CHAPTER II
LITERATURE REVIEW

Definition

The nanoparticle is solid colloidal particle size ranging from 1 to 1000 nm. They can be used therapeutically, for example, as adjuvants in vaccines, bioactive agents, or drug carriers. The active principle (drug or biologically active material) is dissolved, entrapped, or encapsulated in the particles (Kreuter, 1994). Nanoparticles can generally be divided into two groups, i.e. polymeric and lipidic systems.

"SLN" is characterized as lipidic particles of a solid physical state in the nanometer size range. They compose of lipids and physiologically acceptable additives. These carriers provide for the controlled delivery of poorly water-soluble substances such as drugs and other biological materials primarily by the parenteral but also by the peroral, nasal, pulmonary, rectal, dermal and buccal routes of administration (Westesen, and Siekmann, 1998).

Moreover, there are some terms explained the carriers similar to SLN. These carriers are consisted of solid lipids and stabilizers as well as SLN.

The term of "lipid matrix carriers" (LMCs) are announced as globular structures composed of a hydrophobic compound and an amphipathic compound. In this case, a hydrophobic compound is not only specific to solid lipid, but also to liquid lipid in body temperature and as a solid matrix in room temperature. The diameter of these lipid matrix carriers ranges from about 500 nm to 100 μm. These carriers are prepared in a large size that prevents the circulation of carrier from an intramuscular depot (Fountain et al., 1986).

The term of "lipid nanopellets" are disclosed as solid particles comprised of lipids having the melting point between 30°C and 100°C, and a surfactant of which the particle diameter of the nanopellets ranging from 50 to 1000 nm. Thus, they are

the same as SLN. These carriers can be provided with pharmaceutically active substances to make the possible improved biological availability upon peroral administration (Speiser, 1989).

"Solid lipid microspheres" are the term of the particles preparing from dispersed the warm microemulsions of solid lipid, such as triglyceride, in water of 2° C to 10°C. The obtained microspheres have an average diameter smaller than 1 μm and in particularly of between 50 and 800 nm, and a polydispersion comprised between 0.06 and 0.90. So these carriers are one type of SLN (Gasco, 1993).

"Lipospheres" are disclosed in the first times by Domb (1993, 1994, 1995). They are described as suspensions of solid, water-insoluble microparticles that have a layer of a phospholipid embedded on their surface. These spherical structures have average particle diameter between 300 nm and 250 μm. So they mean as the SLN using the phospholipid as stabilizers. They are claimed to provide for the sustained release of entrapped substances controlled by the phospholipid layer. They can be prepared by the melt or by the solvent technique.

The term of "solid fat nanoemulsions" or "emulsomes" are disclosed as particulate nanoemulsions comprising lipid cores composed of lipids which are in a solid or liquid crystalline phase at at least 25°C, stabilized by at least one phospholipid envelope. These carriers are used for the parenteral, oral, ocular, rectal, vaginal, intranasal, or topical delivery of both fat-soluble and water-soluble immunogens. They, having the characteristics of both liposomes and emulsions, provide the advantages of high loading of hydrophobic bioactive compounds in the internal solid lipid core and the ability to encapsulate water-soluble antigens in the aqueous compartments of surrounding phospholipid layers. Their particle size distributions are in the range of 10-250 nm (Anselem et al., 1998).

Ideal properties of SLN for parenteral administration

Lipid matrices would be possible controlled release systems for parenteral and especially intravenous administration if they could be produced with the appropriate properties as follows: (i) small size, i.e. mean diameter in the nanometer range, with a simultaneous low content of microparticle (> 1 μm as limiting factor for intravenous. injectability); (ii) toxicological acceptance by the use of physiological compounds and the absence of toxic residues from the production process, e.g. solvents; (iii) sufficient loading capacity for lipophilic and possible also hydrophilic drugs; (iv) prolonged release of drugs for days or weeks; (v) drug release in the appropriate rate at the site of action; (vi) possible sterilization by autoclaving or gamma irradiation; (vii) long-term stability in aqueous dispersion with regard to coalescence and drug leakage and/or alternatively option to be lyophilized or spray-dried; (viii) tissue specificity by controlled modification of their surface properties; (ix) production on large industrial scale to sufficiently supply the market; and (x) cost acceptable to the health authorities of the customer countries (Müller, Mehnert et al., 1995; Burgess, 1990).

Advantages and Disadvantages of SLN

SLN is an alternative drug delivery carrier for the administration of sustained release drug. It is developed from fat emulsion and polymeric nanoparticles. It has many advantages which better than the other carriers. Its possible advantages are (i) low systemic toxicity; (ii) low cytotoxicity; (iii) possible to produce in large or industrial scale; (iv) possible to avoid the residues from organic solvents by preparing with homogenization technique; (v) possible to sterile by autoclaving; (vi) possible controlled release; (vii) possible to deliver to site specific cells; (viii) increase resistance to hydrolysis or oxidation; and (ix) long term stability (Floyd and Jain, 1996; Schwarz, Mehnert, Luck et al., 1994).

However, this carrier is not the best system. It has some disadvantages that should be avoided or improved. These disadvantages are the difficulty in formulation, difficulty in manufacturing production, discomfort to patient, difficulty to maintain the uniformity and accuracy of dose at time of administration, and it has some problems in the maintenance of physical stability to be the good dispersion if its composition is not suitable (Floyd and Jain, 1996).

SLN ingredients

1. Lipid matrix

The matrix of SLN is constituted by biocompatible hydrophobic materials, which are solid at room temperature and have melting points ranging from approximately 30°C to 120°C. The carrier material must be compatible with the agent to be incorporated. These lipids are as follows: (i) mono-, di- and triglycerides of long chain fatty acids such as tristearin, tripalmitin, trimyristin, trilaurin, tricaprin; (ii) hydrogenated vegetable oils; (iii) fatty acids such as decanoic acid, lauric acid, myristic acid, palmitic acid, stearic acid, and their esters such as ethyl stearate, isopropyl myristate, isopropyl palmitate; (iv) fatty alcohols and their esters and ethers such as cetostearyl alcohol, cetyl alcohol, stearyl alcohol, oleyl alcohol, lauryl alcohol, myristyl alcohol; (v) natural, regenerated, or synthetic waxes such as beeswax, carnuaba wax; (vi) wax alcohols and their esters; sterols such as cholesterol and its esters; (vii) solid hydrogenated castor and vegetable oils; (viii) hard and soft paraffins; or (ix) the mixtures of above mentioned lipids (Domb, 1994; Gasco, 1993; Westesen and Siekmann, 1998).

Because the oil component is insoluble in water, it does not exert an osmotic effect and thus can be used in vivo at higher concentration. However, the purity of the oil is critical when apply to parenteral products and the presence of undesirable substances must be minimized (Floyd and Jain, 1996).

2. Dispersion medium

The dispersion medium that used for preparing SLN is a pharmacologically acceptable liquid not dissolving the agent or mixture of agents. It is selected from the group consisting of water, ethanol, propylene glycol, dimethyl sulfoxide (DMSO), methyl-isobutyl-ketone and mixtures thereof (Westesen and Siekmann, 1998). Water for injection is the most dispersion medium using in SLN preparations for parenteral administration.

3. Stabilizers

Natural and synthetic agents have been considered as possible emulsifying agents because neither of the commonly used oils forms spontaneous emulsions when mixed with water. Amphipathic compounds such as ionic and non-ionic surfactants can stabilize SLN. Suitable stabilizers include but are not limited to the following examples: (i) natural phospholipids such as soybean lecithin, egg lecithin, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, phosphatidic acid, sphingomyelin, diphosphatidylglycerol, phosphatidylserine, phosphatidylcholine, cardiolipin; (ii) synthetic phospholipids such as dimyristoyl-phosphatidylcholine, dimyristoylphosphatidylglycerol, distearoylphosphatidylglycerol , dipalmitoylphospha tidylcholine; (iii) hydrogenated or partially hydrogenated lecithins and phospholipids· (iv) sphingolipids and glycosphingolipids; (v) physiological bile salts such as sodium cholate, sodium dehydrocholate, sodium deoxycholate, sodium glycocholate, sodium taurocholate, sodium taurodeoxycholate; (vi) saturated and unsaturated fatty acids or fatty alcohols; (vii) alkylaryl-polyether alcohols such as tyloxapol; (viii) esters and ethers of sugars or sugar alcohols with fatty acids or fatty alcohols; (ix) acetylated or ethoxylated mono- and diglyceride; (x) polyoxyethylene sorbitan fatty acid esters such as polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monostearate, polyoxyethylene sorbitan trioleate; (xi) polyoxyethylene sorbitol fatty acid esters such as polyoxyethylene sorbitol monooleate, polyoxyethylene sorbitol pentaoleate, polyoxyethylene sorbitol monostearate; (xii) polyoxyethylene glycerine fatty acid esters such as polyoxyethylene glycerol monostearate; (xiii) polyoxyethylene fatty acid esters such

as polyoxyethylene monooleate, polyoxyethylene distearate, polyoxyethylene dioleate; (xiv) polyoxyethylene alkyl ethers such as polyoxyethylene oleyl ether, polyoxyethylene stearyl ether, polyoxyethylene behenyl ether; (xv) polyoxyethylene sterol ethers such as polyoxyethylene cholestanol ether, polyethylene cholesterol ether; (xvi) polyoxyethylene and polyoxypropylene block copolymers; (xvii) polyoxyethylene-polyoxypropylene alkyl ethers such as polyoxyethylene-polyoxy propylene cetyl ether; (xviii) polyoxyethylene castor oil or hydrogenated oil derivatives such as polyoxyetho xylated castor oil (Cremophor®); (xix) polyglycerol fatty acid esters such as decaglycerin dioleate; (xx) amino acids; (xxi) polypeptides and proteins such as gelatin and albumin; and (xxii) a combination of two or more of the above mentioned stabilizes (Anselem et al., 1998; Nakajima, Kohchi, and Tomomasa, 1994; Weiner, Martin, and Riaz, 1989; Siekmann and Westesen, 1994a; Westesen and Siekmann, 1998).

The molecular weight of these surfactants is preferably 1000 dalton or more. When the molecular weight is less than 1000 dalton, the stimulation or irritation to the organisms is strong and, when such surfactants are used in injection preparations, hemolysis is likely to occur (Nakajima et al., 1994).

Natural lecithin, the generally stabilizer used in parenteral fat emulsion, is defined as a mixture of phosphatides, and has been obtained from both animal (egg yolk) and vegetable (soybean) sources. Components of natural lecithin are listed in Table 1. An advantage of using natural lecithin is that it is metabolized in the same way as fat and it is not excreted via the kidneys, as are many synthetic agents. However, there are some studies showed that soybean lecithin was the principle cause of granulomatous lesions in the rats given infusions of olive oil emulsions, and concentrations of soybean lecithin greater than 1% were associated with increased blood pressure. This was attributed to impurities in the lecithin; totally purified lecithins, however, were less satisfactory as emulsifiers. And both natural and purified egg lecithins produced hemolytic and other toxic effects after emulsion storage. This was attributed to the hydrolysis of lecithin to lyso-lecithin, more commonly known as lyso-phosphatidylcholine. However, it is known that phosphatidylcholine and lyso-phosphatidylcholine form complex that may reduce the

Table 1. Major and minor components of lecithin (from Hansrani, Davis, and Groves, 1983)

Major components	Phosphatidylcholine (PC)
	Phosphatidylethanolamine (PE)
Minor components	Lyso-phosphatidylcholine (LPC)
	Sphingomyelin (SP)
	Phosphatidic acid (PA)
	Phosphatidylinositol (PI)
	Phosphatidylserine (PS)
	Cholesterol

toxic potential as well as improve the emulsion stability (Floyd and Jain, 1996).

More recently, stabilizers such as fatty acid esters of sorbitan (various types of span) and polyoxyethylene sorbitan (various types of tween) are now approved by the various pharmacopoeias for parenteral administration. Tween 80 is the most common and versatile stabilizer used in parenteral products marketed in the present. The concentration of tween 80 can be used in parenteral products is 0.01-12% (Nema et al., 1997).

One group of nonionic materials that has shown promise as stabilizers for parenteral preparations is the poloxamer (polyoxyethylene-polyoxypropylene derivatives).

The amount of emulsifier required for surface stabilization of the dispersion of the dispersed particles is higher than in conventional lipid emulsions, for example such as used in parenteral nutrition (Westesen and Siekmann, 1994). This effect can be attributed to the crystallization of the molten lipids after homogenization. Since the lipids typically do not recrystallize or exist in the form of ideal spheres but as anisometric particles, there is a large increase in surface area as

compared to the droplets of the emulsified molten lipids or of conventional lipid emulsions, respectively. The additional surfaces newly generated during recrystallization or polymorphic transitions of the dispersed lipids need to be stabilized immediately on formation to avoid particle aggregation. Therefore, the stable dispersion of SLN requires the presence of a reservoir of stabilizing agents after emulsification.

The choice of stabilizers can not be deduced from compositions and stabilization mechanisms for o/w emulsions but is dependent on the existence of highly mobile stabilizers due to the formation mechanism of the anisometrical particles. For the stabilization of SLN, the diffusion velocity should be significantly high to reach freshly created particle surfaces, especially during recrystallization of the lipid, before particle aggregation can take place, in order to exert a stabilizing action at the lipid/ water interface to prevent particle aggregation. Highly mobile stabilizers can be of ionic or nonionic nature. Typically, these stabilizers dissolve molecularly in the dispersion medium and/or form micelles. Micelles are known to be highly dynamic structures characterized by a fast exchange of molecules between micellar aggregates and the dispersion medium. The monomers in the dispersion medium are immediately available for surface stabilization. In contrast, stabilizing agents that tend to form a separate phase in the dispersion medium are not sufficiently mobile to stabilize freshly created surfaces before particle aggregation can take place. These stabilizers are therefore not suitable as sole stabilizers of dispersions of SLN. Phospholipids are an example of these stabilizing agents. Phospholipid molecules are therefore bound in vesicular structures and are not immediately available to stabilize newly created surfaces during recrystallization of the lipid particles. Consequently, phospholipids alone cannot efficiently stabilize suspensions of SLN. The optimum stabilizer is a surfactant combination of emulsifiers that are present the lipid side, such as phospholipids, and the highly mobile surfactant molecules in the dispersion medium, such as bile salts, tyloxapol and poloxamers (Westesen and Siekmann, 1998).

For SLN prepared by microemulsion method, the co-surfactants are necessarily used to prepare clearly microemulsions. They are selected from the group

comprising: low molecular weight alcohols or glycols such as butanol, hexanol, hexanediol, propylene glycol; low molecular weight fatty acids such as butyric acid, hexanoic acid; esters of phosphoric acid; and benzyl alcohol (Gasco, 1993).

4. Other additives

The dispersion medium may be contained one or more of the following additives: water-soluble or dispersable stabilizers; isotonicity agents; cryoprotectants; electrolytes; buffer; anti-flocculants; and preservatives.

Isotonicity of the parenteral preparations is desired to prevent pain, irritation, hemolytic, and tissue damage at the site of administration. Because emulsified oil exerts no osmotic effect, additives are required to produce isotonic conditions in parenteral preparations. Examples of tonicity agents used in SLN include glycerol, xylitol and mannitol (Nema et al., 1997; Westesen and Siekmann, 1998). Sorbitol is claimed to affect the pH and produce phase separation on autoclaving. Both ionic agents (sodium chloride) and reducing sugar (glucose, dextrose) are unsatisfactory because of interaction with the lecithin-emulsifying agent, resulting in brown discoloration and/or instability problem of the preparation (Hansrani et al., 1983).

Oxidation can lead to unacceptable discoloration of the drug product. Antioxidants are added in the formulation to minimize this degradation by preferentially undergoing oxidation as the result of their lower oxidation potential or by terminating the propagation step in the free radical oxidation mechanism. Examples of suitable antioxidants and their typical concentrations for injectable dosage form are as follows: ascorbic acid (0.02-0.1%), sodium bisulfite (0.02-0.66%), sodium metabisulfite (0.02-0.10%), sodium formaldehyde sulfoxylate (0.075-0.5%), thiourea (0.005%), ascorbic acid esters (0.01-0.15%), butylated hydroxytoluene (0.00116-0.03%), and tocopherols (0.05-0.075%) (Nema et al., 1997). Furthermore, the exposure to oxygen during the manufacturing process should be avoided. This can be accomplished by (i) purging the solvent system and bulk drug product with filtered nitrogen during the manufacturing process, (ii) blanketing the bulk drug

product with filtered nitrogen/argon during the filling operation, and (iii) displacing oxygen from the head space of the filled container with filtered nitrogen.

Flocculation is the phenomena of suspensions that may happen when the particles possess a low zeta potential. In some preparations the flocculation need to prevent caking of the particles. But in SLN, the stable colloidal dispersions are requirement properties of them. Antiflocculants such as sodium pyrophosphate, sodium citrate, sodium dodecyl sulfate and sodium dihydrogen phosphate are used in these preparations (Westesen and Siekmann, 1998). They adsorb on the colloidal particles and enhance the physical stability by increasing the zeta potential. The antiflocculants should be selected by considering the effect of pH shifts, maximum net charge increase, and their toxicological acceptance.

Antimicrobial agents are required for parenteral preparations that are intended for multiple dosing, or single dose vials if the active ingredient(s) does not have bactericidal or bacteriostatic properties or is growth promoting, to protect the product from accidental microbial contamination during clinical usage and to maintain sterility. Some typical preservatives used in parenteral suspensions and their commonly used concentrations are as follows: benzyl alcohol (0.75-5%), methyl paraben (0.05-0.18%), propyl paraben (0.01-0.1%), thimerosal (0.003-0.01%), and chlorobutanol (0.25-0.5%) (Nema et al., 1997).

Lyophilization is the method for reducing chemical instability in the case of drugs susceptible to hydrolysis. It is highly desirable to have a freeze-dried SLN formulation available. For a good reconstitution performance of products, the dispersions of SLN are diluted with the cryoprotectant solution before freezing. These cryoprotectants are sugars or sugar alcohols, such as sucrose, glucose, maltose, and trehalose (Schwarz and Mehnert, 1997; Schwarz, Mehnert, and Müller, 1994).

5. Drugs or bioactive substances

The drugs or bioactive substances can be located in the core of SLN where they are dissolved, solubilized, or dispersed in the matrix, and/or in the

stabilizer layer(s) surrounding the particle matrix, and/or can be adsorbed to the surface of SLN.

Various bioactive substances may be employed in the carrier systems comprised of the lipid and surfactant. Some examples of these are illustrated in Table 2 (Anselem, 1998; Bunjes, Westesen, and Koch, 1994; Cavalli, Morel et al., 1995; Domb, 1993; 1994; 1995; Fountain et al., 1986; Gasco, 1993; Heiati, Tawashi et al., 1997; Olbrich, Kayser, and Müller, 1999; Reithmeier, Göpferich, and Herrmann, 1999; Speiser, 1989; Vyas and Jaitely, 1999; Westesen and Siekmann, 1998; Westesen, Siekmann, and Koch, 1993).

Table 2. Bioactive substances used in SLN carriers

α,β-Blokers	Labetalol; Sulfinalol; Bucindolol
β-Blokers	Timolol; Talinolol; Propranolol; Atenolol; Metoprolol; Nadolol; Pindolol; Oxprenolol; Labetalol; Levobunolol
ACE inhibitors	Captopril, Enlapril; SA-466
Anesthetics and Narcotics	Butanilicaine; Fomocaine; Isobutambene; Lidocaine; Risocaine; Pseudococaine; Prilocaine; Tetracaine; Trimecaine; Tropacocaine; Etomidate
Antiallergics	Ketotifen fumarate; Procaterol; Tiaramide; Tranilast
Antibiotics	Fosfomycin; Fosmidomycin; Rifapentin; Gentamicin; Streptomycin; Tetracycline; Oxytetracycline; Tylosin; Erythromycin
Anticholinergics	Metixen; Profenamine
Anticoagulants	Heparin; Gabexat-Mesilat
Antidepressants; Psychostimulants and neuroleptics	Alimenazine; Binedaline; Perazine; Chlorpromazine; Fenpentadiol; Fenanisol; Fluanisol; Mebenazine; Methylphenidate; Thioridazine; Toloxaton; Trimipramine
Antidiabetics	Glipizide; Gliclazid; Ciglitazone

Table 2. (Continued).

Antiepileptics	Dimenthadion; Nicethamide
Antihypertension agents	Minoxidil; Dihydroergotoxin; Dihydroergotoxin-Mesilat; Endralazin
Antihypotensive agents	Dihydroergotamin; Dihydroergotamin-Mesilate; Gepefrin
Antimigraine agents	Clonidine; Flunarizin; Metergoline; Nadolol; Dopamine antagonists
Antimycotics	Butoconazole; Chlorphenesin; Etisazole; Exalamid; Pecilocine; Miconazole
Antiparkinson agents	Memantin; Piribedil; Mesulergin; Desocryptin; Lisuride hydrogen maleate
Antiphlogistics	Butibufen; Ibuprofen
Antirheumatic agents	Indomethacin; Diclofenac; Ibuprofen; Ibuproxam; Ketoprofen; Pirprofen; Suprofen
Antithrombotics	Suloctidil; Nafazatrom; Picotamid; Heparin oligosaccharides; Antithrombin III
Antiviral agents	Acyclovir; Metisoprenol; Tromantadine; Vidarabine; Vidarabin sodium phosphate; Immunoglobulin; 3′-Azido-3′-deoxythymidine palmitate prodrug
Blood fractions	Albumins; Antithrombins; Factor IX; Factor VIII; Haptoglobin
Bronchodilators	Bamifylline
Bronchospasmolytics	Ipratropium bromide; Chromoglycinic acid; Sobrerol
Calcium antagonists	Diltiazem; Flunarizin; Gallopamil; Verapamil; Nifedipin; Nicardipin; Nimodipine; Nitrendipin; Lidoflazin; Niludipin
Cardiac glycosides	Digitoxin; Digoxin; Methyl digoxin; Acetyl digoxin; K-strophanthin
Cardiovascular drugs	Alprenolol; Butobendine; Cloridazole; Hexobendine; Nicofibrate; Penbutolol; Pirmenol; Prenylamine; Procaine amide; Propatylnitrate; Suloctidil; Toliprolol

Table 2. (Continued).

Cardiovascular drugs	Xibendol; Viquidile
Cephalosporins	Cefamandol; Cefmenoxim; Cefoperazon; Ceftizoxim; Cefalexin; Cefalotin; Cefazedon; Cefotaxim; Cefoxitin; Cefsulodin
Cerebrally acting vasodilators	Dihydroergotoxin; Dihydroergotoxin-Mesilate; Ciclonicate; Vinburin; Vinpocetin; Vincamine
Contraceptives	Binovum; Desogestrel; Triquilar
Cytostatics	Chloromethine; Cyclophosphamide; Melphalan; Chlorambucil; Busulfan; Thio-TEPA; Methotrexate; 5-Fluouracil; Cytarabine; 6-Mercaptopurine; Vincristine; Vinblastine; Vindesin; Actinomycin D; Mytomycin C; Mytramycin; Doxorubicin; Belomycin; Cisplatin; Procarbacin; Estramustine; Thioguanine; Asperline; Chlornaphazine; Mitotane; Taxol; Penclomedine; Trofosfamide; Tributyrin
Diuretics	Triamterene; Hydrochlorothiazide; Furosemide; Piretanide; Metolazone
Enkephalins	Metkephamide; β-Endorphin; Enkephalin
Fibrinolytics	Urokinase; Plasminogenic activator
Growth hormones	Somatropin
Heart protecting	Ubidecarenone; Coenzyme Q_{10}
Hyperemic drugs	Capsaicine; Methylnicotinate
Hypnotic agents; Sedatives	Flurazepam; Nitrazepam; Lorazepam
Immunomodulating peptide	Thymocartin
Insulins	Natural, semi-synthetic, synthetic insulins; Proinsulin
Interferons; Lymphoquins	α-Interferon; β-Interferon; γ-Interferon
Lactation inhibitors	Bromocryptine; Metergoline
Lipid-reducing agents	Beclobrat; Bezafibrat; Etofibrat; Fenofibrat Nicoclonate; Oxprenolol; Pirifibrate; Simfibrate; Thiadenol
Peripherally vasodilators	Buflomedil; Minoxidil; Cadralazin; Propentofyllin

Table 2. (Continued).

Prostaglandins; Prostacyclins	Alprostadil; Carboprost; Epoprostenol; Sulproston
Psycho-pharmaceuticals	Oxazepam; Diazepam; Bromazepam
Radio diagnostic agents	Aminofostin; Misonidazol
Somatostatin	Stilamin; Somastotine and its derivatives
Spasmolytics	Aminopromazine; Caronerine; Difemerine; Fencarbamide; Tiropramide; Moxaverine
Steroid hormones	Testosterone; Testosterone enantate; Testosterone-(4-methylpentanoate); Testosterone undecanoate; Progesterone; Pregnenolone; Corticosterone; Cortisol; Cortisone; Prednisolone; Triamcinolone; Methylprednisolone; Dexamethasone
Systemically acting antimicotics	Ketoconazol; Griseofulvin
Transquilizers	Azaperone; Buramate
Vaccines	Corynebacteria parrum; Hepatitis B vaccine; Lactobacillus vaccine; Staphylococcal enterotoxin B toxoid F; Leishmania lipopeptide antigen; Pneumococcal vaccine; Anti-HIV envelope protein (gp160)
Vasodilators	Molsidomin; Hydralazine; Dihydralazine; Nicorandil
Virustatics	Arildon
Vitamin A derivatives	Retinol; Retinol acetate; Retinol palmitate
Vitamin E derivatives	Tocopherol acetate; Tocopherol succinate; Tocopherol nicotinate

Method for preparing SLN

Many researchers have prepared SLN as colloidal carriers for the controlled delivery of drugs. The different methods for preparing SLN are described.

1. High pressure homogenization method

1.1 Hot melt homogenization method

The SLN can be prepared by an emulsification process of molten material creating liquid droplets which form crystalline anisomerical particles on cooling. The solid lipid or the mixture of lipids is melted and dispersed in an aqueous surfactant solution at temperature above its melting point. The drug is dissolved or dispersed in the melted lipid prior to emulsification. This coarse pre-emulsion obtained is homogenized using high pressure homogenizer or microfluidizer and controlled the temperature above the melting point of the lipid throughout the process. The obtained homogenization product is an o/w emulsion of melted lipid in the aqueous surfactant solution. The oil droplets solidify during cooling to room temperature and form SLN.

1.2 Cold dispersion homogenization method

The solid lipid matrix is ground in liquid nitrogen or dry ice to increase its brittleness with a mortar mill yielding a lipid powder consisting of particles between 50 μm and 100 μm. For drug loaded SLN the drug is dissolved or dispersed in the melted lipid, after solidification of the lipid-drug mixture grinding is performed. The powder is dispersed in an aqueous surfactant solution and homogenized at temperatures of approximately 5°C to 10 °C below the melting point of the lipid, at room temperature or below room temperature using the temperature control unit. This method avoids melting or limits it to a brief softening followed by fast recrystallization. The cavitation and shear forces in the homogenizer gap disrupt the solid microparticles to SLN.

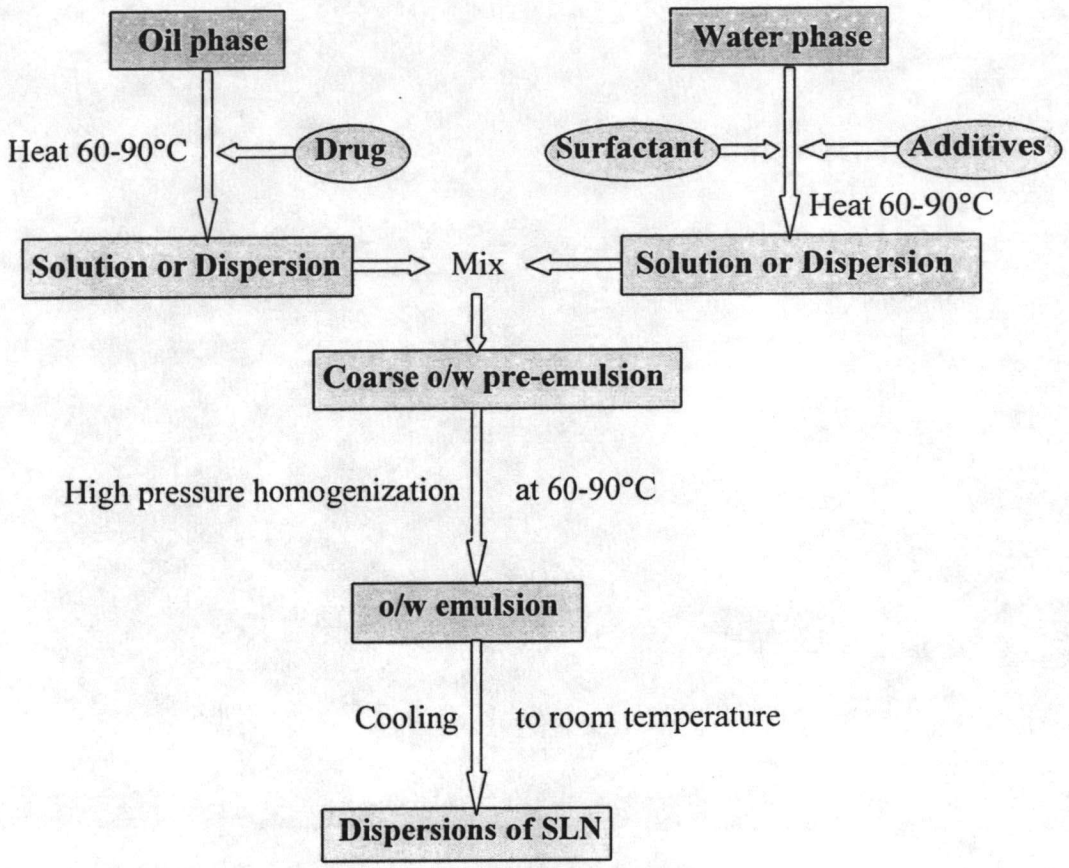

Figure 1. A schematic illustration of hot melt homogenization method.

Considering the gap width of the homogenizer being approximately 25 μm, one could expect a blockage of the gap. However, the cavity during the homogenization process seems to be so effective that a blockage does not occur at concentrations up to 10% solid phase. Blockage of the gap is only observed with some formulations when using a 15% suspension or higher. Dispersing solid-lipids requires more energy, which means that the number of homogenization cycles needs to be increased for obtaining similar small-sized products. This method is appropriate with the drug which is non-stable in high temperature or hydrophilic drugs.

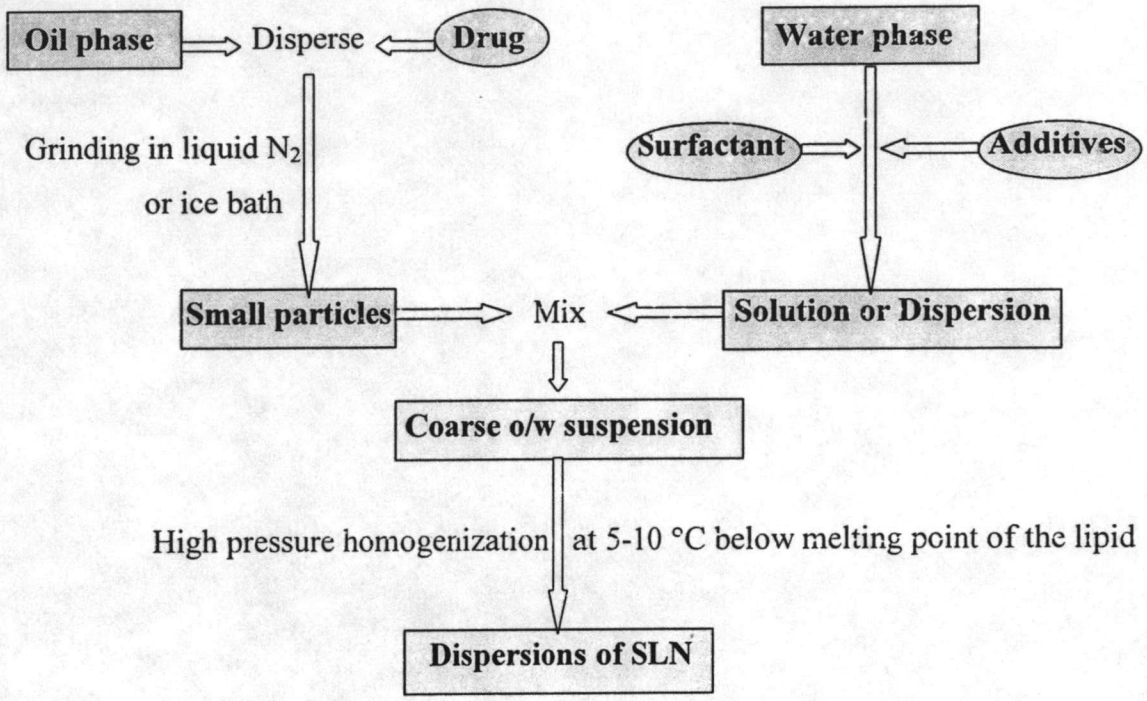

Figure 2. A schematic illustration of cold dispersion homogenization method.

High pressure homogenization is routinely used for the production of emulsions for intravenous administration, liposomes, and SLN. Turbulence, cavity, shearing, collision and intense mixing are among the factors responsible for size reduction. The required particle size can be achieved using various homogenizers or microfluidizers. Their utility on either laboratory or industrial scale must be acknowledged because equipment changes during scale-up may affect the physical and chemical stability and may affect the pharmacological efficacy as well. This step should be carefully controlled by taking sufficient in-process particle size measurements to determine the end point of homogenization (Floyd and Jain, 1996).

In the homogenizer devices, the crude emulsion is forced under pressure through the annular space between a spring-loaded valve and the valve seat. The emulsion may be homogenized in appropriate pressure and number of times in order to achieve the necessary particle size. One type of high pressure homogenizer is illustrated in Figure 3. However, a major concern of the homogenization process is

the inevitable contamination produced from gasket materials, packing, and metal parts. These contaminants also originate from pumps and other metal surfaces. One technology that has demonstrated good performance while minimizing the contamination is microfluidization.

Microfluidization has been used very successfully to produce parenteral emulsion. Microfluidizer processing is based on a submerged jet principle in which two fluidized streams interact at ultrahigh velocities in precisely defined microchannels within an interaction chamber. A combination of shear, turbulence, and cavitation forces results in the energy-efficient production of consistently fine droplets with a narrow size distribution (Floyd and Jain, 1996; Tabibi, 1990). Diagram of the microfluidizer is illustrated in Figure 4.

2. Solvent-emulsification method

Solvent-emulsification is a suitable alternative method to prepare small, homogeneously sized SLN (Siekmann and Westesen, 1996; Sjöström, Bergenståhl and Kronberg, 1993; Sjöström, Kronberg and Carlfors, 1993). The solid lipids and lecithin are dissolved in the organic solvent, such as cyclohexane. For drug-loaded SLN, drugs can dissolve or disperse in the organic phase at the same time. This solution is added to the aqueous phase containing water soluble co-surfactant. Emulsification is accomplished by treatment in colloid mill or other mixing instruments, such as high speed vortexing. The pre-emulsion is homogenized by high pressure homogenizer. The organic solvent in the outlet emulsion is removed at room temperature by evaporation in a rotavapor under reduced pressure (40-100 millibar) and the solid lipids precipitate in the emulsion droplets. The crystalline particles having a mean size below 30 nm can be prepared (Siekmann and Westesen, 1996).

This method allows one to produce a high load of drug in each entity and the emulsifier to drug ratio is relatively low compared to liposomal or micellar preparations. Furthermore, the method allows the surface characteristics and the particle sizes to be altered by the use of a wide range of biocompatible emulsifiers

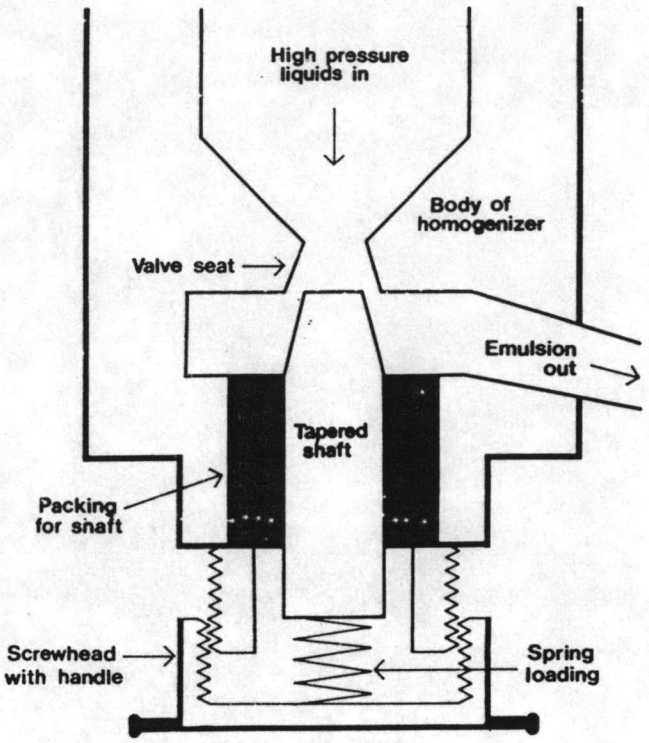

Figure 3. One type of single stage orifice for a high pressure homogenizer (from Hansrani et al., 1983: 148).

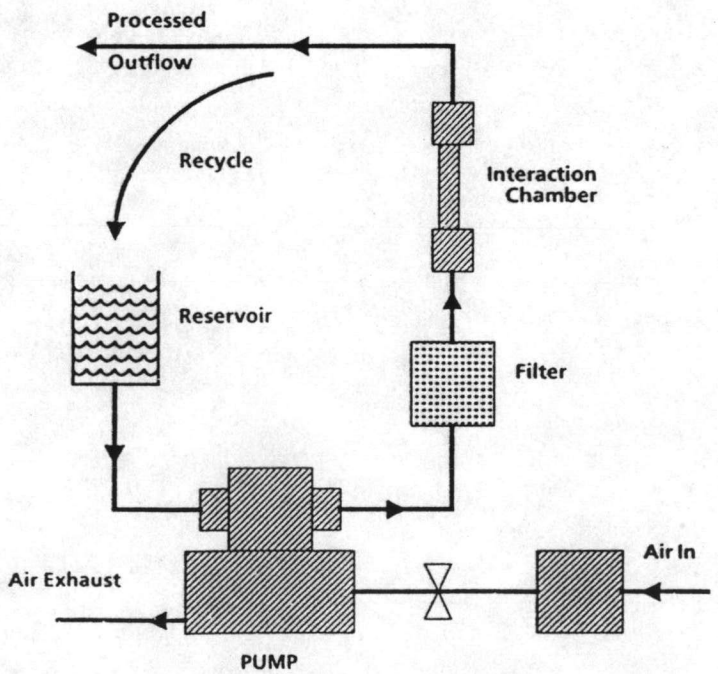

Figure 4. Diagram of the microfluidizer (from Floyd and Jain, 1996: 271).

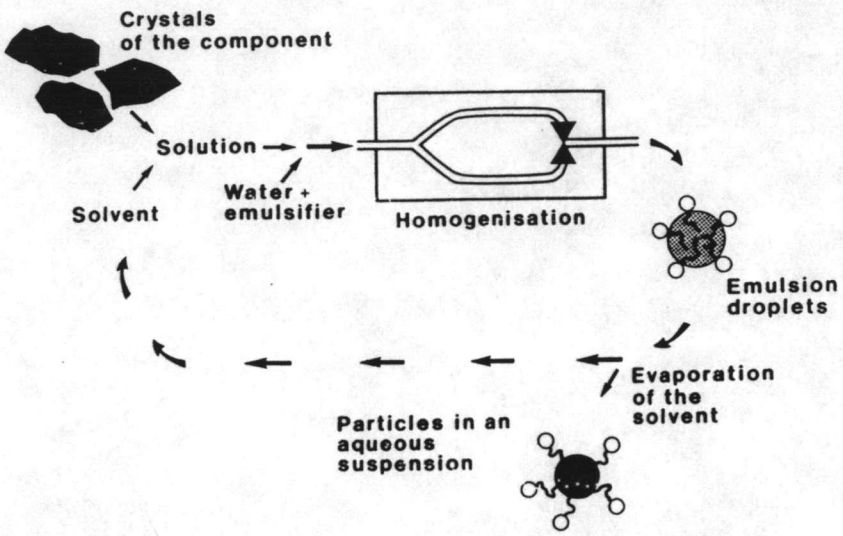

Figure 5. A schematic illustration of solvent-emulsification method (from Sjöström and Bergenståhl, 1992: 55).

(Sjöström and Bergenståhl, 1992; Sjöström, Westesen and Bergenståhl, 1993). Moreover, this process avoids the application of any heat, and can incorporating the temperature sensitive drugs as same as cold dispersion homogenization method while obtain the smaller particle size (Siekmann and Westesen, 1996).

3. Warm microemulsion method

SLN can be prepared by dispersing warm o/w microemulsions in cold water under stirring (Cavalli, Caputo, and Gasco, 1993; Cavalli, Marengo et al., 1996). The internal phase of the microemulsion is composed of lipophilic substances with low melting points. The clear microemulsion is prepared at 65-70°C, a droplet structure is already present in the warm microemulsion system. No energy is required for the formation of the nanoparticles. The microemulsion is poured into the glass syringe and thermostated at the required temperature, and delivered into the cold water in the constant rate. Rapid cooling in the cold aqueous medium is sufficient to obtain a dispersion of the SLN.

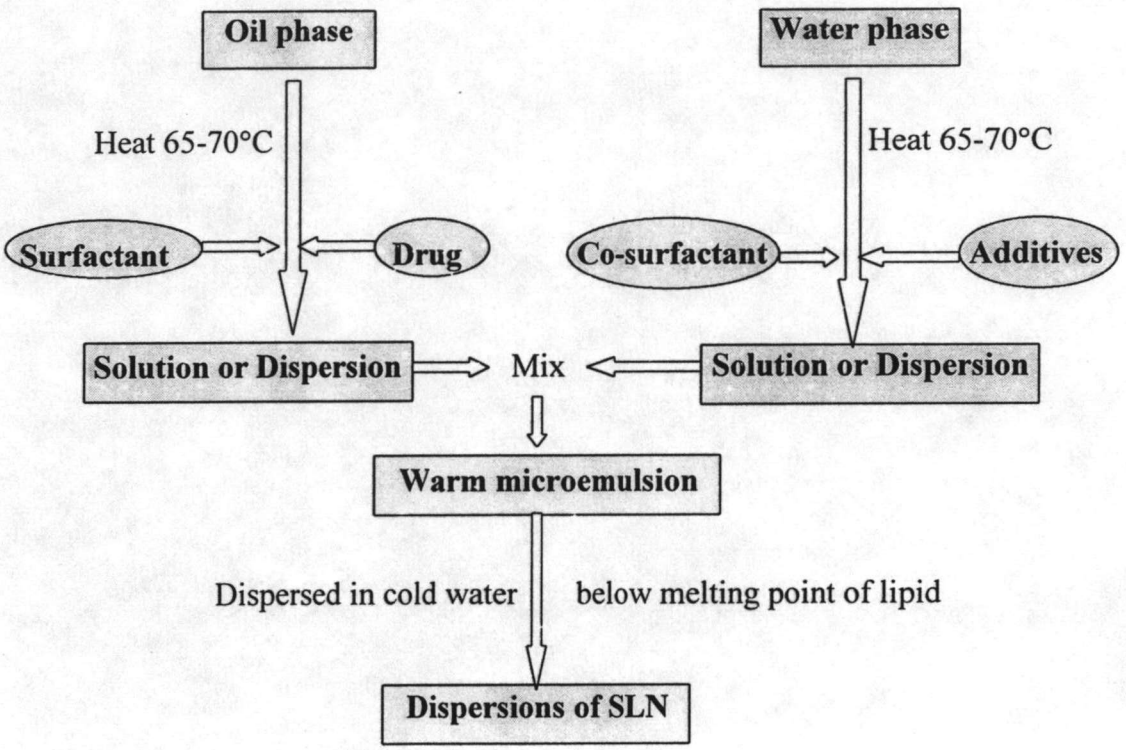

Figure 6. A schematic illustration of warm microemulsion method.

The characteristics of SLN prepared from this method depend on the composition of the microemulsion and the experimental parameters. The differences in the sizes of nanoparticles might be related to the diameters of warm microemulsion droplets and/or to aggregate formation during dispersion. Moreover, the temperature difference between the warm microemulsion and the dispersing water is also important to prepare smaller SLN (Cavalli, Marengo et al., 1996).

Lyophilization of SLN

Lyophilization (or freeze drying) involves the removal of water from products in the frozen state at extremely low pressures. The process is generally used to dry products that are thermolabile and would be destroyed by heat-drying.

Suspensions of SLN can be lyophyllized by freeze-drying to provided a water-free storage system that exhibits a good long-term stability. The lyophilized powder can be reconstituted before use by addition of water, buffer or solution of amino acids, carbohydrates and other infusion solutions directly or can be processed into other pharmaceutical formulations. Various cryoprotectants e.g. carbohydrates and amino acids can protect SLN against the formation of aggregates during freeze drying processes (Cavalli, Caputo, Carlotti et al., 1997; Schwarz and Mehnert, 1997; Schwarz, Mehnert, and Müller, 1994).

Spray-drying of SLN

As an alternative for SLN formulations that cannot be lyophilized successfully, spray-drying can be used as additional method for converting the aqueous dispersion into a dry product for long term storage. The carbohydrates can be used for preventing particle agglomerate are glucose, mannitol, sorbitol, lactose, trehalose, etc. The spraying parameters should be considered are the concentration of nanoparticles and the concentration and nature of carbohydrates. In general, spray-drying appears possible for SLN with sufficiently high melting point (approximately higher than 70°C) when optimizing nanoparticle and carbohydrate concentration (Mü ller, Mehnert et al., 1995).

Sterilization of SLN

SLN for parenteral administration must be sterilized. Many studies have indicated that the optimized preparations of dispersions of SLN can be sterilized by autoclaving at 121°C, 15 psi and 15 minutes (Müller, Mehnert et al., 1995). The SLN in freeze-drying powder products would also be sterilized by autoclaving, and are stable during sterilization and maintained a spherical shape, without any significant increase in size or nanoparticle distribution (Cavalli, Caputo, Carlotti et al., 1997).

Moreover, the development of production of SLN by aseptic process is also proposed (Zimmermann, Schöler et al., 1999). The commercial homogenizer was modified by separating the homogenization tower from the driving unit and placing it underneath a laminar air flow cabinet. The emulsion was prepared aseptically under laminar air flow conditions using sterilized equipments.

Evaluations of SLN for parenteral administration

For parenteral administrations, SLN is generally administered through 19-22 gauge needles, about 1-2 inches long, that have internal diameter in the range of only 300-600 μm. Thus, some properties are necessary to evaluate and control. Moreover, some physicochemical properties of SLN are important factors that can elucidate the distribution of carriers in the body and drug release. The characteristics to be evaluated are as follows:

1. Syringeability

Syringeability describes the ability of the suspension to pass easily through a hypodermic needle or transfer from the vial prior to injection. It includes characteristics such as the ease of withdrawal, clogging and foaming tendencies, and accuracy of dose measurements. Increasing the viscosity, density, particle size, and concentration of solids in suspension hinders the syringeability of suspension. A suitable test is to ensure that the entire suspension passes through a 25-gauge needle of internal diameter 0.3 mm.

2. Injectability

Injectability refers to the performance of suspension during injection and includes factors such as pressure or force required to injection, evenness of flow, aspiration qualities, and freedom from clogging. The syringeability and injectability are closely related to the viscosity and particle characteristics of the suspension.

3. Clogging

Clogging or blockage of syringe needles while administering a suspension may occur because of a single large particle or an aggregation that blocks the lumen of the needle or because of a bridging effect of the particles. It is advisable to avoid particles greater than one-third of the internal diameter of the needle to prevent clogging. Clogging, if observed at or near the needle end, is usually caused by restrictions to flow from the suspension and may involve combination of factors such as vehicle, wetting of particles, particle size, shape and distribution, viscosity, and flow characteristics of the suspension.

4. Drainage

Drainage refers to the ability of the suspensions to break cleanly away from the inner walls of the primary container-closure system and is another characteristic of a well-formulated parenteral suspension. Silicone coating of containers, vials, and plugs with dimethicone can improve the drainage of slightly overflocculated systems as well as of good suspensions.

5. Particle size measurements

The most significant characteristics of colloidal dispersions are the size and the shape of the particles. Particle size of the lipid particles has a direct effect on both toxicity and stability. Particles greater than 4-6 μm are known to increase the incidence of emboli and blood pressure changes. For intravenous injections, particles should be less than 1 μm in diameter. For subcutaneous or intramuscular injections, the particle should preferably be less than 250 μm in diameter. Larger particle sizes can be used for oral formulations. Submicron particles are needed for target drug delivery (Domb, 1993). Particle size measurements are useful in that they allow aggregation or crystal growth to be evaluated. They are technically very difficult because the particle sizes usually extend beyond the limit of detection of any one given instrument. Thus, at least two

complementary techniques should be employed. Many advanced instruments for determining particle size are available. Electron microscopy, laser inspection system and coulter counter methods are used to determine the particle size over than 1 μm. For particle size determinations below 1 μm, proton correlation spectroscopy (PCS) or quasi-elastic laser light scattering (QELS) is useful (Haskell, 1998; Prankerd and Stella, 1990).

6. Zeta potential

Zeta potential determinations can be of great value in the development of suspensions. The determination of the zeta potential of particles in a colloidal system provides useful information of the sign and magnitude of the charge and its effect on the physical stability of the system with time (Nash, 1988; Washington, 1990b). The zeta potential is determined by measuring the migration velocity of the suspension particles with respect to the net effective charges on the surface, called electrophoretic mobility. The Zeta meter® is a microelectrophoretic mobility apparatus used to measure them. Furthermore, zeta potential measurements are typically performed using a Doppler electrophoresis apparatus such as the Zetasizer®. A new technique for measuring zeta potential, electric sonic analysis or ESA, can be performed using a Matec® ESA 8000. This technique allows the zeta potential to be determined for a concentrated dispersion without the typical necessary dilutions that could affect the dispersion stability. The other instrument can be used to measure them is an amplitude weighted phase structuration (Floyd and Jain, 1996).

7. pH

The pH is important for maintenance the desired particle size because of its effect on the surface charge of the particle (Floyd and Jain, 1996). The pH of suspension of SLN is influence to the ionization of drug and surfactants in preparation. This parameter affects to physical and chemical stability. The pH meter is generally instrument to be used for determining the pH of preparation.

8. Osmotic pressure

Osmotic pressure is a colligative property and therefore can be related to the relative molecular mass of the colloidal material. This property is important because it affects directly to the cells, especially red blood cells. The osmotic property is determined by the gradient of some colligative properties, such as freezing point, boiling point, or pressure vapour. Osmomat® O30-D is instrument to determine the osmotic pressure using freezing point depression method.

9. Viscosity

Viscosity describes the resistance to flow with applied stress for a particular system; a more viscous system requires greater force or stress to make it flow at the same rate as a less viscous system. The suspension viscosity can change due to concentration of solid ingredients, particle shape, size, and distribution. This parameter is directly used to describe the syringeability and injectability of suspension for parenteral administration. Many techniques could be used to measure this parameter. Capillary viscometers and the falling ball viscometers are simple instruments for measuring viscosity but only for Newtonian liquids. Rotational viscometers including to coaxial cylinder sensor systems (cup-and-bob viscometers) and cone-and-plate sensor systems are instruments may be used with both Newtonian and non-Newtonian liquids (Martin, 1993; Schramm, 1981).

10. Microscopy

Transmission electron microscopy (TEM) has been particularly valuable for investigation particle size and shape of SLN. Freeze-fractured TEM or cryo-TEM is the technique used to prepare sample observed under microscope (Heiati, Tawashi et al., 1997; Siekmann and Westesen, 1994 (b); Westesen and Siekmann, 1997). Moreover, Scanning electron microscopy (SEM) has been reported for study the surface morphology of lipid micropellets (Eldem, Speiser, and Hincal, 1991).

Atomic force microscopy (AFM) has been applied as a new tool to image the surface of nanoparticles by Mühlen et al. (1994; 1996). This technique utilizes the force acting between a surface and a probing tip resulting in a special resolution of up to 0.01 nm for imaging. A stricking advantage of AFM is the simple sample preparation as no vacuum is needed during operation and the sample does not need to be conductive. Therefore originally hydrated, samples containing aqueous medium can be analyzed. A sufficient sample preparation is placed a drop of a solution or dispersion of a sample on a washed microscope slide or on a mica substrate. The AFM obtains images fast enough (about twenty seconds per image) to allow the observation of in situ processes occurring at interfaces. Thus AFM gained some resent attention in the field of biological and pharmaceutical sciences.

11. Differential scanning calorimetry

The differential scanning calorimetry can be used to determine the thermograms for studying the interaction between two or more components after preparing and its crystal polymorphs (Cavalli, Aquilano et al., 1995).

The recrystallization study of SLN by DSC method was suggested by Bunjes, Westesen, and Koch (1996). The heating curves were recorded with a scan rate of 5 or 10°C/minute, then the samples were kept around 20°C above their melting point for at least 10 minutes and then cooled at 5°C/minute. The melting and recrystallization temperatures were given correspond to the maxima or minima, respectively, in the DSC curves. Supercooling was given as the difference between these two temperatures.

Moreover, differential scanning calorimetry is used to analyze the degree of crystallinity. The recrystallization indices (*RI*) are calculated as follows:

$$RI[\%] = \frac{Enthalpy_{SLNdispersion}[J/g^{-1}]}{Enthalpy_{bulk\_material}[J/g^{-1}] \times Concentration_{lipid\_phase}[\%]} \times 100 \qquad (1)$$

The degree of crystallinity and the shape of DSC curve could be used to describe the physical stability of SLN and gelling formation (Freitas and Müller, 1999).

12. X-ray diffractometry

X-ray powder diffractometry can be used to study the crystalline characteristics of SLN after recrystallization in room temperature (Cavalli, Aquilano et al., 1995).

Several studies reported the using of synchrotron radiation X-ray diffraction to study the crystallization of SLN. The measurements were performed on the double focusing monochromator mirror camera X33 of the EMBL in HASY-LAB on the storage ring DORIS III of the Deutsches Elektronen Synchrotron (DESY) at Hamburg. For the recrystallization studies the nanoparticles were held at about 20°C above their melting point for at least 10 minutes and then rapidly cooled to at least 5° C above the onset of crystallization. They were then further cooled at constant rates between 0.3 and 0.4°C/minute and the scattering pattern is monitored continuously in 1-minute time frames. The melting behavior was investigated by either heating the samples in 3°C steps and monitoring the scattering pattern for each single temperature step or by heating the samples at a constant rate of 1°C/minute and recording the scattering pattern every minute (Westesen, Siekmann, and Koch, 1993; Bunjes, Westesen, and Koch, 1996).

13. Nuclear magnetic resonance spectroscopy

The nuclear magnetic resonance spectroscopy (^{1}H-NMR) is used for determining the physical state of lipid in aqueous dispersion. The magnetic resonance based methods prove themselves as applicable for quantitative non-invasive characterization of SLN yielding unique information on viscosity and polarity at the molecular level. There is no need to dilute the sample or separate the components, permitting serial measurement of the same sample. This technique can

be used to support the data from the other techniques such as DSC or X-ray diffraction method. (Liedtke et al., 1999; and Zimmermann, Liedtke et al., 1999).

14. Incorporation of drug in SLN

For determining the entrapment efficiency of drug in SLN, several studies have used some organic solvents for dissolving drug-loaded SLN and analyzed directly. Methanol and hexane are generally chosen because of they are good solvents for many drugs or substances and are not interfere in analyzed method. Spectroscopy or high performance liquid chromatography is used to measure the quantity of drug (Cavalli, Caputo, Carlotti et al., 1997, Reithmeier et al., 1999).

In some studies, the entrapment efficiency has been determined indirectly by measuring the amount of drug remaining in the supernatant after centrifugation or filtration (Almeida, Runge, and Müller, 1997).

15. *In vitro* drug release

During the last decade there has been a considerable increase in interest in the use of disperse systems as drug carriers. Dissolution characteristic of drugs from these dosage forms is important factor for absorption and bioavailability. Several studies have shown that the dissolution rate is a rate-limiting step for absorption and bioavailability of drugs administered in suspension formulations (Banakar, 1992a). Several types of apparatus have been proposed and implemented in assessing the dissolution rate of suspension or microdisperse systems. One of the common problems associated with most methods is retention of the dissolving material within the confines of the dissolution chamber. Thus, no method is suggested to be the best for determining the dissolution characteristics of microdisperse systems.

The USP paddle method is one method used to study dissolution of SLN (Müller, Mehnert et al., 1995). However, sampling is one problem due to interference of the smaller particles, which often get through the sampling probe.

Employment of filters reduces this problem to insignificant levels, however, clogging of the filter, reducing the effective number of particles for dissolution, often results in the generation of data. Flow-through apparatus has also been employed frequently to determine the dissolution characteristics of suspension (United States Pharmacopeial Convention, 1995). This dissolution cell allows for fresh dissolution medium to be circulated continuously, at a known velocity, surrounding the dissolving particles. However, this method is rather complicate compare to the other methods.

Membrane diffusion technique is the most popular method for studying the dissolution characteristic of microdisperse systems (Kinget and de Greef, 1995; Levy and Benita, 1990; Lostritto, Goei, and Silvestri, 1987; Saarinen-Savolainen et al., 1997; Tukker and de Blaey, 1983; Washington, 1990a). In this approach the carrier disperse phase, suspended in a small volume of continuous phase, is separated from a large bulk of sink phase by a dialysis membrane which is permeable to the drug. The sample and sink are well stirred. The drug diffuses out of the carrier and through the membrane to the sink, wherein it is periodically assayed. Thus, the accumulation of the drug in the sink is controlled by the consecutive rate processed of (non-sink) partition and diffusion of the drug across the membrane. By this method, the *in vitro* membrane permeation systems for transdermal can be applied to separate the sample compartment and receptor compartment by dialysis membrane (Banakar, 1992b; Huang, 1987).

The measurement of release profiles requires good sink condition, implying that release must occur into a large volume of sink medium. It has been recommended as a rule of thumb that the drug concentration in the sink phase in dissolution experiments must be kept below 10% of saturation (Washington, 1990a). This poses a problem since the drug must be assayed in the sink medium, and as the sink volume is increased the concentration of drug being measured decreases. Especially for insoluble drugs, a method of assay is required which is very sensitive to less quantity of drug in solution.

16. Cytotoxicity

For study the cellular uptake and cytotoxicity, flow cytometry can be used to assess the cellular binding capacities of SLN (Olbrich, Kayser, Kiderlen et al., 1999). Erythrocyte solution is incubated with labeled SLN formulations for 30 minutes and erythrocyte binding is determined with a FACSCalibur analytical cytometer equipped with a 15 millivolts, 480 nm air cooled argon ion laser.

Several studies have reported that the *in vitro* uptake of SLN by human granulocytes could be determined by chemiluminescence (CL). Cytotoxicity has been assessed by viability measurements using the dimethylthiazolyl-diphenylytetrazolium test (MTT) (Müller, Maaßen, Schwarz et al., 1997).

Physicochemical characteristics of SLN

The physicochemical properties of SLN are different from that of lipid emulsions. One basic difference of them is their much larger particle surface area as outlined above so that they require a higher amount of surfactants which additionally need to be highly mobile to immediately stabilize the new surfaces created when the molten lipid recrystallizes or transforms into the stable β-polymorph. The second basic difference is that once SLN are formed by recrystallization of the molten lipid any renewed melting of the small particles may result in an instability of the dispersions if there is no excess of mobile stabilizer in the aqueous phase which is not adsorbed to particle surfaces where it is immobilized.

Crystalline characteristics of SLN and their stability

Physical instability is indicated by particle size changes, flocculation, coalescence, caking, or oil separation. After rapid cooling, the oil droplets solidified and formed SLN. The shape of the particles is mainly anisometric which is a consequence of the matrix forming lipids present in a stable β-polymorphic

modification (e.g. β′-, β_1-, β_2-), and not in an amorphous or α-crystalline state. Thus, the product properties will not change significantly during long-term storage due to polymorphic transformations (Westesen and Siekmann, 1998).

However, the optimized composition of the dispersions of SLN being usually physically stable for more than 3 years was reported by Freitas and Müller (1999). Physically stable SLN remained as a mixture of stable β′ with unstable polymorphs (α, and sub α), increasing in crystallinity index during storage was slow and crystallization occurred mainly in unstable modifications. For some formulations tended to form a gel after a certain period of storage time. Especially high temperature, light exposition, and any input of energy were as activation energy caused solidification within a few days (Freitas, Lucks, and Müller, 1994). It was found to be entirely crystalline, i.e. the lipid recrystallized completely in the β′ modification. Dispersions of SLN with a gelling tendency could be stabilized by inhibiting the transitions in lipid modification, e.g. by addition of inhibitors to the lipid matrix.

The kinetic of polymorphic transitions after crystallization of triglyceride nanoparticles was slower for longer-chain than for shorter-chain triglycerides. The presence of triglycerides with longer hydrocarbon chains seemed to catalyze the nucleation of triglycerides with low crystallization tendency. Addition of longer-chain triglyceride such as tristearin might thus help to overcome solidification problems in lower melting triglyceride nanoparticles (Bunjes, Westesen, and Koch, 1996). Moreover, the recrystallization of the colloidally dispersed materials occurred at lower temperatures than that of the bulk material. The increased supercooling of the nanoparticles and their lower melting point was attributed inter alia to the colloidal size of the lipid particles. Nanoparticles of tristearin and tripalmitin were crystalline when stored at room temperature. Under the same conditions, colloidally dispersed trimyristin and trilaurin particles remain in the liquid state for at least several months of storage (Westesen and Bunjes, 1995).

The stabilization of dispersions of SLN required the presence of a highly mobile stabilizing agent in the dispersion medium in such a way that the amount of highly mobile stabilizers in the dispersion medium was, after emulsification, sufficient to stabilize newly created surfaces during recrystallization. SLN stabilized by phospholipids alone, or in combination with non-electrolytic compounds such as glycerol in concentrations used to achieve blood isotonicity, tended to form semi-solid ointment-like gels, whereas the addition of sodium glycocholate to the aqueous phase prevented this gel formation (Westesen and Siekmann, 1997; 1998).

A molar phospholipid to bile salt ratio between 2:1 and 4:1 was reported to be most effective regarding the initial stabilization during homogenization as well as the long-term storage stability of dispersions of SLN. Data thereof suggested that stabilization is most effective if the bile salt was not bound to mixed micelles and that during stabilization of SLN the bile salt molecules were inserted in the phospholipid layer on the surface of the particles (Westesen and Siekmann, 1998).

The effect of increasing phospholipid-triglyceride ratios on SLN characteristics was studied. It was found that the excess phospholipid did not result in the formation of liposomes when the ratio greater than 0.15, but was forming multiple bilayer structure around the lipid core of SLN (Heiati, Phillips, and Tawashi, 1996).

SLN could also be sterically stabilized by nonionic surfactants. Steric stabilization of SLN required, however, a relatively high amount of surfactants with lipid/surfactant ratios up to 1:1. It could be observed in general that the stability of SLN decreased with increasing lipid/surfactant ratio.

Moreover, non-electrolytic compounds used to achieve blood isotonicity such as glycerol turned out to promote the stability of dispersions of SLN (Westesen and Siekmann, 1998).

Particle size and shape of SLN

The particle size of SLN depends on the types and amounts of emulsifier and on the emulsification techniques and conditions. SLN in the nanometer size range are obtained by high pressure homogenization. The particles show a relatively narrow particle size distribution with mean particle sizes by number of approximately 50-300 nm as determined by photon correlation spectroscopy.

The SLN prepared by high pressure homogenization was reported by Schwarz, Mehnert, Luck et al. (1994). It was found that each nanoparticle systems have the differences in the established optimum homogenization conditions. Low homogenization cycles or low pressure can produce high content of particle higher than 5 μm. But many homogenization cycles or high pressure can cause breaking of the emulsions and produce high content of particle higher than 5 μm. The optimum homogenization conditions need to be assessed for each system.

The shape of SLN revealed the disc like structure was reported by Mühlen et al. (1996). The AFM images indicated that the crystalline particles are surrounded by soft layers. The softness of these layers was proved by form alterations, which occurred if they were imaged successively by contact AFM. However, some research reported the shape of SLN in spherical (Cavalli, Caputo,Carıotti et al., 1997). And the mean particle size of SLN was increasing when lipid that had higher melting point was used (Westesen and Siekmann, 1998).

Surface charge

The surface potential plays an important role in the stability of emulsion and suspension through electrostatic repulsion. Preliminary studies suggest that lipid particles with significant surface charge, either positive or negative, are more stable and are cleared from the bloodstream more rapidly than those with neutral charge (Floyd and Jain, 1996).

SLN containing lecithin has negative charges (Schwarz, Mehnert, Lucks et al., 1994). The zeta potential of nanoparticles containing lecithin of 1.2% after autoclaving is approximately –46.7 millivolts, while SLN containing poloxamer 188 of 1.2% was –27.7 millivolts. In the case of poloxamer 188, this zeta potential is not sufficiently high to stabilize the dispersion solely by electrostatic repulsive. However, poloxamer 188 is a steric stabilizer which can easily compensate the missing electrostatic repulsion.

Incorporation of drug and drug release

Drug release from the lipid carriers can be controlled by the composition of the lipid matrix by the choice of stabilizing agents as well as by the size of them. Drug leakage is hindered by the solid state of the carrier due to the restricted drug diffusion. For intramuscular or subcutaneous injection, the rate of drug release from a suspension can be affected by different steps involved in the process. These include diffusion of drug from the particles, perfusion of the area by blood, oil-water partition coefficient, and diffusion through the highly viscous adipose layer to the vascular system (Floyd and Jain, 1996).

The study of characteristics of SLN containing either phenothiazine or nifedipine found that both drugs behaved as co-surfactants and they could be located at the interphases of the microemulsions and probably the remaining part was so soluble in the oil phase that it could not nucleate even under the deep temperature gradient achieved during the nanosphere preparation. Both drugs were in a non-crystalline state within the lipid particles for more than 1 year after their preparation and can be improved their solubility and hence their bioavailability (Cavalli, Aqualino et al., 1995).

The incorporation and release of 3′-azido-3′-deoxythymidine palmitate (AZT-P) in SLN of trilaurin was studied. It was found that the incorporation and the release kinetics of AZT-P are related to the nature and amount of phospholipid surrounding the SLN core. AZT-P as an amphiphilic molecule would appear to preferentially integrate within the phospholipid bilayers. It does not appear to be

incorporated within the triglyceride core of SLN. The principal function of the triglyceride in SLN may therefore be to act as a matrix for the construction of a defined number of phospholipid bilayers capable of incorporating amphiphilic drugs (Heiati, Tawashi et al., 1997).

The physical state of different SLN at body temperature was studied by Westesen and Siekmann (1998). It was found that the different physical states of SLN at body temperature give rise to a different biopharmaceutical behavior with respect to the release of incorporated drugs or bioactive agents. SLN containing hard fat (Witepsol.TM.W35) molten at body temperature display basically the release characteristics typical of conventional lipid emulsions, i.e. the drug can be released relatively fast. In contrast, SLN of tripalmitin, which are solid at body temperature, give rise to sustained release of incorporated drugs. Since the drug molecules are immobilized in the solid matrix drug release is not diffusion-controlled but depends on the degradation of the solid lipid matrix in the body and is therefore delayed.

Characteristics of SLN on cells

In many cases, colloidal drug carriers are developed to deliver drugs, vaccine, and other biologically active agents to site-specific targeting organs. It can enhance the drug's therapeutic efficiency by an increase of the drug concentration at the target site with a simultaneous decrease at non-target sites. Thus, it can decrease the site effects of drugs on the other organs, and the toxicity of some drugs, e.g. anticancer agents, can be diminished.

Recently, passive or active transports of microparticulate carriers after administered intravenously, intraarterially or intraperitoneally have been studied. It is found that particles are rapidly taken up by the mononuclear phagocytes of the reticuloendothelial system (RES). For passive transport to site specific cells, the carriers are taken up by natural physiological processes to achieve the accumulation of particles almost in the liver (60-90%), spleen (2-10%), and less degree in lung and bone marrow (Kreuter, 1994). Typically, 80-95% of the total injected particles is cleared by the RES. This specific interaction opens possibilities for the treatment of

some parasitic and infectious diseases (Singh and Ravin, 1986). Conversely, for active transport to site specific cells, the carriers are generally connected to avoid or reduce carrier uptake by the RES. Due to these facts approaches to drug targeting are the modification of surface characteristics and the reduction of particle size. So that the carriers are not recognized as foreign and will thus evade the RES barrier, and they will have an affinity for a particular target tissue (Prankerd and Stella, 1990). This effect increases circulation times of drugs in the vascular and enhances transporting to tumor cells (Couvreur et al., 1995).

To target specific cells in other organs, colloidal carriers must escape from the vascular compartment. This is generally difficult to achieve, as the plasma membrane is usually continuous and impermeable to large molecules and small particles. Molecules of 5,000 to 10,000 daltons may pass into the interstitial space to be collected by the lymphatic system. Some capillary membranes are fenestrated, containing small holes 50 to 60 nm in size, which allow small particles to pass through. Sinusoidal or discontinuous membranes are present in the parenchymal cells of the liver, spleen, and bone marrow. These membranes have gaps of the order of 150 nm. Fenestration may also occur in areas of tissue inflammation and infection; hence, the uptake of colloid carrier systems may be enhanced in these areas (Burgess, 1990).

The clearance of the particles by RES has been described to depend on particle size as well as on particle surface characteristics such as surface charge and surface hydrophobicity (Westesen and Siekmann, 1998). In generally, small particles are cleared less rapidly from the blood stream than large particles (Davis and Hansrani, 1985). The particles greater than 7 μm in diameter are rapidly filtered by the fine capillary beds of the lung, whereas those with diameter between 100 nm to 7 μm are trapped or taken up by the cells of the RES into the liver and spleen if they are recognized as foreign (Burgess, 1990). Only small particles less than 100 nm are found in the bone marrow (leung et al., 1987), and particles of 50 nm or less are permeated through the liver endothelial system and accumulate at tumor tissues.

The charged particle also significantly affects on their efficient clearance by the RES. Preliminary studies suggest that lipid particles with significant surface charge, either positive or negative, are more stable and are cleared from the blood stream more rapidly than those with neutral charge (Davis and Hansrani, 1985; Davis, Illum et al., 1992). By coating the particles with a non-ionic surfactant, polyoxyethylene-polyoxypropylene copolymer, the zeta potential on the particles is reduced. This causes a significant reduction in liver uptake and an increase in lung uptake (Leung et al, 1987).

Nanoparticle hydrophobicity is also a factor likely to influence distribution in the body. Hydrophobic particles are taken up by macrophages directly, whereas hydrophilic particles must be coated with immunoglobulin G before they can be engulfed (Leung et al, 1987). The relative phagocytic uptake decreases with increasing adsorbed layer thickness of hydrophilic surfactant, such as poloxamer 407, which corresponds in case of steric stabilization effect in an increase in the surface hydrophilicity. Nanoparticles coating by hydrophilic poloxamers is found to reduce considerably nanoparticle opsonization by serum proteins, thus increasing the blood circulation time (Couvreur et al., 1995). More hydrophilicity nature of SLN versus lipophilic cell membranes leaded to reduce SLN-cell interactions. Span 85, the lipophilic surfactant, could induce the particles-cell interactions. This is important to deliver drug to cell targeting, such as antimalarial drugs (Olbrich, Kayser, Kiderlen et al., 1999).

Nanoparticles can cause cytotoxicity by adherence of the particles to the cell membrane, degradation of the adhered nanoparticles and subsequent release of cytotoxic degradation products. Another mechanism is the internalisation of nanoparticles by cells, intracellular degradation and subsequent toxic effects inside the cell.

The effect of SLN on mononuclear cells using *in vitro* culture of peritoneal macrophage was investigated by Schöler et al. (1999). Results of the study demonstrated that poloxamer 188 coated SLN could suppress the cytokine secretion (interleukin (IL-6)) of macrophages, and reduced both cytotoxicity and immune

modulatory effects. The adsorption layers of poloxamer 188 could reduce the phagocytic uptake of SLN by human granulocytes (Müller, Maaßen, Schwarz et al., 1997). This effect leaded to prolong their circulation times in the blood. Furthermore, many studies have indicated that the cytotoxicity of the glyceride SLN is approximately 10-fold below the one of polylactide/glycolide nanoparticles (Müller, Maaßen, Schwarz et al.,1997; Müller, Maaßen, Weyhers et al., 1996; Müller, Rühl et al., 1997).

Applications of SLN in other drug delivery systems

Peroral applications

The intestinal lymphatic transport of particulate matter may offer some potential advantages; indeed, direct lymphatic transport bypasses the liver first passage, and may enable the oral route to be used for some agents whose administration is otherwise limited to the parenteral route. In addition, particulate carrying drugs afford the possibility of targeting drugs to lymph, with a potential application in the treatment of lymphatic cancers. Peroral administration is the oral route of small particles to the lymphatic system by persorption. Persorption, or called endocytosis, is the transport of intact particles through the intestinal mucosa into the lymph and blood circulation.

The lipid nanopellets for peroral administration of poorly bioavailable drugs was disclosed by Speiser (1989). The nanopellets represent drug-loaded fat particles solid at room temperature and small enough to be persorbed. The lipid nanopellets are prepared by emulsifying molten lipids in an aqueous phase by high-speed stirring. After cooling to room temperature the pellets are dispersed by sonication.

The use of SLN as drug delivery by duodenal administration to rats was presented by Bargoni et al. (1998). They found that SLN present in lymph and

blood. The main particles uptake pathway in rats may be via the M-cells overlying the lymphoid follicles and the Peyer's patches and non patches. Particle size is a critical determinant of the fate of microparticles administered orally; larger particles may be retained for longer periods in the Peyer's patches, while small particles are transported to the thoracic duct. Furthermore, the extent of accumulation of particles in the Peyer's patches has been shown to be dependent on the hydrophobicity. SLN has a certain physical stability in the gut lumen and during lymphatic uptake and transport.

Ocular applications

The preparation of colloidal lipospheres containing pilocarpine as ocular formulation was studied (Cavalli, Morel et al., 1995). The aqueous dispersion containing pilocarpine ion pairs entrapped in lipospheres was prepared by warm microemulsion method. The release of drug form the lipospheres was prolonged compare with a simple solution. It was found that this preparation might constitute a promising vehicle for sustained-release ocular formulation.

Topical applications

The main problem of drug delivery through the skin is the barrier properties of stratum corneum. Many researchers have studied the enhancement of substances to increase drug penetration to the deeper skin layers or the circulating systems. The occlusion is one process that can enhance the penetration by increasing the hydration of the stratum corneum. The SLN in conventional cream is suggested in topical applications. Their occlusion effects are produced by film formation of the nanoparticles on the skin.

The influence of SLN on the penetration characteristics of retinyl palmitate, an occlusion sensitive drug, in porcine skin was studied by Jenning et al. (1999). In the case of drug freely dissolved in conventional o/w cream, it released from the conventional o/w cream and was hold back in the stratum corneum because of its barrier properties. When conventional cream containing drug freely dissolved

and drug-free SLN was used, no accumulation over 24 hours could be found. Drug could penetrate through stratum corneum. When conventional cream containing drug encapsulated in SLN was used, drug was slowly released controlling by the lipid matrix. This released amount could pass the stratum corneum. This higher permeability was a result of occlusive effects of SLN. Furthermore, Maia et al. (1999) reported the well local tolerability when the topical glucocorticoids loaded SLN was used.