CHAPTER II

LITERATURE REVIEW

I. *Centella asiatica* (Linn.) Urban

Centella asiatica (Linn.) Urban, the synonym *Hydrocotyle asiatica* Linn., belonging to the umbelliferae family, is a small perennial herbaceous creeper growing to 50 cm with leaves rounded to reinform, the petioles elongated; flowers umbels with three sessile flowers (Figure 1). The leaves are thin and soft, with palmate nerves, hairless or with only a few hair, and measure about 2 to 5 cm in diameter. It has been used as a traditional herbal medicine in Asitic countries for hundred of years. In Thailand, locally known as bua-bok, is widely distributed in open or damp shaded places (Sribusarakum, 1997; Cheng and Koo, 2000).

Figure 1 *Centella asiatica* (Linn.) Urban (Sribusarakum, 1997)

Traditional both the entire plant with root and leaves of *Centella asiatica* can be used medicinally to treat various skin condition ranging from slow healing wounds and lesions, to leprosy. Additionally traditional use of *Centella asistica* include heart disease, high blood pressure rheumatism, fever, nervous disorders, bronchitis, asthma, and syphilis (Duke, 1985).

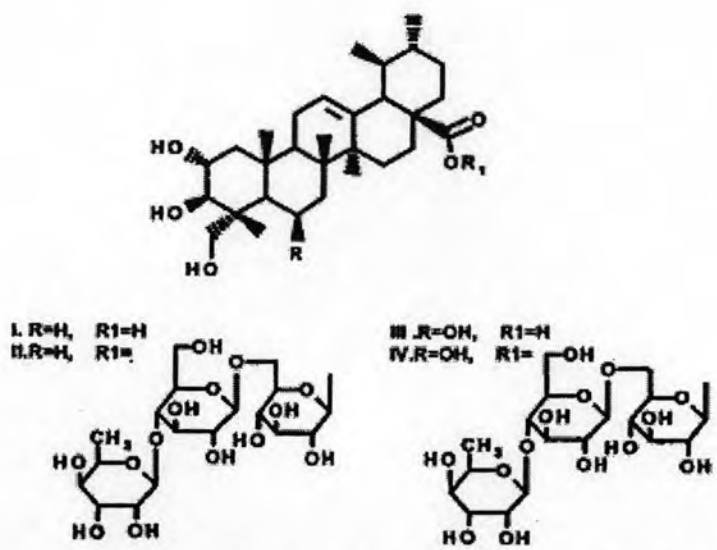
Through decades, there has been increasing interest in various compounds obtained from *Centella asiatica.* The different compounds report may due to places of origin of the material or to the difference in varieties of the plant. At present, the active principles of the plant *Centella asiatica* are asiatic acid, madecassic acid, and asiaticoside. The chemical structures were present in Table 1. Chemically these compound are pentacyclic triterpenes, belonging to the β-amyrin ursolic acid group. These compounds are known their major clinical indications for the treatment of wounds, venous insufficiency of limbs, varicose vein certain mycobacterial infections and cellulitis (Inamdar, Yole, Srivastava, 1996).

The extracts of *Centella asiatica* were studied on the following extracts: tritrate extract of *Centella asiatica* (TECA), total triterpene fraction of *Centella asiatica* (TTFCA). The TECA is combination comprising asiatic acid 30 %, madecassic acid 30 % and asiaticoside 40 %. The TTFCA comprises asiatic acid and madecassic acid 60 % in a ratio that is not clearly defined, in combination with asiaticoside 40 %.

The Centella preparations used in conventional medicine are employed in oral form (tablets and drop), topically (ointment and powder) and in the form of injections (s.c., i.m. and i.v.) (Brinkhaus et al 2000). A summary of commercial products available in the market is shown in Table 2.

Total triterpenic fraction of *Centella asiatica* (TTFCA) is effective in improving venous wall alterations in chronic venous hypertension and protecting the venous endothelium. TIFCA is active on connective tissue modulation, improves the synthesis of the collagen remodulating and other tissue proteins by modulating the

action of fobloblasts in the vein wall, and stimulates collagen remodeling in and around the venous wall. Studies have indicated the role of TTFCA on the synthesis of specific venous wall elements cell cultures of human embryonal fibloblasts. TTFCA is a active on the microcirculation in venous and diabetic microangiopathy. Signs and symptoms of venous hypertension and edema are improved by treatment. The remodeling on collagen synthesis could be one of the possible mechanism of action of TTFCA in the remodeling of echolucent (soft; therefore, with risk of thrombosis and embolization) plaques at the carotid and femoral bifurcation (Incandela et al 2001).



Scheme 1. Structures of asiatic acid (I), asiaticoside (II), madecassic acid (III) and madecassoside (IV).

Figure 2 chemical structure of asisticoside, madecassic acid and asiatic acid

Table 1 Pharmaceutical preparation containing Centella asiatica extract (Martindale, 1993; Sribusarakum, 1997; Brinkhaus et al., 2000)

Name	Manufacturer	Dosage form	Ingredients	Indications
Blastoestimulina®	Funk, Spain	Injectinon, eye drops,topical powder	C. asiatica	wounds, burns, eye disorder, ulcers
Blastoestimulina®	Funk, Spain	ointment, medicated dressing	C. asiatica, neomycin sulphate	burns, ulcers, skin infections, wounds
Blastoestimulina®	Funk, Spain	pessaries	C.asiatica, neomycin sulphate, chlordantoin,metronidazole,	vulvovaginal infections
Blastoestimulina®	Funk, spain	Topical aerosol	C. asiatica, amethocaine	wounds, burns, ulcers
Centellase®	Aventis Pharma, Italy	Ointment, tablets, drops, powder	C. asiatica	Circulatory disorders

Table 1 Pharmaceutical preparation containing Centella asiatica extract (cont.) (Martindale, 1993; Sribusarakum, 1997; Brinkhaus et al., 2000)

Name	Manufacturer	Dosage form	Ingredients	Indications
	Rhone-Poulenc Rorer,France	ointment	asiaticosid	Skin disorders
Madecassol®	Roche, Belgium	ointment, powder, tablet	C. asiatica	burns, cellulitite, keloids,ulcers, venous insufficiency
Madecssol C®	Sanofi Winthrop, Mexico	-	C.asiatica, metronidazole, nitrofurazone	Bacterial vaginosis, tricomoniasis
Madecassol Neomycin Hydrocortisone®	Nicholas, France	ointment	C. asiatica, neomycin. sulphate, hydrocortisone acetate	wounds, skin disorders ulcers
Madecassol Tulgras®	Nicholas, France	ointment	C. asiatica	venous, ulcers

Pharmacodynamics

The pharmacodynamic effects of *Centella asiatica* have been investigated in numerous animal experiments and in vivo studies. Demonstrated actions include, in particular, a wound healing, ulcers-protective, psychoneuro-pharmacological, antimicrobial and antiviral effect of the Centella or asiaticoside extract. TTFCA has important pharmacological characteristics as a modulator of collagen synthesis .and has been used in traditional oriental medicine for the treatment of vascular disease and in plastic and dermatological surgery. Microcirculatory methods can be used to evaluate the changes in the microcirculation induced by TTFCA in venous and diabetic microangiopathy. TTFCA may also modify the tone of veins by a structure action on the collagen of vein walls (Cesarone et al 2001)

Brinkhaus et al (2000) reviewed the investigation of the effect of TECA on skin lesions induced by repeated mechanical stressing of skin in rat. The orally administered (TECA) was shown to accelerate wound healing. At the same time, TECA suppressed the post-traumatic edema of the tissue observed to occur after repeated mechanical injury of the skin. The mechanical underlying this phenomenon is possible the regulative effect of the mixture of asiaticoside on the metabolic processes taking place in abnormal skin condition. However, Dermaderosiaun and Beutler (2002) reported that the topically applied TECA had a greater effect on wound healing than oral administration. Moreover, they found that the glycoside madecassoside has anti-inflammatory properties, while asiaticoside appears to stimulate wound healing.

Furthermore, the whole plant of Centella asiatica has been shown to be beneficial in improving memory and is reported to improve the general mental ability of mentally retarded children. Phytochemical have long been recognized to posses many properties including antioxidant. According to the oxidative stress hypothesis of ageing, the senescence-associated loss of functional capacity in due to the accumulation of molecular oxidative damage by toxic free radicals produced during normal respiration. Oxidative damage may contribute to the ageing process and to the

neuropathogenesis of several disease including stroke, Parkinson's disease and Alzheimer's disease (Subathra et al 2005).

Kumar and Gupta (2003) reported that *Centella asiatica* has recently been shown to be effective in reducing oxidative damage in the central nervous system, which is attributed to its antioxidant properties. Furthermore, *Centella asiatica* has been shown to improve the mental capabilities function of rodents as well as humans.

In pharmacological studies, the *Centella asiatica* extract has shown to have central nervous depressant activity and also to improve the maze learning in rats. *Centella asiatica* has recently reported to have antilipid peroxidantive and anti epileptic activities in the lithium pilocarpine model of status and was beneficial in improving memory in normal rats (Rao et al 2005) while Wattanatorn et al (2007) reported that the *Centella asiatica* can modulate only the reaction time of spatial memory and % accuracy of numeric working memory in the healthy elderly.

II. Alzheimer's disease

Alzheimer's disease (AD) is a complex, multifactorial, heterogenous mential illness, which is characterized by and age-dependent loss of memory and an impairment of multicognitive function. AD is associated with presence of intracellular neurofibrillary tangles (NFTs) and extracullular amyloid beta (Aβ) plaques, loss of neuronal subpopulations, synaptophysin immunoreactivity of presynaptic terminals, loss of cholinergic fibers, proliferation of reactive astrocytes and micloglia, and mithocondrial dysfunction with human life span increasing and decreasing cognitive functions in elderly individuals with AD related dementia.

AD occurs in both familial and sporadic forms. In familial AD (FAD) mutation in the amyloid precursor protein, pre sinilin 1, and presinilin 2 genes are the currently known causal factors. These genetic mutation inherit in a autosomal dominant fashion. FAD constitutes only 2-3 % of the total number of AD patients and it has an early age of onset (younger than 65 years of age). Sporadic AD (SAD)

constitutes the vast majority of AD case, and it has a late age of onset (65 years of age and older). The case of SAD are still unknown. Histological, pathological, molecular, cellular, and gene expression studied of AD have revealed that multiple cellular pathways are involve in AD progression. Pathologically, there no differences between FAD and SAD, pathological changes including A-beta production and deposits, NFTs, synaptic damage, and neuronal loss occur latter than in patient with FAD.

In FAD, recent molecular, cellular, and animal model studies have provided evidence that a 4 kDa peptide, a cleavage product of APP due to β and γ secretases, is a key factor in AD development and progression. The formation of the 4 kDa Aβ peptide in the brains of AD patients is a progressive and sequential process. Initially, soluble monomeric and oligomeric forms of 40-42 aminoacid residues (Aβ 1-40, shorter form; and Aβ 1-42 longer form) accumulate and later became insoluble fibrils and Aβ deposits.

In recent studies of triple transgenic mice that express 3 trans genes related to AD (AD-PS1, AD-APP, and FID-tau), Aβ plaques were found in mice at 5 months of age, and NFTs were found at 12 months, suggesting that Aβ production is critical and many facilitate tau pathology. Further, synaptic changes that occur in the triple transgenic mouse line have been directly associated with Aβ production. Aβ production critical for subsequent cellular changes seen in these mice, including the synaptic damage, hyperphosphorylation of tau and NFTs (Oddo et al 2004).

The Aβ plaques in the AD transgenic mice were also found to be associated with activated microglia and astrocytes and to trigger inflammatory response. However, astrocytes and microglia were found to proliferate in the vicinity of Aβ and to clear Aβ deposits. Yet in other studies, interactions among Aβ, glia and astrocytes were found to cause inflammatory in the AD brain, which can lead to altered neuronal homeostasis and oxidative injury. In the present, there are only four drugs that the Federal Drug Administration (FDA) has proved and that are currently available for treating AD patients in the United state. Three of drugs, Tacrine (Cognex®), Donepezil (Aricept®), and Rivastigmine (Reminyl®), inhibit acetylcholine-esterase

either selectively or non-selectively, but they have resulted in various adverse effect (Allian et al 2003).

In two recent studies, AD patients treated with Donepezil showed rescued APP metabolism or a slow down in the progression of hypocampal atrophy, a surrogate of disease progression. Thus, Donepezil was shown to provide neuroprotective effects (Hashimoto et al 2005), (Zimmermann et al 2005). Memantin (Namenda®), the fourth and most recently approved drug, non-competitively inhibits NMDA receptor, prevent glutamate excitotoxicity, and shows minimal adverse drug effects in AD patients. However, these drug do not modified the disease mechanism in the long run. Thus, when patients no longer take the drugs, their symptoms of ad return. The paucity of drugs currently available for treating AD and their limited targets in AD pathology call for the development of a new generation of drugs that not only affect cholinergic functions associated with AD but also target other cellular pathways in AD pathogenesis.

Thus, the quality of recent studied suggest that herbal drug and AD pathology are at a new crossroad. However, there are problems surrounding the preparation of herbal drug. *Centella asiatica* is poorly water-soluble. Solubility is an essential factor for drugs. It also poses a major challenge for pharmaceutical companies developing new pharmaceutical product, since near half the active substance being identified through the new paradigm in high-throughput screening are either insoluble or poorly soluble in water. A limiting factor for in vivo performance of poorly water soluble drugs, increasing the dissolution rate of poorly water soluble substance is thus important for optimizing bioavaibility. Nanoparticle engineering enables poorly water soluble drugs to be formulated as nanosuspension alone, or with a combination of pharmaceutical excipients.

III. Solid lipid nanoparticle (SLN)

Solid lipid nano particle represents an alternative colloidal drug carrier system with mean particle diameter ranging from 50 up to 1000 nm. SLN is characterized as

lipid base carrier system of solid physical state. It consists of biodegradable lipids and physiologically acceptable additives. These carrier provide sufficient loading capacity for lipophilic and possibly also hydrophilic drugs. SLN can be administered by parenteral, transdermal and oral route. By varying production parameters and the excipients, a desired mean particle size can be produced in a controlled way (Westensen and Siekman, 1996).

Solid lipid as matrix material for drug delivery is well-known from lipid pellet for oral drug delivery. The first attempts to develop SLN dated back to decades ago when the first parenteral lipid emulsions became commercially available. The use of solid lipid as carrier matrix can combine the advantages of polymeric nanoparticles and lipid emulsions. SLN possesses obvious advantages which is superior than other carrier. Their benefits are (i) biocompatibility and biodegradability of lipid carriers (ii) the possibility of controlled drug release and drug targeting (iii) avoidance of physical instability (iv) reduction of drug leakage (v) avoidance of the toxic residues (vi) no problem with respect to sterilization by autoclaving (vii) increasing of drug resistance to hydrolysis or oxidation and possibility to administer drugs through most routes of administration including parenteral, oral, transdermal and pulmonary (Schwarz et al 1994).

However, the difficulty in formulation, in manufacturing production and reproducibility problem are the obstacles that limit the development plans in particulated drug delivery systems. Therefore, the study of the process of preparation, characteristics and drug release of SLN is currently increasing attention during recent years (Floyd and jain 1996).

Excipient and formulation consideration

In general, SLN includes therapeutic agent, pharmaceutical acceptable lipid matrix, stabilizer, other additives and water. Special attention should be given into two major ingredients in SLN formulation, lipid matrix and stabilizer, especially in preparation intended for parenteral application. Potential toxicity, physical stability, chemical in compatibility and physiochemical property must be taken into consideration.

Lipid matrix

The variety of solid matrices used in the formulation of SLN include fatty acids, partial glycerides, triglycerides steroids, waxes which are solid at room temperature. Lipid consist of different chemical structures that have the melting ranging from approximately 30-120 °C. Some lipid used for preparation of SLN are shown in table 3. (Danisco, 2001, Freitas and Muller, 1998, Heiati, Tawashi et al. 1996, Lukowski et al. 2002, Zimmermann, Muller, and Madder, 2000)

Using the hot homogenization, it has been found that the average particle size of SLN dispersions increased with higher melting lipid. These results are in agreement to the general theory of high pressure homogenization and can be explained by the higher viscosity of the dispersed phase. In addition, other critical parameters for nanoparticle formation will be different for different lipids. The reasons include the velocity of lipid crystallization, lipid hydrophilicity, the shape of the lipid crystals and the surface area.

It is also noteworthy that most of the lipids used represent a mixture of several chemical compounds. The composition might therefore vary from different supplier and might even vary for different bathes from the same supplier. However, small differences in the lipid composition might considerably impact on the quality of SLN dispersion e.g. by changing the zeta potential, retarding crystallization process. For example, lipid nanodispersions made with cetyl palmitate from different supplier had different particle sizes and storage stability (Mehnert and Madder, 2001). The influence of lipid composition on particle size was also confirmed on SLN produced via high shear homogenization. The average particle size of Witepsol W 35 SLN was found to be significantly smaller (117.0±1.8 nm) than the size of Dynasan 118 SLN (175±1.8 nm). Witepsol W 35 contains shorter fatty acid chains and considerable amounts of monoglycerides and diglycerides which possess surface active properties.

Previous work has indicated that the stability of SLN after autoclaving depened on the nature of lipid. It was found that poloxamer 188 was the most efficient for stabilizing cetyl palmitate SLN, but little effective in syncrowax® HRC SLN (Muller et al., 1995).

Muhlen, Schwarz, and Mehnert (1998) have pointed out that controlled adjustment of drug release could be achieved by modification of chemical nature of lipid matrix.

Lipids exhibit a pronounced polymorphism. Depending on the conditions, glycerides may crystallize in three different polymorphic forms-alpha (α), beta prime (β') and beta (β). These polymorphic modifications characterized by the particular carbon chains packing may differ significantly in their properties such as solubility, melting point and thermal stability. The β form, a triclinic subcell structure, is the melting and thermodynamically stable polymorph. Where as α is the least stable with a loosely packed hexagonal subcell structure. The α form therefore has a tendency to be quickly transformed to a form with a better chain packing β' and β (Eldem, Speiser, and Altorfer, 1991). This transformation is accompanies by a change of physiochemical properties. Early study has revealed that the polymorphic transition in glycerol behenate SLN changed from β' to β after continuation of the drying process (Jenning, Schadfer-Korting, and Gohla, 2000).

Table 2 Lipids used for preparation of SLN (Danisco, 2001, Freitas and Muller, 1998, Heiati, Tawashi et al. 1996, Lukowski et al. 2002, Zimmermann, Muller, and Madder, 2000)

Lipid	Melting range (°C)	Tradename (Manufactacturer)
Glycerides		
• Glyceryl tricaprinate	31-32	Tricaprin® (Fluka)
• Glyceryl trilaurate	46.5	Trilarin® (Fluka)
• Glyceryl trimyristate	55-58	Dynasan® 114(CONDEA)
• Glyceryl tripalmitate	61-65	Dynasan® 116(CONDEA)
• Glyceryl tristearate	70-73	Dynasan® 118(CONDEA)
• Glyceryl palmitostearate	53-57	Precirol ® ATO 5 (Gattefossé)
• Glyceryl monostearate	54-64	Imwitor® 900 (CONDEA)
• Glyceryl behenate	69-74	Compritol® 888 ATO (Gattefossé)
• Hydrogenated coco-glyceride	42-44	Softisan® 142 (CONDEA)
• Hydrogenated coco-glyceride	33.5-35.5	Witepsol® W 35 (CONDEA)
• Hydrogenated coco-glyceride	33.5-35.5	Witepsol® W 35 (CONDEA)
• Hydrogenated coco-glyceride	41-43	Witepsol® W 42 (CONDEA)
• Hydrogenated coco-glyceride	42-44	Witepsol® W 48 (CONDEA)
• Monostearate monocitrate diglyceride	64	Grindsted CITREM® N12 (Danisco)
Fatty acids		
• Palmitic acid	63-64	Palmitic acid (Fluka)
• Stearic acid	69-71	Stearic acid (Fluka)
• Behenic acid	77-80	Behenic acid (Fluka)
Waxes		
• Cetyl palmitate	46-51	Cutina® CP (Cognis)

Stabilizer

Natural and synthetic agents have been onsidered for use as possible stabilizers because none of oils typically employed form a spontaneous emulsion when mixed with water. Many stabilizers have shown a high potential to stabilize SLN in a long period of time. The choice of the stabilizers and their concentrations is of great impact on the quality of SLN dispersion. Table 3. demonstrates stabilizers and method used for production of SLN (Almeid, Runge, and Muller, 1997, Cavalli, Marego et al., 1996, Floyd, 1999, Terreno et al., 1998, Seikmann and Westesen, 1996).

Only the limited number of stabilizers is commonly regarded as safe to use for parenteral administration of which the most important is lecithin. Lecithin, the most commonly used emulsifier in lipid emulsions, is defined as a mixture of triglycerides of stearic, palmitic, and oleic acid, liked to the choline ester of phosphoric acid. It has been obtained from both animal (egg yolk) and vegetable (soybean) sources. Lecithin can be totally biodegraded and metabolized since it is an integral part of biological membranes. It is regarded as a well tolerated and non toxic compound which is expressed by Generally Recognised As Safe (GRAS) approved by the FDA, making is suitable for long term and large dose in fusion.

The production of SLN is similar to that of lipid emulsions. During the preparation of SLN by hot homogenization method, an emulsion of the lipid melt in the aqueous phase is intermediately created before the lipid droplets solidify to form solid lipid nanoparticles. However, it has been observed that the preparation of lecithin stabilized tripalmitate SLN with a composition similar to lipid emulsions resulted in the formation of gel. Westesen and Siekmann (1997) reported that melt homogenized tripalmitate dispersions containing exclusively the phosphotidylcholine rich soybean lecithin product, Lipoid® S100, as a stabilizer became semisolid immediately on cooling of the hot emulsion. Whereas dispersions stabilized by the egg lecithin, Lipoid ® E80, formed gel within several hours after preparation. In tripalmitate suspensions stabilized by the cruder soybean lecithin, Lipoid® S75, transformation into semisolid product was obviously retarded but not prevented. The less pronounce gelation tendency of the Lipoid® S75 stabilized systems compared to those stabilized by Lipoid ® E80 may be

explained an improved but still not sufficient steric or electrostatic stabilization caused by the minor components of the cruder lecithin mixtures, such as glycolipids. According to the manufacturer of Lipoid® S75, the lecithin may contain up to 15 % glycolipids. The different commercially available lecithins are shown in Table 4.

Table 3 Stabilizers and methods used for preparation of SLN (Almeid, Runge, and Muller, 1997, Cavalli, Marego et al., 1996,.Floyd, 1999, Terreno et al., 1998).

Stabilizers	Methods
Natural stabilizers	
• Soybean lecithin	Hot homogenization/ Microemulsion
• Egg lecithin	Hot homogenization/ Microemulsion
Synthetic stabilizers	
• Poloxamer 188	Hot homogenization/ Cold homogenization
• Poloxamer 182	Cold homogenization
• Poloxamer 407	Hot homogenization
• Poloxamine 908	Hot homogenization
• Tyloxapol	Hot homogenization /Solvent emulsification and evaporation
• Polosorbate 20	Microemulsion
• Polosorbate 60	Microemulsion
• Polosorbate 80	Hot homogenization/ Cold homogenization
• Sodium glycocholate	Cold homogenization
• Taurocholic acid sodium salt	Hot homogenization/ Cold homogenization /Solvent emulsification and evaporation
• Taurodeoxycholoc acid sodium salt	Microemulsion
• Burtanol	Microemulsion
• Butyric acid	Microemulsion
• Dioctyl sodium sulfosuccinate	Microemulsion
• Monooctylphosphoric acid sodium	Microemulsion

Table 4 Composition and source of commercially available lecithins (Westesen and Siekmann 1997)

Components	Lipoid® S100	Lipoid®S75	Lipoid®E80
Phosphatidylcholine	min.94.0	66.0-70.0	80.0-85.0
Phosphatidylethanolamine	n. sp.	7.0-10.0	7.0-9.5
N-Acyl phosphatidylethanolamine	max. 1.0	n. sp.	n. sp.
Phosphatidylinositol	max.0.1	max. 0.5	n. sp.
Lysophospholipids	max.3.0	max. 3.5	max. 3.5
Triglycerides	max.2.0	max. 3.0	max. 3.0
Free fatty acids	max.0.5	max.0.5	max.0.05
Sphingomyelim	n. sp.	n. sp.	2.0-3.0
Cholesterol	n. sp.	n. sp.	max. 1.5
Dl-ά-Tocopherol	0.15-0.25	0.1-0.2	0.05-0.1
Source	soybean	soybean	egg yolk

However, the gel formation in the preparation of lecithin stabilized tripalmitate SLN can be avoided by the addition of a cosurfactants such as glycocholate or tyloxapol. These observations point to basic physiochemical difference between similarly composed lipid emulsions and solid lipid nanoparticles.

In contrast to Westesen and Siekmann, Ugazio et al. (2000) stated that SLN using lecithin (Epikuron ® 200) as emulsifier could be prepared by microemulsion method. They also found that the mean diameters of SLN were in nanometer range and the mean particle sizes after autoclaving showed similar results to those before autoclaving. The difference in SLN product using lecithin as stabilizer resulted from differently experimental condition, type of lipid matrix and quantity of lecithin in formulation.

Recently, many synthetic stabilizers continue to receive attention. The group of nonionic materials that has shown promise as stabilizers for parenteral applications is the poloxamers. Poloxamers consist of neutral synthetic polyoxyethylene-polyoxypropylene block co-polymers. Poloxamer 188 are well suited for small volume parenterals but large volume or long term administration are associated with overloading syndrome. Jumaa and Muller (1998) demonstrated that the using of poloxamer 188 as stabilizer was superior to other nonionic stabilizer including polyoxyethylene glycol sorbitan monooleate (tween 80), polyoxyethylene 660 hydroxy stearate (Solutol® H 15 and polyoxyethylene 35 ricinoleate (Cremophore® EL) upon autoclaving. They explained the results on basic of high cloud point of poloxamer 188, resulting in more resistance against dehydration during autoclaving and subsequently no stabilizer damage.

Other investigators continue study the fatty acid esters of sorbitans (various type of spans) and polyoxyethylene sorbitans (various type of tween) that are approved by the various pharmacopoeias for the parenteral administration and have been included in parenteral formulation (Nema et al., 1997). Both tween 20 and tween 80 are used as pharmaceutical excipients in available commercial parenteral products Calcijex® and Codarone® X IV, respectively. Many studies revealed that using a combination of stabilizers are superior to those formed using single stabilizer. The combination of stabilizers can produce more flexible interfacial films necessary to form stable system. Lundberg (1994) found that a suitable stabilizer is the mixture of purified egg yolk phosphotidylcholine and tween 80 in ratio 4:0.12.

Investigating the influence of stabilizer concentration on the particle size of glycerol behenate SLN dispersions, Muhlen (1998) obtained best results with 5 % sodium cholate or poloxamer 188. Bathes produced with lower concentrations of the stabilizer contained higher amounts of microparticles.

Different stabilizer compositions might require different homogenization parameters. For example, the maximum degree of dispersing was obtained with 500 bar and three cycles for poloxamer 188 stabilized systems. Homogenization with

pressure of 1000 or 1500 bar did not result in further reduction of the particle. In contrast, pressure of 1500 bar proved to be the best for lecithin (Lipoid® S75) stabilized systems. A possible explanation for this observation is the different velocity of the coverage of the new liquid surfaces.

Aqueous phase

The dispersion medium of SLN may contain one or more of following additive: isotonic agent, preservative, antiflocculant, cryoprotectant.

Isotonic agent

Normally, emulsified oil exerts no osmotic effect, hence isotonic adjustment is needed to adjust the physiological tonicity for large volume parenterals. The osmolalty should be in range of 280-300 mOsmol/L in order to prevent any hemolysis, pain, irritation and tissue damage at the site of administration. Glycerol has been proved to be vary efficient in this respect. While sorbitol and xylitol are also used as isotonic agents. Siekmann and Westesen (2001) found that the use of glycerol could promote the stability of SLN. Nevertheless, this consideration may not pay much attention in small volume parenteral.

Preservative

All colloidal dispersions for small volume parenteral should include an antimicrobial agent because the aqueous is most vulnerable to inadvertent contamination. These agents can be dissolve in the aqueous phase prior to emulsification. Suggested preservatives include the methyl and butyl derivatives of p-hydroxybenzoic acid. Quaternary ammonium compounds are useful because of their high aqueous solubility and limited tendency to partition into the oil phase. Thimerosal in concentration of 0.01 % was used as preservative for SLN (Floyd 1999).

Cryoprotectant

Previous study has been shown that particle sizes of aqueous SLN dispersions might be stable over 12-36 months. However, this stability is not a general feature of SLN dispersions and in most cases, an increase in particle size will be observed in a shorter period of time. Lyophilization is one approach to increase chemical and physical SLN stability over extended periods of time. However, the addition of cryoprotectors is necessary to decrease SLN aggregation and to obtain a better redispersion of the dry product. Typically, cryoprotective agents are sorbitol , lactose, mannose, trehalose, glucose, and polyvinylpyrrolidone. Schwarz and Mehnert (1997) investigated the lyophilization of SLN in great detail. Best results were obtained with the cryoprotectors glucose, mannose, maltose and trehalose in concentrations between 10 and 15 %. The observations were in agreement with the results of Muller et al. (1995) in that glucose and trehalose were proved to be the most suitable cryoprotectant.

Drugs

Many different drugs have been incorporated in SLN, examples are given in Table 6. A very important point to judge the suitability of a drug carrier system is its loading capacity. Factors determining the loading capacity of drug in lipid are (i) solubility of drug in melted lipid (ii) miscibility of drug melt and lipid melt (iii) chemical and physical structure of solid lipid matrix (iv) polymorphic state of lipid material. The prerequisite to obtain a sufficient loading capacity is a sufficiently high solubility of the drug in the lipid melt. Typically, the solubility should be higher than require because it decrease when cooling down the melt and might even be lower in the solid lipid. To enhance the solubility in the lipid melt one can add solubilizers. In addition, the presence of monoglycerides and diglycerides in the lipid used as matrix material promotes drug solubilization. The chemical nature of the lipid is also important because lipids which from highly crystalline particles with a perfect lattice such as monoacid triglycerides lead to drug expulsion (Westesen, Bunjes, and Koch,

1997). More complex lipids being mixtures of monoglycerides, diglycerides and triglycerides and also containing fatty acids of different chain length form less perfect crystals with many imperfections offering space to accommodate the drugs.

Crystalline structure is a key factor to decide in whether a drug will be expelled or firmly incorporated in the long term. Therefore, for a controlled optimization of drug incorporated and drug loading characterization of the physical state of lipid particles by nuclear magnetic resonance (NMR) and X-ray powder diffractometry is highly essential.

The polymeric form is also a parameter determining drug incorporation. Crystallization of the lipid in nanoparticles is different to the bulk material, lipid nanoparticles recrystallize at least partially in the α form, whereas bulk lipids tend to recrystallize preferentially in the β' modification and transforming rapidly into the β form (Westesen, Siekmann, and Koch, 1993). With increasing formation of the more stable modifications the lattice is getting more perfect and the number of imperfections decrease, that mean the β' to β transition promote drug expulsion. In general the transformation is slower for long chain than short chain triglycerides (Bunjes, Westesen, and Konch 1996).

SLN production

Many researchers have prepared solid lipid nanoparticle by various techniques.

1. High shear homogenization and ultrasound

The lipid nanopellets developed by Speiser (1989) are produced by dispersing a melted lipid in a surfactant solution by high shear homogenization and ultrasound. Both methods are widespread and easy to handle. However, dispersion quality is often compromised by the presence of microparticles Furthemore, metal contamination has to be considered if ultrasound is used. By using ultrasonication,

speiser obtained lipid nanopellets in range of 80-800 nm constituted mainly of fatty acids and glycerides.To preferentially obtain nanoparticles relatively high surfactant concentrations are employed. However, during the production of lipid particles, surfactant is also incorporated into the lipid phase. The more surfactant is present, the more it is incorporated leading to a reduced crystallinity of the lipid particles. Higher surfactant concentrations might be acceptable for oral administration but might cause some problems for other administration routes such as intravenous.

2. High pressure homogenization

High pressure homogenization (HPH) has emerged as a reliable and powerful technigue for the preparation of SLN. The high pressure homogenizers may in principle be attributed to either on of two types according to the geometry of the interaction device (i) machines with a ring-shapeed gap valve and (ii) machines based on an interaction chamber where two liquid streams are forced to interact with each other. Homogenizers of different sizes are commercially available from several manufacturers e.g. Micron Lab 40, Gaulin lab 60, Microfluidizer 110, Nanojet, Kavitator (Brandl, 1998.).

HPH has been used for years for the production of lipid emulsions for parenteral nutrition. In contrast to other techniques, scaling up represents no problem in most cases. High pressure homogenizers push a liquid with high pressure through a narrow gap in range of a few microns. The fluid accelerates on a very short distance to very high velocity over which is 1,000 km/hr. Very high shear stress and cavitation forces disrupt the particles down to the submicron range. Typical lipid contents are in the range 5-10% and represent no problem to the homogenizer. Even higher lipid concentrations up to 40% have been homogenized to lipid nanodispersions (Lippacher, Muller, madder, 2000).

In this study, The EmulsiFlex® C5 (Avestin, Canada) used to prepare SLN is shown in Figure 1. It has a capacity of 1-5 liter/hr. A sample as 7 ml can be processed with a hold back volume of less than 2 ml. The homogenizing pressure can be adjusted from 500 to 30,000 psi. The aqueous dispersion is pushed by a high pressurepump which is connected compressed air supply line. The EmulsiFlex® C5 consistedof two different homogenizing valves as shown in Figure 3. The static valve's pressure is controlled by varying the flow rate through the homogenizing valve.

The greater the flow rate, the greater the pressure is. The clogging might occur during operation. However, the static valve can easily be disassembled for cleaning and inspection. While dynamic homogenizing valve is fully adjustable through its maximum homogenizing pressure range. Pressure is independent from flow rate and will remain at the set value over the process time. During homogenization the process is discontinuous, therefore the system needs to be dismantled and the dispersion poured back into the cylinder body for next homogenizing cycle. For multiple cycling, the particle size distribution becomes narrower which is due to the effect reducing the coarse material (Avestin, 2000).

Two general approaches of the homogenization step, The hot and cold homogenization techniques can be used for the production of SLN. In both cases, a preparatory step involves the drug incorporation into the bulk lipid by dissolving or dispersing the drug in the lipid melt. Schematic procedure of hot and cold homogenization techniques for SLN production is shown in Figure 4.

Table5.Examples of drugs incorporated in SLN (Cavalli, Morel et al.,1995, Cavalli, piera et al., 1999, Heiati, Phillips et al., 1996, Jenning, Gysler et al., 2000, Morel, Ugazio et al., 1996, Morel, Terreno et al., 1998, Westesen, Bunjes et al., 1997, Yang, Zhu et al., 1999, Zhang, et al., 2000,)

Drug	Research group
• Deoxycorticosterone • Doxorubicin • Gadolinium (lll) complexes • Hydrocortisone • Idarubicin • Paclitaxel • Pilocarpine • Progesterone • Thymopentin • Timolol	Gasco
• Coenzyme Q 10 • Retinol • Retinyl palmitate • Vitamin A palmitate	Gohla
• Prednisolone • Tetracaine • Etomidate	Mehnert
• Cyclosporine	Muller
• 3'-Azido-3'deoxythymidine palmitate	Phillips
• Betamethasone valera • Cortissone • Menadione • Oxazepam • Prednisolone • Retinol	Westesen

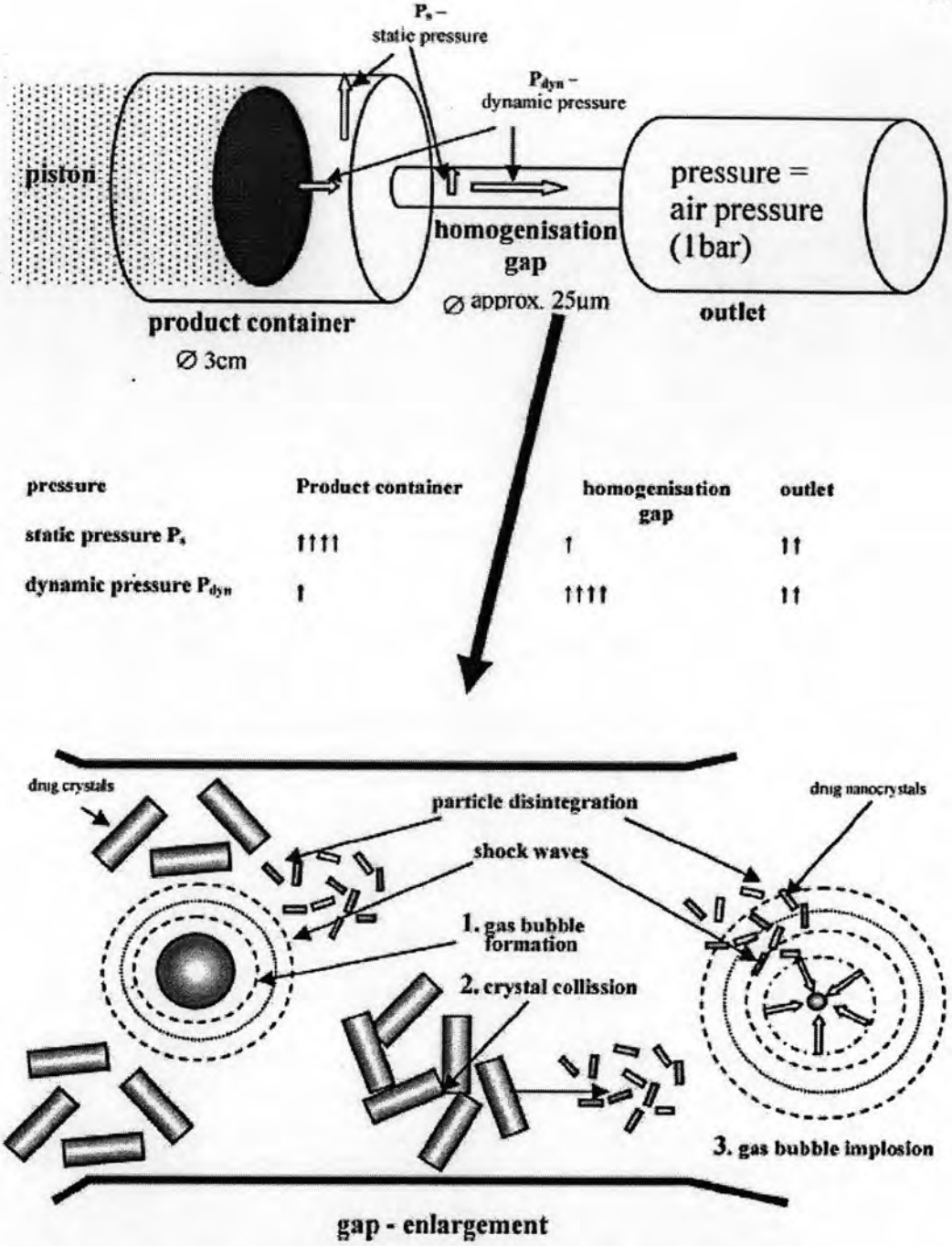


Figure 3 The schematic of static homogenizing (Muller and Keck 2004)

2.1 Hot homogenization

Hot homogenization is carried out at temperatures above the melting point of the lipid and can therefore be regarded as the homogenization of an emulsion. A pre-emulsion of the drug loaded lipid melt and the aqueous phase at the sametemperature is obtained by high shear mixing device e.g. Ultra-Turrax®. The

obtained coarse pre-emulsion is then homogenized using high pressure homogenizer. Cooling down of this emulsion to room temperature will lead to lipid crystallization and formation of the solid lipid nanoparticle. The hot homogenization technique can be applied to lipophilic and insoluble drugs. Many heat sensitive drugs can be processed because the exposure time to higher temperatures is relatively short. However, in case of highly temperature sensitive compounds the cold homogenization technique can be applied. The hot homogenization technique is not suitable for incorporating hydrophilic drugs into SLN. During the homogenization of the melted lipid phase the drug will partition to the water phase resulting in a too low entrapment efficiency (Siekmann and Westesen, 2001).

The quality of the pre-emulsion affects the quality of the final product to a large extent and it is desirable to obtain droplets in the size range of few micrometers.HPH of the pre-emulsion is carried out at temperatures above the melting point of the lipid. In general, higher temperatures result in lower particle sizes due to thedecreased viscosity of the inner phase. However, high temperatures may also increase the degradation rate of the drug and the carrier. The homogenization step can be repeated several times. Typically, HPH increases the temperature of the sample approximately 10°C for 500 bar. In most case, 3-5 homogenization cycles at 500-1500 bar are sufficient (Jahnke, 1998). Furthermore, it was found that the small particle size and the presence of stabilizers, lipid crystallization may be highly retarded and the sample may remain as a supercooled melt for several months (Bunjes, Siekmann, and Westesen, 1998).

2.2 Cold homogenization

Cold homogenization has been developed to overcome the following problems (i) temperature-induced drug degradation (ii) drug distribution into the aqueous phase during homogenization (iii) complexity of the crystallization step of the emulsion leading to several modifications and/or supercooled melts. In the first preparatory step, the drug is dissolved in the melt lipid. The drug containing melt lipid is solidified in dry ice or liquid nitrogen and milled using a mortar mill or

ball mill. The high cooling rate flavors a homogeneous distribution of the drug within the lipid matrix. Typical particle sizes obtained by means of mortar mill or ball mill are in range 50-100 microns. Then the obtained lipid microparticles are dispersed in a cold aqueous surfactant solution and the dispersion is homogenized at room temperature or below. The cavitation and shear forces in the homogenizing gap are sufficiently high to break the microparticles and to yield solid lipid nanoparticles. In general, compared to hot homogenization, larger particle sizes and a broader size distribution are observed in cold homogenized samples of the same lipid at identical homogenization parameters. To further reduce the mean particle size and to minimize the size distribution, a higher number of homogenization cycles can be applied. The method of cold homogenization minimizes the thermal exposure of the sample, but it does not avoid it due to the melting of the lipid/drug-mixture in the initial step.

3. Microemulsion

Gasco (1993) developed SLN preparation techniques which are based on the dilution of microemulsions. Microemulsions are clear or slightly bluish solutions being composed of a lipophilic phase, surfactant, co-surfactant and water. To form amicroemulsion with a lipid being solid at room temperature, the microemulsion needs to be produced at a temperature above the melting point of the lipid.The lipid is melted, a mixture of water, the susrfactant and co-surfactant are heated to the same temperature as the lipid and added under mild stirring to the lipid melt. A transparent, thermodynamically stable system is formed when the compounds are mixed in the correct ration for microemulsion formation. This microemulsion is then dispered in a cold aqueous medium (2-3°C) under mild mechanical mixing, thus ensuring that the small size of the particles is due to the precipitation and not mechanically induced by a stirring process. Surfactants include lecithin, polysorbate 20, polysosrbate 60, taurodeoxycholate sodium salt and co-surfactants consist of butanol, sodium microemulsion to cold water are in the range of 1:25 to 1:50. The dilution process is critically determined by the composition of the microemulsion.

Considering microemulsions, the temperature gradient and the pH value fix the product quality in addition to the composition of the microemulsion. High temperature gradients facilitate rapid liquid cryustallization and prevent aggregation (Cavalli, Marengo et al., 1996) Large scale production of SLN by the microemulsion technique also appears feasible and is at present under development at Vectorpharma (Trieste, Italy). The microemulsion is prepared in a large, temperature-controlled tank and then pumped from this tank into a cold water tank for the precipitation step (Muller, Mader, and Gohla, 2000).

4. Solvent emulsification and evaporation (precipitation in o/w emulsions)

Sjostrom and Bergenstahl (1992) described a production method to prepare nanoparticle dispersions precipitation in O/W emulsions. The lipophilic material is dissolved in a water-immiscible organic solvent e.g. cyclohexane, chloroform, methylene chloride, diethyl ether, petroleum ether. This solution is then emulsified in an aqueous phase. Upon evaporation of the solvent a nanoparticle dispersion is formed by precipitation of the lipid in the aqueous medium. The mean diameter of the obtained particles was 25 nm with cholesterol acetate as model drug and by using a lecithin/sodium glycocholate blend as emulsifier. The reproducibility of these results is confirmed by Siekmann and Westesen (1996). They prepared nanoparticles of tripalmitin by dissolving the triglyceride in chloroform. This solution was emulsified in an aqueous phase by HPH. The organic solvent was removed from emulsion by evaporation under reduced pressure. The mean particle size ranges from approximately 30 t 100 mm depending on the lecithin/co-surfactant blend. Particles with average diameters as small as 30 nm were obtained by using sodium glycocholate as co-surfactant. The advantage of this procedure over the homogenization process is the avoidance of any thermal stress. However, a clear disadvantage is the use of organic solvent.

Analytical characterization of SLN

An adequate characterization of SLN is a necessity for the control of the quality of the product. The characterization methods should be sensitive to the key parameters of SLN performance and should avoid artifacts. However, characterization of SLN is serious challenge due to the colloidal size of the particles and the complexity of the system which includes also dynamic phenomena. Many analytical tools do not permit direct measurement in the undiluted SLN dispersion. Possible artifacts caused by sample preparation e.g. the removal of stabilizer from particle surface by dilution, the induction of crystallization processes, the changes of lipid modifications. Therefore, several parameters have to be considered.

1. Particle size and Zeta potential

The measurement of the zeta potential allows predictions about the storage stability of colloidal dispersion. Generally particle aggregation is less likely to occur for charged particles due to electric repulsion. A reduction in the electrical charge is known to increase the rate of flocculation and coalescence (Floyd and Jain, 1996) However, this rule cannot strictly applied for systems which contain steric stabilizers because the adsorption of steric stabilizer will decrease the zeta potential due to the shift in the shear plane of the particle. In this observation, zeta potentials were determined using a ZetaSizer ZS. In ZetaSizer ZS, zeta potential measurements are performed using a laser Doppler anemometry (LDA). LDA allows fast determination of the electyrophoretic mobility using laser light scattering. The zeta potential is calculated from the electrophoretic mobility , the electric field strength applied, the viscosity and the dielectric constant of the dispersion medium at a given temperature. Zeta sizer ZS measured the fluctuation of the intensity of the scattered light which was caused by particle movement. Since small particles suspended in a fluid exhibit random Brownian motion as a consequence of molecular bombardment. The more massive the particle, the less significant this effect is. Thus measurement of the random motion can yield size.

Transmission electron microscopy (TEM) can be used for direct examination of particle in the size range 1 nm.-5 μm. Both Freeze-Fracture Transmission electron microscopy (FF-TEM) and Cryo-Transmission electron microscopy (Cryo-TEM)) were used to investigate particle shape and size of SLN (Cavalli, Gasco et al., 2001, Sznitowska et al., 2001, Zhang et al., 2000). Scanning electron microscopy (SEM) has also been reported to study the surface morphology of lipid micropellets (Eldem, Speiser, and Alfoter,1991). In this study, Cryo-Scanning electron microscopy (Cryo-SEM) was used to investigate particle shape of SLN. Cryo-SEM is provided with two stages- a specimen treatment stage in the Cryo-chamber and a cooling stage in the SEM specimen chamber. Both stages are constantly cooled with liquid nitrogen. A fracture knife, an etching heater and an evaporator are built into the Cryo chamber. The Cryo-SEM construction diagram is shown in Chapter 3. The Cryo-SEM method is to physically fix water (i.e., freeze into ice). After The sample is transferred to cooling stage, the specimen was fractured with the built-in knife. The particle shape and size can be observed.

Sznitowska et al (2001) investigated cetyl palmitate SLN stabhilized by alkyl glucoside (Plantacare 2000) by TEM. The electron micrographs suggest the spherical form of particles. On contrary, different SLN shapes such as platelet-like pattern were reported for SLN made of triglycerides with high purity (Siekmann and Westesen,1998). The chemically homogenous lipid tends to form more or less perfect crystals with the typical platelet-like pattern of the ß modification. The use of chemically heterogeneous lipids in combination with heterogeneous surfactants favors the formation of ideally spherical lipid nanoparticles.

2. Degree of crystallinity and lipid modification

Special attention must be paid to the characterization of the degree of lipid Crystallinity and the modification of the lipid, because these parameters are strongly Correlated with drug incorporation and release rates.

Differential scanning calorimetry (DSC) is a method which measures the difference in energy between a reference and a sample. It is widely used to investigate the status of the lipid because different lipid modifications possess different melting points and melting enthalpies (Bym, Pfeiffer, and Stowell, 1999). Freitas and Muller (1999) studied the correlation between long-term stability of solid lipid nanoparticles and crystallinity of the lipid phase using DSC. They found that the destabilizingfactors factors light, temperature and shear forces cause a distinct increase in the recrystallization index by transformation of the lipid to the ß modification being accompanied by gel formation. In addition, the crystalline and amorphous nature of drug dispersed in SLN can be determined using DSC (Clas, Dalton, and Hancock, 1999). Cavalli, Peiraq et al. (1999) found that hydrocortisone and progesterone are dispersed in lipid matrix in an amorphous form.

X-ray diffractometry is widely used to study for the identification of solid phases. The X-ray diffraction pattern of every crysrtalline form of a compound is unique, making this technique particularly suited for the identification of the polymorphic forms of a compound (Suryanaraynan, 1995). The X-ray diffraction pattern also allows to differentiate between crystalline and amorphous material. Using the X-ray diffractometry and H NMR, Bunjes, Siekmann et al. (1998) revealed that dispersed trimyristin in SLN remained in liquid and does not form a solid amorphous phase at room temperature. Hoowever, the colloidally dispersed trimyristin could crystallize by cooling down the temperature below its critical temperature.

Infrared spectroscopy (IR) is very useful for analysis of solid. It is extremely sensitive to the structure and thus is a powerful method for the characterization and identification of different solid forms. In SLN, IR was used to study chemical interaction occurred between the lipid matrix and drug. Zhang et al. (2000) found that no any shift after encapsulation of cyclosporine A to stearic acid. Hence, there was no chemical reaction occurred in cyclosporine A loaded stearic acid SLN

3. Coexistence of additional colloidal structures

The coexistence of additional colloidal structures e.g. micelles, liposomes, mixed micelles, supercooled melts has to be taken into account for all SLN dispersions Unfortunately, this aspect has been ignored in the majority of the SLN literature. Stabilizing agents are not localized exclusively on the lipid surface, but also in the aqueous phase. Therefore, micelle forming surfactant molecules will be present in three different forms (i) on the lipid surface (ii) as micelle (iii) as surfactant monomer. Lecithin will form liposomes, which have also been detected in lipid emulsions for parenteral nutrition. Mixed micelles have to be considered in glycocholate/lecithin stabilized and related systems. The characterization and quantification are a conscientious challenge due to the similarities in size combined with the low resolution of PCS to detect multimodal distributions. Anyway, nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR) are powerful tools for investigating dynamic phenomena and the characteristics of nocompartments in colloidal lipid dispersions.

Simple ^{1}H NMR spectroscopy permits an easy and rapid detection of supercooled melts due to the low linewidths of the lipid protons. This method is based on the different proton relaxation times in the liquid state give sharp signals with high signal amplitudes, while semisolid/solid protons give weak and broad NMR signals under these circumstances.

EPR spectroscopy was used to investigate the incorporation of drugs into SLN in order to establish their location, the entrapment efficiency and to follow the stabilization of SLN dispersion during storage. Ahlin et al. (2000) synthesized spin-labeled derivertives of fatty acid as the model lipophillic drug for their study. They have shown that model lipophilic drug distribute between the solid glyceride core and the phospholipids layers and the distribution depends on the type of lipid matrix and on the phospholipids concentration.

5. Drug incorporation and drug release

A large number of drugs have been studied with regard to their incorporation into SLN as shown in Table 4. Drug loading might result in strong changes of the SLN characteristics – particle size distribution, Zeta potential, lipid modification. The modification of drug and lipid could be characterized by DSC, X-ray diffractometry and NMR. However, there are distinctly less data available about drug release especially information about the release mechanisms. Due to the colloidal size, release studies are not trivial experiment. The choice of a suitable model of drug release nanoparticles is still problematic. Membrane diffusion technique is the most widely used to study the in vitro drug release from SLN (Yang et al., 1999). The USP paddle method and flow-thouh diffusion Franze cell have also been employed to determine the release kinetics from SLN (Jenning, Thunemann, and Gohla, 2000, Muller, Mehmnert et al., 1995). The release experiments were conducted under everal conditions. Therefore, it is not easy to compare the results.

At the beginning of SLN development, burst release was observed. It seemed that the system is not feasible for a prolonged drug release. The breakthrough was indeveloping the first SLN, which showed a prolonged in vitro drug release up to 5-6 weeks. To develop controlled release SLN, the understanding of the drug release is necessity. Muller, Lippacher et al. (2000) proposed four different models of internat structure SLN to explain the drug release profiles.

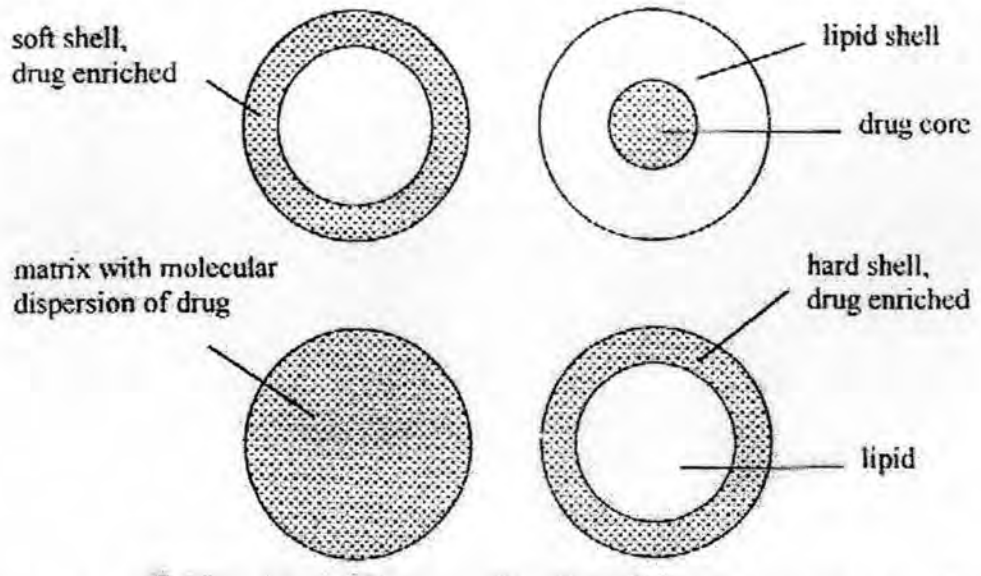


Figure 5 Proposed mofels for the internal structure of SLN: (1) Soft drug-containing shell surrounding a lipid core (upper left) (2) Homogeneous particle matrix with molecular dispersion of drug (lower left) (3) A drug core surrounded by a lipid shell being drug-free or of low drug content (upper right) (4) Drug-free lipid core surrounded by a hard shell composed of lipid-drug mixture (lower right) (Muhen et al.1998).

1. Soft drug-containing shell core model

Muhen et al. (1998) studied the release profile of drugs from SLN. They found that the burst release was observed when incorporating tetracaine and etomidate into SLN. It was also found that the burst release diminished with increasing particle size and prolonged release could be obtained when particles were sufficiently large i.e.lipid microparticles. From the data, it was concluded that the drug was enriched in an outer shell of the particles. The drug has a relatively short distance of diffusion and will be released in a burst.

The formation of the shell is explained by the stepwise crystallization process of the drug-lipid mixture. After the hot homogenization step the produced O/W emulsion is cooled, the lipid precipitates first forming a more or less drug-free lipid core. The remaining liquid drug-lipid mixture will enrich continouously in drug

content until the eutecticum is reached. Reaching the eutecticum leads to the simultaneously crystallization of lipid and drug, forming an outer shell surrounding the drug-free lipid core as depicted in Figure 6. The soft drug-containing shell core model is shown in Figure 5, upper left.

In addition, it must be considered that surfactant is present. This surfactant will interact with the outer shell and affect its structure. The existence of a shell can be proven by atomic force microscopy (AFM) measured. With special technique, noncontact imaging, the hardness of the particle. The force required to press the cantilever of the AFM instrument into the particle. The force required to press the cantilever into the particle is a measure of the viscosity of the particle matrix. It can be shown that there is an outer shell of relatively low viscosity that is composed of lipid, drug, and partially incorporated surfactant (Muhlen, Muhlen et al., 1996).

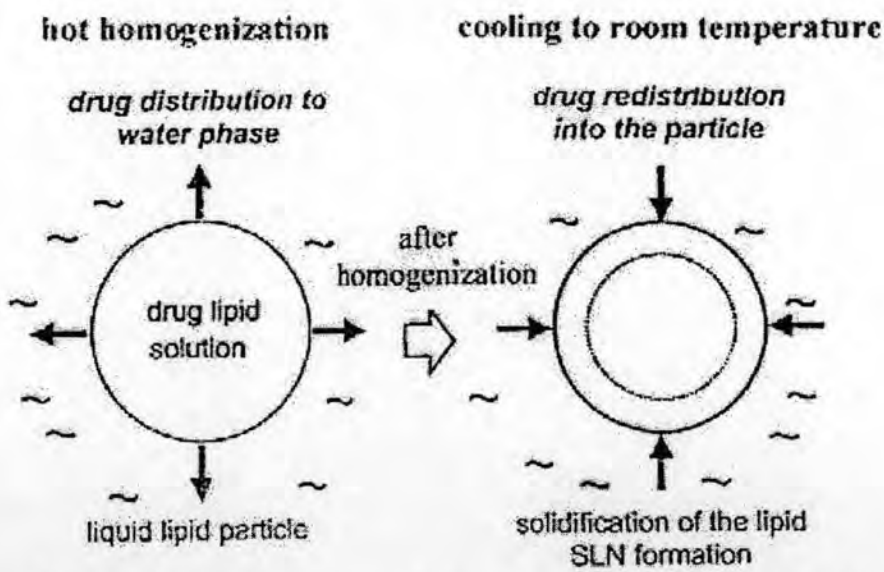


Figure 6 Partitioning effects on during the production of SLN by the hot homogenization technique. Left:Partitioning of drug from the lipid phase to the water phase at increased temperature. Right: Re-partitioning of the drug to the lipid phase during cooling of the produced O/W emulsion (Muhen et al.1998).

2. Solid dispersion model

In contrast, the prolonged release over a period of 5 weeks was observed from prednisolone loaded glyceryl behenate SALN (Muhlen, Schwarz et al., 1998). The SLN system was produced by cold homogenization method. The prolonged release can be explained by molecular distribution of the drug in the lipid matrix. This is very likely because cooling the drug-containing lipid will lead to the formation of a solid dispersion. This solid dispersion was just milled by high pressure homogenization, which means that no or limited melting occurred. The particles were just broken down and retained their structure of a solid dispersion. Although, there will be a warming upof the dispersion by approximately 20°C. However, this does not lead to a melting in lipid because melting point of the lipid is sufficient high. Based on these results, the solid dispersion model was proposed as depicted in Figure 4, lower left.

3. Drug core/lipid shell model

A drug-enriched core will be found in case the drug precipitates first before the lipid recrystallizes. This should be obtained when dissolving a drug in the lipid melt at or close to its saturation solubility. Cooling of the emulsion will lead to a supersaturation of drug in the melted lipid and subsequently to drug crystallization prior to lipid crystallization. Further cooling will finally lead to the recrystallizatrion of the lipid surrounding the drug core as a membrane. This lipid membrane will contain only drug in such a concentration cosrresponding to the saturation solubility of the drug at the recrystallization temperature of lipid. That means it will result in a drug-enriched core surrounded buy a lipid shell as shown in Figure 5, upper right.

4. Drug-free core/hard drug-containing shell model

Recently, it was discovered that there is additionally the hard shell core model of SLN. Within an industrial product development, the SLN was loaded with coenzyme Q10. The coenzyme Q10 loaded SLN was routinely investigated by

contact AFM. It was assumed that a solid dispersion of coenzyme Q10 in lipid would be present. Contact AFM revealed that there was an outer shell of increased rigidity, the core was distinctly less rigid. The coenzyme Q10 was released relatively fast.

Possibly coenzyume Q10 and the lipid had structural properties such that they fitted together very well to form a solid structure like brick layers. It could be possible that the molecule coenzyme Q10 fitted into the imperfections of the lipid, leading to a more solid structure. Due to the location of coenzyme Q10 in the outer shell. the drug release was fast but the presence of coenzyme Q10 led to a more solid state of the lipid leading to a firm outer shell. The proposed model of drug-free lipid core surrounded by a hard shell composed of lipid drug mixture is shown in Figure 4,lower right.

Applications of SLN for drug delivery

The applications of SLN are manifold. Basically, the SLN can be employed for any purpose for which nanoparticles have a distinct advantage.The application range from topical to parenteral.

1. Topical administration

Regarding the regulatory aspect, topical application is relativel unproblematic. The major advantages for topical products are the protective properties of SLN for chemically labile drugs against degradation and the occlusion effect due to film formation on the skin. Stability enhancement was reported for coenzyme Q10 and also for the very sensitive retinol. An enhancement of occlusiveness can be achieved by adding SLN of suitable composition to light O/W day creams, thus increasing the moisturing effect without having the glossiness of a night cream.

The parameter to assess the ability of a delivery system is its effect on active ingredient penetration into skin and consequently its therapeutic effect and in

cosmetic applications the effect on skin appearance. A range of active ingredients e.g. coenzyme Q10, retinol, vitamin E and its derivatives have been incorporated into SLN. The skin caring properties of a commercial retinol cream have been compared to the same cream containing retinol loaded SLN, reference was untreated skin. Parameters assessed were skin elasticity, moisture state and skin roughness as standard read out parameters.

The moisture level of the SLN containing formulation and SLN free cream were raised by 33% and 23%, respectively after a 1 week period of treatment compared to untreated skin. Besides this the cream containing retinol loaded SLN improved the skin smoothness by 10.3%, the SLN free cream achieved only 4.1% (Muller, Mader et al., 2000).

2. Peroral administration

The application of SLN as drug delivery by oral administration was presented by Yang, Zhu et al. (1999).They produced camptothecin containing SLN from stearic acid (2%), lecithin (1.5%) and poloxmer 188 (0.5%). The encapsulation efficiency of camptothecin was 99.6%. The plasma levels and body distribution were determined after administration of camptothecin loaded SLN versus a camptothecin solution. Two plasma peaks were observed after administration of camptothecin loaded SLN. The first peak was attributed to the presence of free drug, the second peak can be attributed to controlled release or potential gut uptake of SLN. These two peaks were also found in the total camptothecin concentration-time profiles of all measured organs. It was also found that the incorporation into SLN protecsted camptothecin from hydrolysis. The conclusion from this study was that SLN was the promising sustained release system for camptothecin and other lipophilic drugs after oral administration.

3. Parenteral administration

For parenteral applications, SLN had to be easily drawn into a syringe through A 20-25 gauge needle (syringeability) and readily ejected from syringe into the patient (injectability). The range of inside diameters of 20-25 hypodermic gauge size is Shown in Table 6 (Terumo, 2001). However, the amount of microparticles is the limiting factor for SLN to be acceptable for intravenous administration by the regula authorities. The injection f the relatively high content of microparticles larger than 5 μm could bring about the danger of capillary blockage resulting in fat embolism. The pharmacopoeia differs very much regarding their specifications. The monographs regarding fat emulsinons for intravenous administration might be a guideline to judge the SLN. The European Pharmacopoeia 1979 required that the particle diameter should not be larger than 5 μm, the German Pharmacopoeia demands only a determination of the particle size. There is a lack of obligatory precise specification.In addition, one has to consider that a toxicity study with the parenteral new product has to be made. To formulate parenteral SLN, surfactants accepted for parenteral administration can be used e.g. lecithin, tween 80, poloxamer 188, polyvinyl pyrrolidone, span 85. For the intravenous route it is recommended to focus on the i.v. accepted surfactants e.g. lecithin, tween. 80, poloxamer 188, sodium glycocholate (Műller, Mader et al., 2000).

Studies usin intravenously administered SLN have been performed by various groups. Bocca et al. (1998) produced stealth and non-stealth solid lipid nanoparticles and studied them in cultures of macrophages and also after loading them with paclitaxel in vivo. The i.v. administered SLN to higher and rolongedplasma levels of paclitaxel. Both no-stealth and stealth SLN showed a similar low uptake by the liver and the spleen macrophages, a very interesting point was the increased uptake observed in the grain. This study demonstrates the potential of SLN to achieve prolonged drug plasma levels. The observee similar low uptake by the liver and spleen macrophages might be explained by a similar low surface hydrophobicity of both types of particles avoiding the adsorption of any blood proteins mediating the uptake by liver and spleen macrophages. The uptake of the SLN by the brain might be

explained by adsorption of a blood protein mediating the adherence to the endothelial cells of the blood brain barrier.

Table 6. The range of inside diameters of 20-25 hypodermic gauge size (Terumo, 2001).

Gauge size	Designated metric size	Inside diameter of tubing (millimeters)					
		Regular wall		Thin wall		Ultra wall	
		min.	max.	min.	max.	min.	max.
25 G	0.5	0.23	0.28	0.29	0.34	0.29	0.34
24 G	0.55	0.28	0.33	0.35	0.39	0.35	0.39
23 G	0.6	0.33	0.38	0.36	0.41	0.38	0.43
22 G	0.7	0.39	0.44	0.44	0.49	0.46	0.51
21 G	0.8	0.48	0.54	0.53	0.58	0.54	0.61
20 G	0.9	0.56	0.62	0.61	0.67	0.63	0.69

Pharmacokinetics studies of doxorubicin incorporated into SLN showed higher blood levels in comparison to a commercial drug solution after i.v. injection in rats. Concerning the body distribution, SLN was found to cause higher drug concentrations in lung, spleen and brain, while the solution led to a distribution more into liver and kidneys (Zara et al., 1999).

Yang, Lu et al.(1999) reported on the pharmacokinetics and body distribution of camptothecin after i.v. injection in mice. In comparison to a drug solution, SLN was found to lead to much higher AUC/dose and mean residences time especially in brain, heart and reticuloendothelial cells containing organs. The highest AUC ratio of SLN to drug solution among the tested organs was found in the brain.

Toxicity aspects

The status and toxicity of SLN are a major issue for the use of a delivery system particularly in parenteral administration. For parenteral administration,

information about the interaction of SLN with phagocytic cells is a prerequisite. Phagocytic cell such as mononuclear phagocytes and granulocytes which are the first cells that interact with particles in the blood stream and thereby represent the first line of defence of the immune system. (Scholer, Hahn et al., 2002). Interaction of phagocytic cells with foreign bodies such as drug delivery systems may result in phagocytic uptake and uncontrolled release of pro-inflammatory cytokines such as interleukin 1 (IL-1), interleukin 6 (IL-6), interleukin 12 (IL-12), tumor-necrosis-factor ά (TNF-ά) (Schöler, Olbrich et al.,2001). Uncontrolled secretion of these molecules may lead to a cascade of adverse reactions and subsequently cell death. In order to evaluate the performance and toxicological acceptance of drug delivery systems, knowledge on what causes change in the production of these pro-inflammatory cytokines is of utmost importance.

The interaction of SLN with phagocytizing cell has been studied in vitro on human granulocytes. A luminal-based chemiluminescence was use to compare SLN with polymer particles and to compare SLN composition on the phagocytosis rate. Műller, Maassen et al. (1997) found that phagocytosis rate of poloxamer stabilized glycerol behenate and cetyl palmitate SLN was lower in comparison to polystyrene nanoparticles.

Furthermore, they also concluded that the cytotoxicity of the glyceride SLN was about 10-fold below the one of polylactide/glycolide nanoparticles. The results of cytotoxicity studies assessed by the 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyl-tetrazolium bromide (MTT) test indicated that glycerol behenate and glyceryl myristate SLN were less toxic than polyalkylcyanoacrylate and polylactic/glycolic acid nanoparticles (Műller, Rűhl et al, 1997). The cytotoxicity of SLN determined by viability measurements proved to be very low (Műller, Maassen et al., 1997). The viability of human granulocytes was 84% after incubation with 1.2% poloxamer 188 stabilized cetyl palmitate SLN and 72% after incubation with 5% poloxamer 188 stabilized glycerol behanate SLN. Poloxamer stabilized polylactide polyglycolide particles reduced the cell viability to 50 % at a concentration of 0.1 % Higher

concentrations of polylactide/polyglycolide particles up to 0.5% led to complete cell death.

Recently, in vivo toxicity study with i.v. injected glycerol behenate and cetyl palmitate SLN was performe Bolus injections of 1.33 g lipid/kg body weight were aministered every two days in mice, a total of six injections. Despite of the cetyl palmitate being a wax, these SLN were very well tolerated without increase in liver and spleen weighst. Glycerol behenate SLN showed an increase in liver and spleen weight accompanied by histological changes e.g. infiltration of macrophages. However, these side effects were reversible and could be avoided by lowering the dose of glycerol behenate.