CHAPTER II

LITERATURE REVIEW

1. Nanoparticles

Nanotechnology is becoming increasingly more important in the many fields including, pharmaceutical and cosmetic lines. This is primarily due to the fact that particles made at nanoscale have different and in many cases more desirable physical, chemical, and biological properties than larger particles. Nanoparticles are solid colloidal particles consisting of macromolecule substances with a mean diameter between 1 to 1000 nm (Lockman *et al.*, 2002). In this system, the active principle (drug or biologically active material) is dissolved, entrapped, encapsulated, or attached to the nanoparticle matrix (Kreuter, 1994). The nanoparticulate delivery systems broadly comprise of polymeric nanoparticles, nanocapsules, solid lipid nanoparticles, and nanogels (Date and Patravale, 2004). There are two principle basic types of nanoparticles; nanocapsules and nanospheres. Nanocapsules are defined as vesicular systems in which the drug is encapsulated within the cavity, which is an oil or aqueous core, surrounded by a unique polymer membrane. Whereas, nanospheres or monolithic matrix-type particles are defined as matrix systems in which the drug is physically and uniformly dispersed throughout the particles or entrapped into the polymer network. The schematic diagram of cross-section nanoparticles is shown in Figure 2-1 (Allémann *et al.*, 1996; Kreuter, 1994).

Matrix system was chosen rather than reservoir systems because of the ease of manufacture and the relatively high loading. For a poorly soluble drug this latter requirement usually leads to bulky dosage forms particularly with respect to reservoir systems (Wong, Gilligan and Po, 1992).

Figure 2-1 Nanoparticles (a) nanocapsule type and (b) nanosphere or monolithic type (Kreuter, 1994).

Nanoparticles made from lipid material represent an alternative coilloidal drug carrier system since 1990s period. This carrier combines the advantages but avoids the disadvantages of other traditional colloidal carriers such as emulsions, liposomes and polymeric microparticles. The proposed advantages include protection of incorporated labile drugs from degradation, controlled release capacity, low cytotoxicity, excellent tolerability and high entrapment efficiencies, especially for water-insoluble drugs. At the same time, this lipid-base nanoparticles also avoid the problem of organic solvent and biotoxicity of carrier (Mehnert and Mäder, 2001; Wissing, Kayser and Müller, 2004). The additional advantage of SLN may be the possibility of freeze-drying. Freeze-drying can be a promising way to increase chemical and physical stability of drugs and drug carries over extended period of time (Lim and Kim, 2002).

2. Production of lipid-based nanoparticles

2.1 High shear homogenization and ultrasonication

High shear homogenization and ultrasound are dispersing techniques which were initially used for the production of solid lipid nanodispersions. Both methods are widespread and easy to handle. However, dispersion quality is often compromised by the presence of microparticles leads to physical instabilities such as particle growth upon storage. This could be improved by higher surfactant concentrations, which might be correlated with toxicological problem. Furthermore,

metal contamination has to be considered due to ultrasonication (Mehnert and Mäder, 2001; Wissing, Kayser and Müller, 2004).

2.2 High pressure homogenization

High pressure homogenization (HPH) can be performed at elevated temperature (hot HPH technique) or at below room temperature (cold HPH technique). The particle size is decreased by cavitations and turbulences. Briefly, for the hot HPH technique, the lipid and drug are melted at approximately 5 °C above the melting point of the lipid and combined with and aqueous surfactant solution having the same temperature. A hot pre-emulsion is formed by high speed stirring. The hot pre-emulsion is then processed in a temperature controlled high pressure homogenizer. The obtained nanoemulsion recrystallises upon cooling down to room temperature as nanoparticles. The cold HPH is a suitable technique for processing the temperature labile drugs or hydrophilic drugs. Here, lipid and drug are melted together and then rapidly ground under liquid nitrogen forming solid microparticles. A pre-suspension is formed by high speed stirring of the particles in a cold aqueous surfactant solution. This pre-suspension is then homogenized at or below room temperature forming nanoparticles (Wissing, Kayser and Müller, 2004).

2.3 Solvent emulsification-evaporation or diffusion

In the solvent emulsification-evaporation, the lipid is dissolved in a water-immiscible organic solvent (e.g. toluene, chloroform) which is then emulsified in an aqueous phase before evaporation of the solvent under reduced pressure. Upon evaporation of the solvent, the lipid precipitates forming nanoparticles. An important advantage of this method is the avoidance of heat during the preparation, which makes it suitable for the incorporation of highly thermolabile drugs. Problems might

arise due to solvent residues in the final dispersion (Wissing, Kayser and Müller, 2004).

2.4 Water-in-oil-in-water double emulsion method

Recently, a novel method based on solvent emulsification-evaporation for the preparation of solid nanoparticles loaded with hydrophilic drugs has been introduced. Here, the hydrophilic drug is encapsulated – along with a stabilizer to prevent drug partitioning to the external water phase during solvent evaporation – in the internal water phase of a w/o/w double emulsion. However, the average size was in the micrometer range so that the term "lipospheres" in the sense as a term for nanopaticles is not used correctly for these particles (Wissing, Kayser and Müller, 2004).

2.5 Microemulsion techniques

The microemulsion precursor technology is based on the natural and spontaneous formation of a microemulsion that can easily be used as a template to form nanoparticles from within the dispersed oil droplet phase and using a properties of lipid being solid at room temperature. This method to obtain nanoparticles requires two step, formulation of warm o/w microemulsion and formation of solid nanoparticles (Cavilli *et al.*, 1997). The use of microemulsion as a template for producing nanoparticles is considered as follow:

2.5.1 Warm oil-in-water microemulsion process

The microemulsion needs to be produced at a temperature above the melting point of the lipid. The lipid is melted, and then a mixture of water, co-surfactant(s) and the surfactant are heated to the same temperature as the lipid and

added under mild stirring to the melted lipid. A transparent, thermodynamically stable system is formed when the compounds are mixed at the proper ratio.

Lawrence (2004) described microemulsions as clear, thermodynamically stable, fluid dispersions of oil and water stabilised by a surfactant or surfactants; usually in combination with a cosurfactant and formed without excessive mixing (spontaneous process). Microemulsion can have characteristic properties such as ultralow interfacial tension, large interfacial area and capacity to solubilize both aqueous and oil-soluble compounds. The diameter of the disperse phase of microemulsion is in the range of 10 to 100 nm. According to the content of water and oil, microemulsion can be classified into three types; oil-in-water, water-in-oil and bicontinuous microemulsions as shown in Figure 2-2.

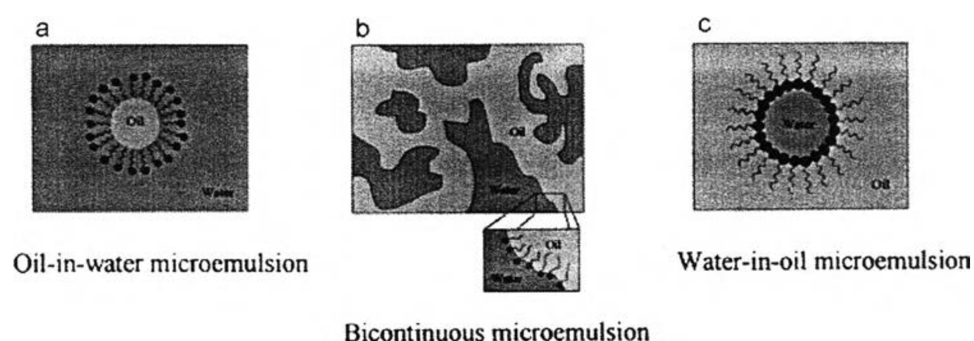

Figure 2-2 Schematic representation of the three most commonly encountered microstructure (a) oil-in-water, (b) bicontinuous and (c) water-in-oil microemulsion (Lawrence and Rees, 2000).

The choice of components for pharmaceutical microemulsions is often a balance between compounds, which are able to form microemulsions and non-toxic. As a general guideline, nonionic and hydrophilic zwitterionic surfactants are less toxic than ionic ones. Depending on the physicochemical properties of the constituents and composition, the stability of the microemulsion may be affected by

the addition of buffers, electrolytes, preservatives, polymers and drugs, which may alter the microemulsion structure and region or range of area of microemusion existence. Microemulsion systems based on nonionic surfactants are generally less affected by additives and changes in pH than ionic surfactants (Kreilgaard, 2002).

2.5.2 Formation of nanoparticles from microemulsion

To obtain nanoparticles, Gasco (1997) was firstly reported the preparation of nanoparticles from warm microemulsion by using a dilution method. The warm microemulsion is added to a cold aqueous medium (2-3°C) in the range of 1:25 to 1:50 volume ratio of microemulsion to medium and the precipitation of the lipid phase forming fine particles is obtained. The size of nanoparticle is affected by the composition of the microemulsion system, particularly the surfactant and co-surfactant used. However, due to the dilution step, achievable lipid contents forming particles are considerably low.

Oyewumi and Mumper (2002) have recently developed method to produce small and stable nanoparticles from microemulsion systems. Upon the addition of defined amounts of a suitable polymeric surfactant material (such as emulsifying wax, phospholipids, and/or polymeric surfactant) and water, a clear and stable oil-in-water (o/w) microemulsion is formed. Simple cooling of the warm microemulsion in the same container results in the formation of nanoparticles. This strategy has been reported to have advantages; since a variety of biocompatible ingredients can be used, well-defined and uniform nanoparticles may be reproducibly made without the use of expensive and/or damaging high-torque mechanical mixing, and no organic solvents are required. In addition, high entrapment efficiencies will be achievable, especially for water-insoluble drugs.

2.5.3 Influence of ingredient composition on nanoparticles

The importance of the emulsifier for the quality of the lipid nanodispersion was also demonstrated on microemulsion based solid lipid nanoparticles (SLN) dispersions (Mehnert and Mäder, 2001). In Cavalli *et al.* (1999) used stearic acid as the lipid phase and compared an ionic surfactant/cosurfactant system composed of Epikuron 100, taurodeoxycholate and monooctylphosphate with a nonionic system composed of Tween 80 and butanol. The particle size of the SLN dispersion produced with the ionic surfactants was considerably smaller (70±62 nm) compared to the nonionic formulation (200±65 nm).

2.5.4 Scale up feasibility

Large scale production of solid nanoparticles 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. Important process parameters during the scaling up are e.g. the temperatures of the microemulsion and the water, but also temperature flow in the water medium and the hydrodynamic of mixing which should change as little as possible during scaling up to maintain the same product characteristics.

3. Coenzyme Q_{10}

Coenzyme Q_{10}, also know as ubidecarenone, or Co Q_{10}, was first isolated in 1957 as fat-soluble crystals from the bovine heart muscle and first used in human illness in 1967 by Yuichi Yamamura. Coenzyme Q_{10} belongs to a family of substances called ubiquinones. Coenzyme Q_{10} is a naturally occurring vitamin-like compound found in the inner mitochondrial membrane throughout the body, especially, in organ with high rate of metabolism such as the heart, kidney and liver

and present in small amouts in a wide variety of food. Coenzyme Q_{10} is a strongly lipophillic compound, that exists in two redox form, namely ubiquinone and ubiquinol. The biological activity of Coenzyme Q_{10} results from its ability for reversible redox conversion (Crane, 2001).

3.1 Chemistry and physicochemical properies

Chemical name:	2, 3-dimethoxy, 5-methyl, 6-polyisoprene parabenzoquinone
Molecular formula:	$C_{59}H_{90}O_4$
Molecular weight:	863.36
Melting point:	Approximately 48°C
Appearance:	Yellow to orange, crystalline powder Odorless and tasteless
Solubility:	Very soluble in chloroform and carbon tetrachloride, freely soluble in dioxane, ether and hexane, sparingly soluble in acetone, and practically insoluble in water, methanol and ethanol
Stability:	It is gradually decomposed to dark color when exposed to light.

Structural formula :

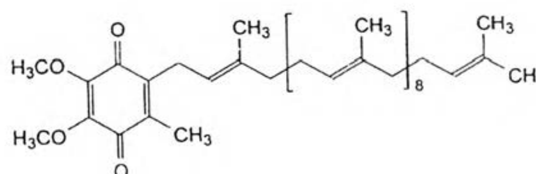

Figure 2-3 The structure of Coenzyme Q_{10}, comprising a quinone ring and a side chain of 10 isoprene units (BP, 1998).

The basic structure of ubiquinones consists of a benzoquinone "head" and a terpinoid "tail." The "head" structure participates in the redox activity of the

electron transport chain. The functional group is the quinone ring. However, the fully substituted quinone ring does not allow addition reactions with thiol groups in the cell such as glutathione, thioredoxin or thioctic acid. By reduction of the quinone to quinol a carrier of protons and electrons is produced. The percent in quinol form in various membranes and serum ranges from 30% to 90%, depending on the metabolic state of the cell. The quinone ring is ideally suited for electron and proton transfer. The coenzyme Q_{10} found in humans has a polyisoprene chain containing 10 isoprene units (5 carbons each) or a total of 50 carbons, structurally similar to vitamin K. The all-*trans*-polyisoprene ensures an affinity for the interior of cell membranes. The two methoxy groups contribute to the specificity in enzyme action as may the methyl group (Bayer, 1992; Crane, 2001; Ernster and Dallner, 1995). The electron and proton transfer functions of the quinone ring are of fundamental importance to all life forms; ubiquinone in the mitochondria of animals, plastoquinone in the chloroplast of plants, and menaquinone in bacteria (Langsjoen and Langsjoen, 1998).

3.2 Biosynthesis

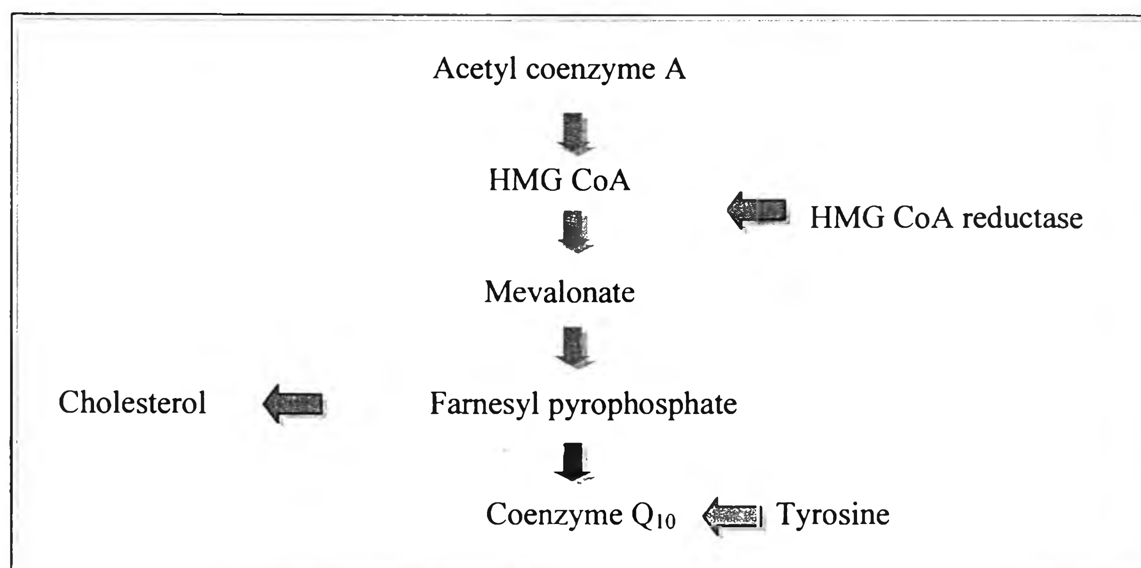

Figure 2-4 The pathway of Coenzyme Q_{10} synthesis (Langsjoen and Langsjoen, 1998).

The biosynthesis of CoQ_{10} from the amino acid tyrosine is a multistage process requiring at least eight vitamins and several trace elements as demonstrated in Figure 2-4. The biosynthesis of Coenzyme Q_{10} is multifold, with the isoprenyl side chain deriving from mevalonate, the benzoquinone ring structure from tyrosine, and condensation of these structures through polyprenyl transferase enzyme activity. The primary regulation of Coenzyme Q_{10} biosynthesis is the 3-hydroxy-methyglutaryl Coenzyme A (HMG-CoA) reductase action, which is similar in cholesterol synthesis (Langsjoen and Langsjoen, 1998).

3.3 Function of Coenzyme Q_{10}

3.3.1 The bioenergetic effect or mitochondria energy coupling properties

Coenzyme Q_{10} is an essential part of the cellular machinery used to produce ATP (adenosine triphosphate), which provides the energy for muscle contraction and other vital cellular functions. The major part of ATP production occurs in the inner membrane of mitochondria, where Coenzyme Q_{10} is found. The Coenzyme Q_{10} has a unique function since it transfers electrons from the primary substrates to the oxidase system at the same time that it transfers protons to the outside of the mitochondrial membrane. This transfer results in a proton gradient across the membrane. As the protons return to the interior through the enzymatic machinery for making ATP, they promote the formation of ATP. The Coenzyme Q_{10} is bound to the oriented enzymatic protein complexes. It is oxidized and releases protons to the outside and picks up electrons and protons on the inside of the mitochondrial membrane as shown in Figure 2-5 (Ernster and Dallner, 1995; Langsjoen and Langsjoen, 1998).

The bioenergetic properties are believed to be the fundamental importance in Coenzyme Q_{10} clinical application, particularly as patient relatively to

cell with high metabolic demand such as cardiac monocytes. The cellular effect of Coenzyme Q_{10} may be especially important inpatient with cardiac disease such as coronary artery disease and congestive heart failure, which is often, characterized by an energy depletion status that has been associated with low endogenous Coenzyme Q_{10}. Moreover, it is an integral part in the regeneration and rejuvenation of the skin (Hoppe *et al.*, 1999; Tran *et al.*, 2001; Yalcin *et al.*, 2004).

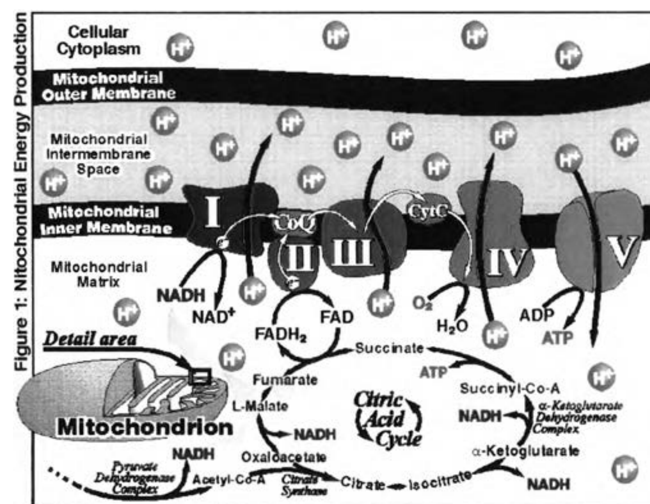

Figure 2-5 The role of Coenzyme Q_{10} in the electron transport chain of mitochondria (Dean and Fowkes, 1996).

3.3.2 Potent-lipid soluble antioxidant

The second fundamental property of Coenzyme Q_{10} involves its role as a potent lipid soluble antioxidant. The long polyprenyl side chain of Coenzyme Q_{10} assures its location within the lipid environment of mitochondrial membranes which are presented in most cell including heart and epidermis. Coenzyme Q_{10} can react with reactive oxygen species has resulted in scrutiny for systematic use of many diseases especially for cardiovascular disorder such as heart disease and atherosclerosis (lipid peroxidation protection) (Langsjoen and Langsjoen, 1998).

Moreover, it has been believed that the antioxidant activities of Coenzyme Q_{10} may make it useful as treatment in aged skin and photoaging which are the conditions that usually increased in cellular oxidation. A study by Hoppe *et al.* (1999) demonstrated that Coenzyme Q_{10} penetrated into the viable layers of the skin and significantly suppressed the expression of collagenase in human dermal fibroblasts following UVA irradiation. They also reported in a reduction of wrinkle depth following Coenzyme Q_{10} application. It has also been suggested that Coenzyme Q_{10} has function synergistical with other antioxidants such as vitamin E. Ubiquinol is known to be closely linked to vitamin E and serves to regenerate the reduced (active) alpha-tocopheral form of vitamin E, which can further help the skin fight free radical damage (Baumann and Weisberg, 2002; Hoppe *et al.*, 1999; Lupo, 2001).

Furthermore, Portakal *et al.* (2000) found that reactive oxygen species increased in malignant cells and may cause over expression of the consumption of Coenzyme Q_{10}. Administration of Coenzyme Q_{10} by nutrition may be related with the susceptibility of cells to carcinogenic agents and the response of tumor cells to the chemotherapeutic agents. This study showed the induction of protective effect by Coenzyme Q_{10} on breast tissue. Carmia (2004) also suggested that dietary antioxidants such as Coenzyme Q_{10} and vitamin E act as cancer preventive agents.

3.3.3 The maintenance of membrane integrity and fluidity

Coenzyme Q_{10} is involved in the maintenance of membrane integrity and fluidity emerge from the reduction of free radicals that may cause damage to structural proteins and lipids found in membrane. It has been also evidence that a decrease in blood viscosity with coenzyme Q_{10} supplement (Langsjoen and Langsjoen, 1998).

3.3.4 Induction of apoptosis

Carmia (2004) proposed that some dietary antioxidant may have potential as adjuvant in cancer therapy by their ability to induce programmed cell death (apoptosis). And recently, Narain *et al.* (2004) found that Coenzyme Q_{10} exerted an inhibitory effect on melanoma cells, which was related to the role of Coenzyme Q_{10} in the intrinsic pathway of apoptosis, however no adverse effects were observed on normal cell. Studies have demonstrated that the intrinsic pathway of apoptosis is often altered in melanoma due to mitochondrial abnormalities. They also found that the tumor mass was significant reduced (inhibit tumor growth) when topically applied with liposome encapsulated of Coenzyme Q_{10}. Moreover, Guttman (2005) also reported in the same way that topically applied Coenzyme Q_{10} has exciting potential as a safe and effective anti-tumor agent for treating and preventing recurrence of skin cancer. Coenzyme Q_{10} consistently induces apoptosis of the malignant cells but has no adverse effect on survival of normal cell. In addition, animal treated topically with a Coenzyme Q_{10} –containing cream had a 55 percent decrease in tumor mass.

3.4 Coenzyme Q_{10} preparation

Several experiments on Coenzyme Q_{10} formulation are focused almost exclusively for improvement of Coenzyme Q_{10} bioavailability via oral route. The examples of Coenzyme Q_{10} oral formulation reported include solubilized system with blend of surfactant (Chopra, *et al.*, 1998), self-emulsifying systems (Kommuru *et al.*, 2001), solid dispersion (Nazzal, *et al.*, 2002), and nanoemulsified composite systems (Carli, *et al.*, 2005). However, oral administration is one of the least effective modes of Coenzyme Q_{10} delivery due to uncertainties of absorption and inactivation in the gastrointestinal system. In contrast, a topical delivery is more pharmacologically advantageous due to a more specific and directs delivery to the point of interest, resulting in a higher absorption/dosage ratio of Coenzyme Q_{10}. Moreover, due to its

comfortable; the topical use will increase patient compliance. At the time of publication of this research there were several over-the-counter cosmeceutical products on the market containing Coenzyme Q_{10} especially, in form of cream. Recently, Narain *et al.* (2004) has developed liposome-encapsulated Coenzyme Q_{10} for used as an anti-cancer on skin. However, Mehnert and Mäder (2001) suggested that liposome has some problem in chemical and physical stability that might lead to liposome aggregation and drug degradation during storage.

Incorporation of Coenzyme Q_{10} into the solid matrix of nanoparticles can protect them against chemical degradation (Müller, Radtke and Wissing, 2002). Nanoparticles facilitate the topical delivery of drug to underlying tissues due to the small size of nanoparticles is in close contact with the stratum corneum that can increase the amount of encapsulated agents penetrating into the viable skin. These ultrafine particles also show an occlusive effect which promotes the penetration of active ingredients into the upper part of the epidermis. Moreover, due to their solid matrix, sustained drug release is possible. Furthermore, nanoparticles seem to be well suited for use on damaged or inflamed skin because they are based on non-irritative and non-toxic lipids. (Dingler *et al.*, 1999; Jenning, Schafer-Korting, and Gohla, 2000; Mei *et al.*, 2003).

4. Physicochemical characterization of nanoparticles

An adequate characterization of nanoparticles is a necessity for the control of the quality of the product. The characterization methods should be sensitive to the key parameters of nanoparticles performance and should avoid artifacts. However, characterization of nanoparticles is serious challenge due to colloidal size of the particles and the complexity of system, which includes also dynamic phenomena. Many analytical tools for investigation and characterization of colloids are used in

drug development and thus may be found in pharmaceutical laboratories. Size is their most prominent feature, although other parameters have to be considered as it may affect the release pattern, such as density, molecular weight, crystallinity and degradation properties. The surface charge, hydrophilicity, and hydrophobicity can significantly influence the interactive with biological environment after administration.

4.1 Particles size measurement

Photon-correlation spectrometry (PCS) and laser diffractometry (LD are the most powerful techniques for determination of nanometer sized colliods. PCS determines 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. PCS gives information about the mean diameter of the bulk population and about the width of the distribution via the polydispersity index (PI). The PI is necessary to characterize the particle size distribution of nanoparticles. The small values of PI corresponds a narrow size distribution and a monodisperse system, whereas the larger PI values indicate a broad size distribution. LD by Fraunhofer diffraction is more useful for larger, micrometer-sized particles that based on the dependency of the diffraction angle on particle radius. However, PCS and LD are not probably a direct particle measurement. They detect light scattering effect which is used to calculate particle size. Moreover, small amounts of different particle species can skew the measurements. Especially larger particles, such as dust, accidental microbial contamination, crystallization of ingredients, or secondary particle agglomerates can lead to erroneous results. Therefore, additional techniques might be performed (Attwood, 2002; Wissing, Lippacher and Müller, 2001).

4.2 Electron microscopy

Electron microscopy is used to verify the results obtained from light scattering technique. The electron microscopy that is normally used is transmission electron microscopy (TEM) and scanning transmission electron microscopy (SEM).

TEM produces two dimensional images from which particle shape interpretation is difficult; however, size may be determined because of the high resolution. Transmission electron microscopy without or with staining is a relatively easy method of particle size determination. This method is limited in some nanoparticles which are not dense in electron. The system which cannot be stained and melted when irradiated by the electron beam and therefore cannot be visualized by this method.

SEM produces three-dimensional images. The specimen is scanned with a focused fine beam of electrons and the surface characteristic is obtained from secondary electrons emitted from the specimen surface. This technique is performed in high vacuum. For visualization, the particles have to be dried and coated with gold (Attwood, 2002; Wissing, Lippacher and Müller, 2001).

4.3 Thermal analysis

Differential scanning calorimetry (DSC) is a thermal analytical technique, which measures heat flow associated with transitions in materials as a function of temperature. DSC results provide useful information about the physical and chemical changes that involve endothermic or exothermic process or change in heat capacity. The thermo-analysis provides information about melting and recrystallization behavior, the timing of polymorphic transitions and enthalpy (Cui, Hsu and Mumper, 2003; Heurtault *et al.*, 2003; Hou *et a.l*, 2003). The crystalline and

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amorphous nature of drug dispersed in nanoparticles can be determined by this method. Cavalli *et al.* (1999) found that hydrocortisone and progesterone are dispersed in lipid matrix in an amorphous form. DSC is also widely used to investigate the status of the lipid because different modifications possess different melting points and melting enthalpies (Müller, Mäder and Gohla, 2000).

5. Drug incorporation in nanoparticles

A very important point to judge the suitability of a drug carrier system is its loading capacity. The loading capacity is generally expressed in percent related to the lipid phase; lipid and drug (Müller, Mäder and Gohla, 2000). There are basically three different models for the incorporation of active ingredients into solid nanopaticles which depends on the composition and the production conditions.

5.1 model of drug incorporation

5.1.1 Homogeneous matrix model

A moleculary dispersed drug or drug being present in amorphous cluster is thought to be mainly obtained when incorporating very lipophilic drugs in solid nanoparticles.

5.1.2 Drug-enriched shell model

An outer shell enriched with active compound can be obtained when phase separation occurs during the cooling process from the liquid oil droplet to the formation of a solid lipid nanoparticle. According to the TX diagram, the lipid can firstly precipitate forming a practically compound-free lipid core. At the same time, the concentration of active compound in the remaining liquid lipid increases

continuously during the forming process of the lipid core. Finally, the compound-enriched shell crystallises comparable to the eutecticum in the TX diagram.

5.1.3 Drug-enriched core model

A core enriched with active compound can be formed when the opposite occurs, which means the active compound starts precipitating first and the shell will have distinctly less drug. This leads to a membrane controlled release governed by the Fick's law of diffusion (Müller, Radtke and Wissing, 2002).

5.2 In vitro release study of drug

In vitro release study, in principle, should be useful for quality control as well as for the prediction of *in vivo* kinetics of nanoparticulate systems. In addition, it can be very useful for evaluation of the influences of process parameters on the release rate of active compounds from nanoparticles (Allémann *et al.*, 1996). Drug may be released by (Kreuter, 1994)

1) Desorption of surface-bound drug
2) Diffusion through the nanoparticle matrix or monolithic device (Figure 2-6 a)
3) Diffusion through the polymer wall or reservoir device (Figure 2-6 b)
4) Nanoparticle matrix erosion
5) A combined erosion diffusion process or eroding monolithic device (Figure 2-6 c)

Analysis of nanoparticle release profiles frequently shows a biphasic release pattern that can be described by a biexponential function as follows

$$C_t = Ae^{-\alpha t} + Be^{-\beta t}$$

where; C_t is the concentration of compound remaining in the nanoparticles at a given time t; A and B are system-characteristic constants, and α and β are rate constants that can be obtained from semi-logarithmic plots (Kreuter, 1994).

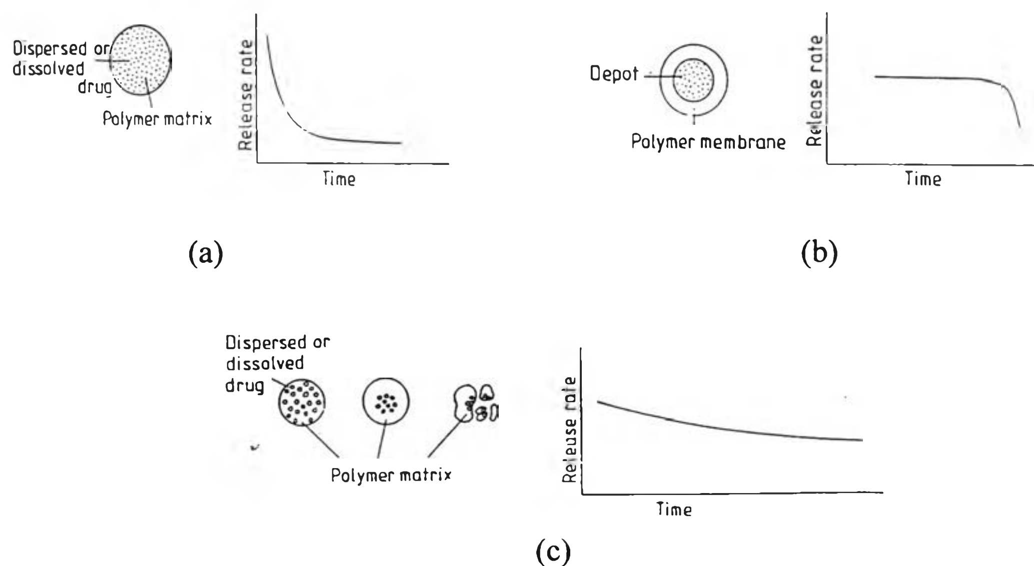


Figure 2-6 the release of drug from nanoparticles and plot of drug release rate versus time of (a) monolithic device, (b) reservoir device and (c) eroding monolithic device (Kreuter, 1994).

It could be monitored that the release profiles are not or only slightly affected by the particle size. Dominant factors for the shape of the profiles are the production parameters (surfactant concentration, temperature) and also the nature of the lipid matrix (Müller, Mäder and Gohla, 2000).

The characterization of the *in vitro* drug release from a colloidal carrier is difficult, because it is not possible to effectively and rapidly separate the particles from the dissolved or released drug in the surrounding solution due to their very small size of this dose form. The choice of a suitable model of drug release from

nanoparticles is still problematic. There are several methods used for *in vitro* release study such as side-by-side diffusion cells with artificial or biological membranes, dialysis bag diffusion, reverse dialysis, ultracentrifugation, ultrafiltration and centrifugal ultrafiltration (Kreuter, 1994).

For the sample and separation technique used for release study, the nanoparticles are suspended into a sink medium maintained at a constant temperature under agitation. Ideally, the sink medium should be a buffer solution with a pH of 7.4, but some active substances require other pH values or the addition of a surfactant or organic solvent to reach sink conditions. Usually, the working temperature is 37° C. Sampling is carried out at various time intervals. The continuous phase is then separated from the dispersed phase by ultracentrifugation or centrifugal ultrafiltration, and the release drug is assayed.

The centrifugal ultrafiltration technique has been developed by Millipore Corp. The device, mainly based on an eppendorf centrifuge tube separated from an enclosed tube by an ultrafiltration membrane, allows for separation of nanoparticles from microliter volumes of aqueous dispersion medium by a centrifuge, this technique has been successfully to evaluate the in vitro release profile of indomethacin from polylactic nanocapsules (Klang and Benita, 1998).

6. Stability of nanoparticulate system

In colloidal dispersions frequent collision between the particles occur as a result of Brownian movement. Whether these collisions result in permanent contact of the particles (coagulation), which leads eventually to the destruction of the colloidal system as the large aggregates formed sediment out, or temporary contact (flocculation), or whether the particles rebound and remain freely dispersed (a stable colloidal system), depends on the forces of interactive between the particles. These

forces can be divided into three groups: electrical forces of repulsion, forces of attraction, and forces arising from salvation. An understanding of the first two (DLVO theory) explains the stability of lyophobic systems, and all three must be considered in a discussion of the stability of lyophilic dispersions (Attwood, 2002).

6.1 The process of instability

6.1.1 Flocculation

Attraction forces between droplets cause them to cluster. The aggregates have the structure in which the particles remain a small distance apart from one another. This may be a secondary minimum phenomenon in the DLVO theory or a consequence of bridging by a polymer or polyelectrolyte. The processes are commonly used to remove suspended matter from water because the flocculate finally sediments. It is a reversible phenomenon (Attwood, 2002; Heurtault *et al.*, 2003).

6.1.2 Coalescence

Coagulation signifies that the particles are closely aggregated and difficult to redisperse (a primary minimum phenomenon). Coalescence is an irreversible rupture of the emulsion leading to phase separation. On collision between two drops, the interfaces distort, up to a certain critical thickness before it finally ruptures and allowing droplets to merge. Rigid solid particles are expected to be stable against coalescence. In contrast, solid nanoparticles dispersions tend to cream or gel after particle contact (Attwood, 2002; Heurtault *et al.*, 2003).

6.1.3 Creaming and sedimentation

The reversible creaming process describes how emulsion droplets tend to rise to the top of a vial or to sink to the bottom as sedimentation.

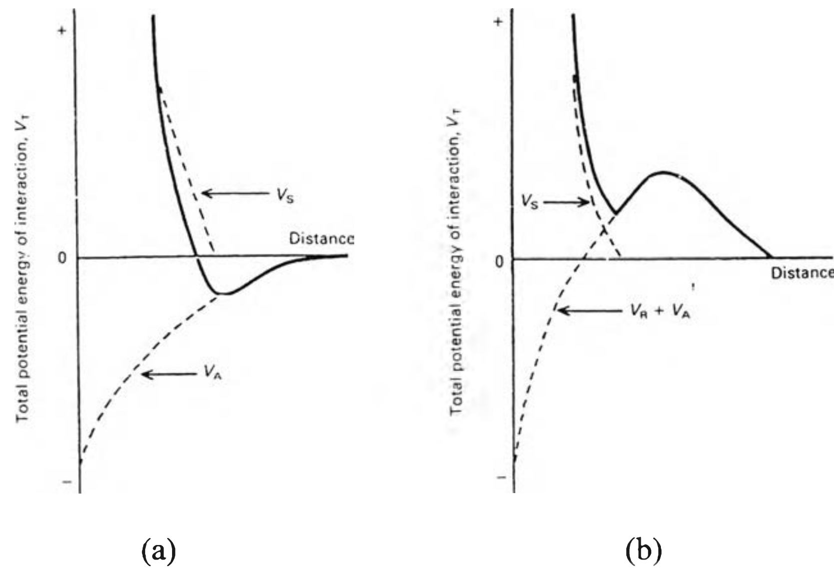
Competition between Brownian agitation and gravity can lead to non-homogeneous dispersion. It is generally due to the density difference between the two phases (Heurtault *et al.*, 2003).

6.1.4 Ostwald ripening

This phenomenon depends on both the granulometry and on the Laplace surpressure being higher for lower size droplets. A species flux occurs from small to large droplets via the continuous phase. The average diameter consequently increases. For solid nanoparticles, it corresponded to a particle size increase due to the dissolution of smaller crystals and deposition of the dissolved material on larger surfaces, resulting in the growth of large particles at the expense of smaller ones (Heurtault *et al.*, 2003).

6.2 Steric stabilization

It has long been known that non-inonic polymeric materials adsorbed at the particle surface can stabilize a lyophobic colloid even in the absence of a significant zeta potential. The approach of two particles with absorbed polymer layers results in a steric interaction when the layers overlap, leading to repulsion. In general, the particles do not approach each other closer than about twice the thickness of the absorbed layer, and hence passage into the primary minimum is inhibited. An additional term has thus to be included in the potential energy of interaction for what is called steric stabilization (Attwood, 2002).



(a) (b)

Figure 2-7 Schematic curves of the total potential energy (V_T) of the interaction versus distance for two particles, showing the effect of the steric stabilization, V_S (a) in the absence of electrostatic repulsion (V_R), the solid line representing $V_T = V_A + V_S$. V_A is the attractive force; (b) in the presence of electrostatic repulsion, the solid line representing $V_T = V_R + V_A + V_S$ (Attwood, 2002).

Steric stabilization consists of two forces, osmotic or salvation forces and entropic effects. For salvation force, it can be explained that when two droplets come in close contact, hydrophilic chains of surfactant start to overlap. It leads to an osmotic gradient in solution between concentrated polymer solution in an overlap region and a dilute solution in bulk solution. Then, water enters into the concentrated polymer region in an attempt to dilute it, resulting in force chains or droplets apart. For entropic effect, the loss of freedom of motion of polymer chain and the loss of entropy when droplets come together and polymer chains overlap. This phenomenon is thermodynamically unfavorable, resulting in force droplets apart again (Lawrence, 2004).

The effect of steric stabilization (V_S) on the potential energy against distance between particles curve is seen in Figure 2-8, showing that repulsion is generally seen at all shorter distances provided that the electrostatic (V_R) adsorbed polymeric material does not move from the particle surface (Attwood, 2002).

Steric repulsion can be explained by reference to the free energy changes that take place when two polymer-covered particles interact. The change in free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) are related according to:

$$\Delta G = \Delta H - T\Delta S$$

,where T is the absolute temperature.

The second law of thermodynamics implies that a positive value of ΔG is necessary for dispersion stability while a negative value indicating that the particles have aggregated. A positive value of ΔG can arise in a number of ways, for example when ΔH and ΔS are both negative and $T\Delta S > \Delta H$. Here the effect of the entropy change opposes aggregation and outweighs the enthalpy term; this is termed ***entropic stabilization.*** Interpenetration and compression of the polymer chains decreases the entropy as these chains become more ordered. Such a process is not spontaneous; work must be expended to interpenetrate and compress any polymer chains existing between the colloidal particles, and this work is a reflection of the repulsive potential energy. The enthalpy of mixing of these polymer chains will also be negative. Stabilization by these effects occurs in non-aqueous dispersions.

Again, a positive ΔG occurs if both ΔH and ΔS are positive and $T\Delta S < \Delta H$. Here enthalpy aids stabilization, entropy aids aggregation. Consequently, this effect is termed ***enthalpic stabilization*** and is common with aqueous dispersions, particularly where the stabilization polymer has polyoxyethylene chains. Such chains

are hydrated in aqueous solution due to H-bonding between water molecules and the 'either oxygens' of the ethylene oxide groups. The water molecules have thus become more structured and lost degrees of freedom. When interpenetration and compression of ethylene oxide chains occurs there is an increased probability of contact between ethylene oxide groups, resulting in some of the bound water molecules being released as shown in Figure 2-9. The released water molecules have greater degrees of freedom than those in the bound state. For this to occur they must be supplied with energy, obtained from heat absorption, i.e. there is a positive enthalpy change. Although there is a decrease in entropy in the interaction zone, as with entropic stabilization, this is overridden by the increase in the configurational entropy of the released water molecules.

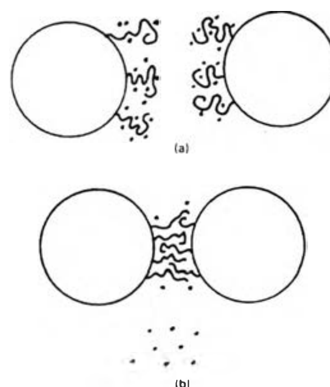


Figure 2-8 Enthalpic stabilization (a) particles with stabilizing polyoxyethylene chains and H-bonded water molecules and (b) particles with stabilizing chains overlap and water molecules released (Attwood, 2002).

At low polymer concentration (and hence low particles surface coverage), bridging flocculation may occur as shown in Figure 2-10. Bridging is a consequence of the adsorption of segments of an individual polymeric flocculants molecule onto the surface of more than one particle (Attwood, 2002). Generally,

steric forces are dependent upon length of polymer chains with longer the chains, greater the stabilization (Lawrence, 2004).

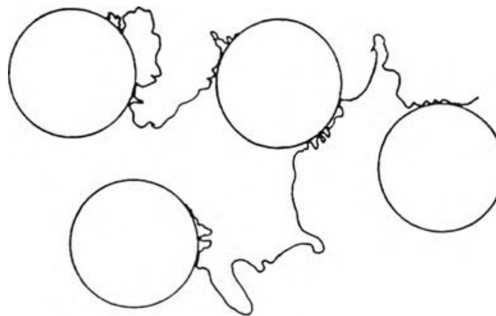


Figure 2-9 Bridging flocculations (Attwood, 2002).

6.3 Freeze-dry process (Lyophilization)

Storage stability involves chemical and physical aspects and includes the prevention of degradation reactions such as hydrolysis and the preservation of the initial particle size. It requires that the solid nanoparticles ingredients have sufficient chemical stability and that the particles have a very narrow size distribution to avoid crystal growth by Ostwald ripening. The solid naoparticles formulation should be resistant to temperature changesupon storage. It has been shown that particle sizes of aqueous solid nanoparticles dispersions might be stable over 12–36 months. However, this stability is not a general feature of solid nanoparticles dispersions and in most cases, an increase in particle size will be observed in a shorter period of time. Degradation of polymer, drug leakage, and/or drug degradation may occur if nanoparticles are kept in an aqueous medium during storage.

Lyophilization is a promising way to increase chemical and physical stability over extended periods of time. Transformation into a solid form will prevent Ostwald ripening and avoid hydrolysis reaction. Lyophilization also offers principle possibilities for solid nanoparticle incorporation into various preparations. However,

two additional transformations between the formulaions are necessary which might be the source of additional stability problems. The first transformation (from aqueous dispersion to powder) involves the freezing of the sample and the evaporation of water under vacuum. Freezing of the sample might cause stability problems due to the freezing out effect which results in changes of the osmolarity and the pH. The second transformation (resolubilization) involves, at least in its initial stages, situations which favor particle aggregation (low water and high particle content, high osmotic pressure).

The addition of cryoprotectors will be necessary to decrease solid nanoparticles aggregation and to obtain a better redispersion of the dry product. Typical cryoprotective agents are sorbitol, trehalose, dextrose, lactose, mannitol and polyvinylpyrrolidone. They decrease the osmotic activity of water and crystallization and favor the glassy state of the frozen sample. Cryoprotectors prevent the contact between discrete lipid nanoparticles. Furthermore, they interact with the polar head groups of the surfactants and serve as a kind of 'pseudo hydration shell' (Allémann *et al.*, 1996; Mehnert and Mäder, 2001).

7. Application of nanoparticles

Nanoparticles have been introduced as a novel drug delivery system for pharmaceutical and cosmetic active agent in various application routes such as parenteral, oral, topical, ocular, pulmonary and rectal (Müller, Mäder and Gohla, 2000).

7.1 Topical application

This carrier is considered as being the next generation of delivery of pharmaceutical and cosmetic formulations. The lipid-based nanoparticles posses some features which make them promising carriers for topical applications:

7.1.1 The protection of labile compounds against chemical degradation and the possibility to modulate drug release due to their solid state of the particle matrix has been shown. (Müller-Goymann, 2004; Jenning *et al.*, 2000). It has been reported that incorporation of Coenzyme Q_{10} into the solid lipid nanoparticles matrix protected them against chemical degradation (Müller, Mäder and Gohla 2000).

7.1.2 They are composed of well-tolerated excipients. Lipid-based nanoparticles seem to be well suited for use on damaged or inflamed skin if they are based on non-irritative and non-toxic lipids (Dingler *et al.*, 1999).

7.1.3 Lipid-based nanoparticles show a UV-blocking potential as they act as physical sunscreens on their own and can be combined with molecular sunscreens in order to achieve improved photo-protection (Wissing and Müller, 2002 a and b).

7.1.4 A white pigment effect that covers undesired colors of compounds or their degradation products (Wissing, Lippacher and Müller, 2001).

7.1.5 Adhesiveness is a general property of a very fine particle, leading to film formation on the skin. This film of ultrafine particles provides an occlusive effect which promotes penetration of active ingredients into the upper part of the epidermis, mainly the stratum corneum. Due to an occlusion effect, water evaporation from skin to the atmosphere is decreased and water is thus retained within the skin (reduced transepidermal water loss; TEWL). The stratum corneum swells (hydration), thus enhancing the cosmetic effect and pharmaceutical efficiency of

incorporated ingredients and also providing smoothing effect on skin wrinkles. Moreover, penetration might also be affected by the nanoparticles carrier itself, the high specific surface area of nanometer size facilitated close contact of encapsulated drug with the stratum corneum (Mei *et al.*, 2003; Wissing, Lippacher and Müller, 2001). It is assumed that nanoparticles dispersion, when administered directly onto skin in a small but sufficient quantity, would cause fewer side effects, if any, than the currently available formulations.

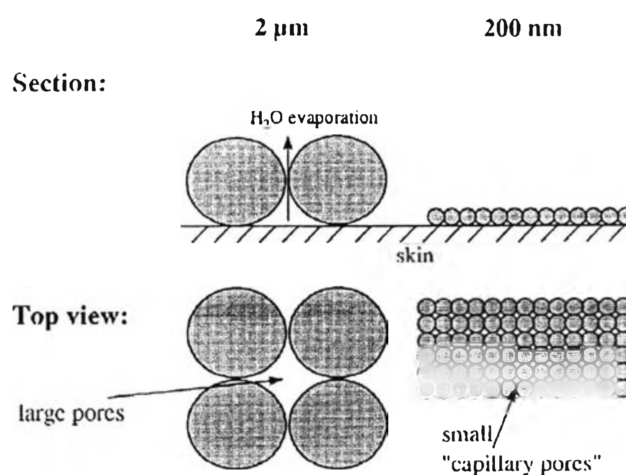


Figure 2-10 Model of occlusive effect depending of size of the particles, 2μm (left) and 200 nm (right) (Müller, Radtke and Wissing, 2002).

A first model of occlusive effect and film formation by solid nanoparticles was developed by Dingler (1999), suggesting that the occlusive effect is strongly dependent on the paticles size. Figure 2-11 shows the difference between large particles microparticles and small nanoparticles. The ‘holes’ in between the microparticles applied to the skin are relatively large and favour the evaporation of water hydrodynamically. In contrast, only tiny nanosized pores exist in the monolayer of solid nanoparticles thus evaporation of water is hydrodynamically unfavourable.

Water condensates in the pores due to their small size and reduced vapour pressure (La Place equation).

In vitro studies were performed to confirm the occlusivity of nanoparticles or so-called 'occlusive factor' (Müller, Radtke and Wissing, 2002). The *in vitro* model by Vringer consisted of a beaker of water covered by a filter paper. The formulation was spread on a filter paper; a reference control was a beaker with a filter only. An occlusion factor was calculated by the formula:

$$F = 100 * \frac{(A-B)}{A}$$

where, F is the occlusive factor,

A is the water loss without sample (reference) and

B is the water loss with sample

From this study, an occlusive factor of zero means no occlusive effect compared to reference; the maximum occlusive factor is 100 (Müller, Radtke and Wissing, 2002). Moreover, the systematic study of occlusion was performed by Wissing, Lippacher and Müller (2001) who investigated the chemical nature of the lipid, crystallinity of the lipid matrix, and particle size. The study of particle size showed that one needs to have really small-sized nanoparticles; lipid micocroparticles have no or little occlusive effect. The study also showed the superiority of 200 nm nanoparticles over 4 μm microparticles.

An advantage of solid nanoparticles is a simple performed incorporation into various topical formulations (Amselem and Friendman, 1998). Solid nanoparticles can be admixed to an already commercially available and established topical formulation Ad-mixing the solid nanoparticles leads to an increase

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in occlusivity while still maintaining the 'light character' of the day cream and avoiding the glossiness of more occlusive night creams.

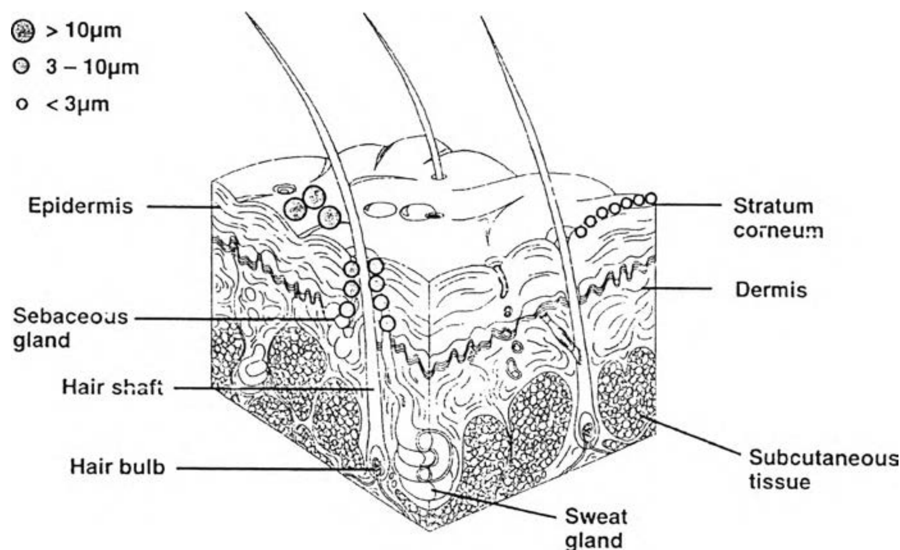

Figure 2-11 Illustration of the proposed influence of particle size on cutaneous penetration pathways (Amselem and Friendman, 1998).

The observed improved efficacy of the solid nanoparticles formulation could be explained based on skin structure. The skin barrier, which prevents penetration of external substances, is localized in the outer horny layer (stratum corneum). It consists of several layers of dead epithelial cells and the intracellular space which is filled with non-polar lipids. These lamellar lipid layers are assumed to be a primary barrier against penetration of exogenous compound. Amselem and Friendman (1998) explained direct influence of the particle size on its penetration into skin. It was found that the parcutaneous penetration pathway of polymeric microspheres is size dependent. Particles below 3 μm were randomly distributed into the stratum corneum and hair follicles. The main penetration pathway of these microspheres was the transepidermal route since the outer surface of the follicular orifice is only 0.1% of the total skin surface area. The larger microparticles

(>10-20 μm) did not penetrate the skin and remained on the stratum corneum outer surface as shown in Figure 2-12.

7.2 Oral administration

Oral administration of nanoparticles is possible as aqueous dispersion or alternatively after transform into a traditional dosage form such as tablets, pellets, capsules or powders in sachets. An example for orally administered nanoparticles is Camptothecin (CA)-loaded nanoparticles using stearic acid (2%), lecithin (1.5%) and poloxamer 188 (0.5%) as ingredients (Yang *et al.*, 1999). Cyclosporin has also been formulated as nanoparticles. A loading capacity of 20% cyclosporin in the lipid Imwitor 900 was achieved. The nanoparticles were stabilized using a mixture of Tagat-S and sodium cholate (Müller, Mäder and Gohla, 2000).

7.3 Parenteral administration

Basically nanoparticles can be used for all parenteral applications, ranging from intra-articular to intravenous administration. Studies using 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 intravenous administered SLN led to higher and prolonged plasma levels of paclitaxel (Müller, Mäder and Gohla, 2000).

7.4 Other Applications

In addition to topical, oral and parenteral administration, the use of nanoparticles for nasal (Illum *et al.*, 2001), pulmonay and ophthalmic delivery of drugs (Müller, Mäder and Gohla, 2000; De Campos *et al.*, 2001) has been

investigated. Nanoparticles have enabled crossing the blood-brain barrier that represents an insurmountable obstacle for a large number of drugs, including antibiotics, antineoplastic agents, and a variety of drugs of central nervous system active drugs, especially neuropeptides (Kreuter, 2001; Schroeder *et al.*, 1998). Nanoparticles are also used as immune adjuvants including tetanus toxoid and influenza virus (Amselem and Friendman, 1999).