. However, this assumption is not applicable for all colloidal dispersion, especially the dispersion which contains steric stabilizers. The ZP value of −30 mV is enough for good stabilization of nanodispersion (Bunjes et al., 2003). The ZP of the nanodispersions can be determined by zetameter using electrophoretic mobility.

Shape and Morphology

Scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) are very useful techniques to determine the shape and morphology of lipid nanoparticles. These techniques can also determine the particle size and size distribution. SEM utilizes electron transmission from the sample surface, whereas TEM utilizes electron transmission through the sample. In contrast to PCS and LD, SEM and TEM provide direct information on the particle shape and size. Several SEM and TEM study showed spherical shape of the lipid nanoparticles. Although normal SEM is not very sensitive to the

nanometer size range, field emission SEM (FESEM) can detect nanometer size range. Cryogenic FESEM might be helpful in this case, where liquid dispersion is frozen by liquid nitrogen and micrographs are taken at the frozen condition. AFM technique is also gaining popularity for nanoparticle characterization. AFM provides a three-dimensional surface profile unlike electron microscopy which provides two-dimensional image of a sample. AFM directly provides structural, mechanical, functional, and topographical information about surfaces with nanometer- to angstrom-scale resolution. In this technique, the force acting between a surface and a probing tip results in a spatial resolution of up to 0.01 nm for imaging. Direct analysis of the originally hydrated, solvent-containing samples is possible as no vacuum is needed during operation and the sample does not need to be conductive. AFM and SEM were compared and reported same particle size of the nanoparticles by both methods (Muhlen et al.,1998).

2.3. 6. Stability of the Drug and Solid Lipid Nanoparticles

Stability considerations relevant to SLNs include the chemical stability of the drug and the physical stability of the SLNs. Prevention of degradation reactions such as hydrolysis is an important chemical stability parameter and examples for physical stability issues include the prevention of particle size growth and polymorphic changes of the solid lipid. Lipids and surfactants must be chosen carefully and should be mutually compatible to improve chemical stability.

The particle size distribution of the SLN formulation dictates the biodistribution, shelf-life, reticulo-endothelial system (RES) clearance and the route of administration. The SLN dispersion should possess a narrow size distribution to avoid particle size growth due to Ostwald ripening. Ostwald ripening is a thermodynamically driven process, in which smaller particles dissolve and redeposit onto the surface of larger particles. This process occurs because smaller particles have larger surface area and higher surface energy and hence higher Gibbs free energy than the larger particles. All systems tend to attain lowest Gibbs free energy. In other words, larger particles are more energetically stable and favored over smaller particles. Ostwald ripening can be reduced by minimizing polydispersity in the particle size but it cannot be prevented.

There are three other types of instabilities in SLN dispersions: creaming, flocculation and coalescence. Creaming is a process in which the less dense phase migrates to the top of the dispersion under the influence of buoyancy or centripetal force. Creaming brings the SLN particles close to each other aiding Ostwald ripening, flocculation and coalescence. This is of significance to the formulator since a centrifuge is usually used to separate the nanoparticles from the liquid SLN dispersion. Creaming can be prevented by matching the density of the lipid and aqueous phases. Flocculation is a process in which the nanoparticles are held together in loose associations by weak van der Waals forces. Coalescence is a process in which the nanoparticles fuse to form larger particles. The electrostatic repulsion and steric hindrance between particles produced in the

presence of surfactants have been found to inhibit flocculation .Electrostatic repulsion produces an electrical double layer around each nanoparticle in SLN dispersion. The electrical double layer comprises of two parts: an inner region (stern layer), in which the ions are tightly bound and an outer diffuse region, in which the ions are less firmly attached. A notional boundary forms between particles and ions within this diffuse layer. Ions within the boundary move with the particle and the ions outside the boundary do not move with the particle. This notional boundary is called as slipping plane. The potential at the slipping plane is known as zeta potential. The magnitude of the zeta potential is an important determinant of the stability of SLN dispersions. As the zeta potential increases, the magnitude of electrostatic repulsion between the particles also increases, hence the particles will tend to repel each other and there is no tendency to flocculate. Colloidal dispersions with a zeta potential more positive than +30 mV and more negative than -30mV are considered to be stable [Muller et al.,1995].

Steric effects also play an important role in the stability of SLN dispersion by hindering the particles from coming close to each other and thus preventing flocculation and coalescence. The polyoxyethylene chain present in nonionic surfactants extends in the aqueous medium in the form of a coil and providing steric hindrance. Optimum surfactant concentration and sufficient chain length (≥ 20 ethylene oxide units) will impart steric effect mediated formulation stability . For long-term

stability a balance between electrostatic repulsion and steric effect must be obtained.

Lipid crystallization is important for the stability of lipid nanoparticles. It significantly affects the drug incorporation and release rates. Polymorphic transition is the ability to form a different unit cell structure in crystals due to different molecular conformations and packing patterns. SLNs do not completely crystallize during their storage and contain various polymorphic forms such as α, β' and β. The main difference between the polymorphic forms is the molecular distance. "α" form is unstable and is characterized by the hexagonal structure with the largest molecular distance. "β" form is stable and is characterized by the tightest triclinic packing pattern. Presence of residual liquids in lipid nanoparticles promote the crystallization of the stable form because unstable crystals may redissolve and recrystallize to the more stable form [Muller et al.,1995]. Increase in particle size, change in particle shape, and drug expulsion occurs when lipids undergo polymorphic modifications. An increase in thermodynamic stability and decrease in the drug incorporation rate was observed in the following order :

supercooled melt < α-modification < β'-modification < β-modification.

Differential scanning calorimetry (DSC) and X-ray scattering are widely used to study lipid polymorphic transitions. Different lipid forms possess different melting points and enthalpies and thus can be detected by DSC. X-ray scattering can be used to detect the length of long and short spacings of the lipid lattice [Bunjes et al.2005].

2.3. 7. Lyophilization or freeze drying of solid lipid nanoparticles

The stability of SLN dispersions has been reported to be in the range of 12 to 36 months . But in most formulations the particle size increases within a short period of time and hence lyophilization is a way to increase the stability of SLNs. Ostwald ripening as well as hydrolysis can be avoided by lyophilization. Moreover it also makes SLNs feasible to be incorporated into various dosage forms such as tablets, capsules, pellets, parenteral redispersion, etc.

Lyophilization involves freezing the SLN dispersion followed by the evaporation of the water under vacuum. The lyophilization parameters to be considered are freezing out effect which leads to changes in osmolarity and pH. Low water and high particle content produces high osmotic pressure which in turn favors particle aggregation and hence the lipid content of the SLN dispersion should not exceed 5% [Ohshima et al]. Cryoprotectants such as mannitol, sorbitol, trehalose, glucose and polyvinylpyrrolidone are usually added to decrease particle aggregation and to obtain better redispersion of the lyophilizates. Cryoprotectants help in SLN stability by decreasing osmotic activity of water and crystallization and favoring the formation of glassy state of the frozen sample [Kamiya et al.,]. Cryoprotectants prevent direct contact between lipid particles and they also interact with the polar groups of the surfactants and serve as a pseudo hydration shell [49]. Diglycerides has been reported to give the best results as cryoprotectant for SLN

lyophilization in comparision to monoglycerides. Cryoprotectants are usually used in a concentrations of 5-10% [Ohshima et al.,].

Addition of cryoprotectant prior to homogenization helps in reducing the increase in the particle size. Better particle size results are obtained when SLN lyophilizates are redispersed using a bath sonicator as opposed to simple hand shaking. The removal of water and increase in particle concentration during lyophilization compromises the protective effect of the surfactant and hence favors particle aggregation. Mehnert et al. recommends a sugar/ lipid weight ratio of 2.6-3.9 [8] .

Extensive research has been done in optimizing the lyophilization procedure of SLN dispersions. Results on the rate of freezing (Slow freezing in a deep freeze at -70°C, rapid freezing in liquid nitrogen) are ambiguous and hence the procedure has to be optimized on a case-by-case basis. Thermal treatment (2 h at -22°C followed by 2 h at -40°C) of the frozen SLN dispersion has also been reported to improve the results [Freitas and muller,].

Rapid cooling helps to decrease freezing out effects by forming small and heterogeneous crystals.

Spray drying

Although rarely used, spray drying is another technique that can be used to transform an aqueous SLN dispersion into a dry product. The production cost is lower with spray drying when compared to lyophilization. Spray dryers utilize hot gases and atomizers or spray

nozzles to disperse the SLN dispersion and hence cause aggregation and partial melting of the SLN particles. It is suggested that the use of high melting point lipids (>70°C), low lipid content in the dispersion, ethanol-water mixtures (10/90 v/v) as the dispersion medium, addition of about 20-30 % carbohydrates such as trehalose to control particle aggregation during spray drying (Freitas and Muller.,).

2.3. 8. Drug Loading

Hydrophilic as well as lipophilic drugs can be incorporated into the SLN system. Ideally a nanoparticulate system should have high drug loading and long-term incorporation for efficacy and efficiency reasons. Factors affecting the drug loading are drug/lipid ratio and solubility, partition coefficient, chemical and the physical structure of the solid lipid matrix and polymorphic state of the lipid (Muller et al., 1995). The drug gets positioned between the lipid layers, imperfections or fatty acid chains. In a lipid matrix the drug is located either in the core or the shell or is molecularly dispersed throughout the matrix. However, drug can also be entrapped in micelles, mixed micelles, liposomes, super-cooled melts and other lipid modifications [8]. The drug loading capacity is generally expressed as percentage and is calculated as the ratio of drug to the lipid phase. The drug should be highly soluble in the lipid to obtain sufficient loading capacities. The solubility of the drug decreases when cooling down the melt and is lower in the solid lipid (Mehlen et al., 1998). Solubilizers can also be added to increase the solubility. The chemical properties of the lipid are an important factor affecting loading capacity

as the drug gets expelled from highly crystalline particles with perfect lattice. The presence of mono-, di- and triglycerides in the lipid will form less perfect crystalline structures with many imperfections and possess higher loading capacities.

2.3. 9. Drug release

The release profiles from SLNs can be modulated to obtain burst release, prolonged release (with no initial burst release) and different percentages of burst release followed by prolonged release (Hou et al., 2003). The release rate of the SLN can be modified by proper choice of the lipid type, surfactant concentration and production parameters. One important application of SLNs is the sustained and controlled delivery of the drugs. A major limitation with SLNs is the observance of burst release kinetics. Burst release was found to be independent of the production technique. Controlled release of the drug was first reported by Müller when incorporating prednisolone into SLN produced by high pressure homogenization (Muller et al., 2011). The drug partitions from the liquid lipid phase to the aqueous phase when hot homogenization is the production technique. As the water solubility of the drug increases, the amount of the drug partitioning into the water phase also increases. The saturation solubility of the drug in the water phase increases with increasing processing temperature and surfactant concentration. As the temperature of the system decreases the solubility of the drug in the water decreases and the drug tends to repartition into the lipid phase. A solid lipid core containing the drug in it starts forming at the

recrystallization temperature and the hydrostatic pressure on the drug further increases the drug repartition into the lipid phase. As the system continues to cool the solid lipid core is not accessible to the drug and the drug molecules tend to solubilize more in the liquid lipid outer shell of the SLN. The drug in the lipid core exhibits prolonged release kinetics whereas the drug in the shell exhibits burst release kinetics. Mehnert et al. proposed three drug incorporation models: 1. Solid solution, 2. Drug enriched shell/lipid core model and 3. Drug enriched core/ lipid shell model (Mehnert and Madar, 2001). Controlled release kinetic profile is obtained with solid solution model. In the cold homogenization technique the drug is molecularly dispersed in the solid lipid and mechanical force is used to reduce the particle size thus SLNs produced by this technique typically exhibit controlled release. SLNs produced by hot homogenization technique exhibit drug enriched shell/lipid core model and show burst release profiles. Drug enriched core/ lipid shell model is exhibited when the drug precipitates first before the lipid crystallizes (Muhlen et al., 1998). In such systems when the solubility of the drug is close to its saturation solubility prolonged drug release is observed.

2.3. 10. SLN in Lymphatic Targeting

Drugs with poor oral bioavailability due to low solubility in GI tract or pre-systemic hepatic metabolism (first-pass effect) can be incorporated into SLN. The lipid core of SLN may mimic chylomicron formation by enterocytes, which dissolve and assimilate lipophilic drug molecules and promote the absorption of water-insoluble drugs into intestinal

lymphatics by the transcellular mechanism of lipid absorption, thereby reducing hepatic first pass metabolism by allowing transportation of drug via the intestinal lymphatics, which directly drain into thoracic duct, finally into the venous blood, thus by passing the portal circulation (Porter and Charman, 2001; Paliwal *et al*., 2009). Previous studies demonstrate that SLN as carrier for bioactives through lymphatic regions following oral administration prolong the systemic circulation time and increase the bioavailability of drugs including Methotrexate, Repaglinide and Lopinavir etc (Paliwal *et al*., 2009; Rawat *et al*., 2011 Bargoni *et al*., 1998; Alex *et al.*, 2011).