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A review on Solid Lipid Nanoparticles (SLN) for controlled and targeted delivery of medicinal agents

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ABSTRACT

Solid lipid nanoparticles (SLN) were introduced as alternative carrier system to traditional colloidal carriers, such as emulsions, liposomes and polymeric micro- and nanoparticles. SLN combine advantages of the traditional systems but avoid some of their major disadvantages. Nanoparticles are sub-nanosized colloidal structures composed of synthetic or semi synthetic polymer. The colloidal carriers based on biodegradable and biocompatible polymeric system have largely influenced the controlled and targeted drug delivery concept. Nanoparticle loaded bioactive could not only deliver drug to specific organ within the body but delivery rate in addition could be controlled as being bystanders, burst, controlled pulsatile or modulated. This paper reviews the present methods and techniques regarding production of SLN, drug incorporation, loading capacity and drug release, especially focusing on drug release mechanisms. Relevant issues for the introduction of SLN to the pharmaceutical market, such as status of excipient, toxicity/tolerability aspects and sterilization and long-term stability including industrial large scale production are also discussed. The potential of SLN to be exploited for the different administration routes is highlighted.

Keywords: Colloidal drug carrier; Nanotechnology; Solid lipid nanoparticles; Targeted drug delivery.

1. INTRODUCTION

Particulate drug carriers investigated for many years include oil-in-water (O/W) emulsions, liposomes, microspheres and nanoparticles based on synthetic polymers or natural macromolecules. The O/W emulsions have been introduced successfully to the clinic for parenteral nutrition in the fifties. The only intention of these emulsions was to reduce drug side effects, e.g. pain of injection and inflammation at the injection site (e.g. diazepam). Despite the excellent tolerability of these O/W emulsions the number of products on the market is relatively low, indicating their limited success [1]. One of the reasons preventing a broader introduction of emulsions for drug delivery is the physical instability which can be caused by the incorporated drug. In addition, the registered oils such as soybean oil, MCT and LCT and mixtures thereof show an insufficient solubility for drugs of possible interest to be incorporated into emulsions. Phospholipid vesicles rediscovered as `liposomes' in 1965 by Bangham found their way to the cosmetic market in 1986.

It was the anti-aging product Capture (Dior) which smoothed the way for liposome-based pharmaceutical products. Having the first liposome product on the market strengthened at least the morale of researchers in the pharmaceutical area working intensively for so many years with this delivery system [2]. Finally, the first pharmaceutical products came to the market at the end of the eighties and beginning of the nineties, and include the synthetic lung surfactant Alveofact for pulmonary instillation, Epi-Pevaryl, a topical product for anti-mycotic therapy (drug: econazole) and other products for intravenous injection (e.g. Ambi-some with amphotericin and cytotoxic-containing formulations like Doxil and Daunosome). However, the total number of products on the market is still limited. One of the reasons for this apart from possible technological problems is the non-availability of a `cheap' pharmaceutical liposome [3].

The number of products based on polymeric micro-particles on the market is limited. After the introduction of the first wave of products (e.g. Enantone Depot, Decapeptyl Depot etc.), there was only a limited increase in the number of micro-particulate products. There are quite a few well-known reasons for this, of which two should be highlighted: the cytotoxicity of polymers and the lack of a suitable large scale production method. Polymers accepted for use as implants are not necessarily also of good tolerability in the form of nanoparticles. In the nanometer size range and having a size of a few micrometers, the polymer can be internalized by cells (e.g. macrophages) and degradation inside the cell can lead to cytotoxic effects, e.g. as reported for polyester polymers. A hundred percent mortality was found in cell cultures when incubating the cells with 0.5% PLA/GA nanoparticles [2]. A prerequisite to introducing a product to the pharmaceutical market is the availability of a suitable large scale production method, suitable means a method being cost-effective and leading at the same time to a product having a quality being acceptable by the regulatory authorities. There are still problems in the production of polymeric nanoparticles on large scale.

Since the beginning of the nineties attention from various research groups has focused on an alternative to polymeric nanoparticles, the solid lipid nanoparticles (SLN) [3]. The use of solid lipids as a matrix material for drug delivery is well-known from lipid pellets for oral drug delivery (e.g. Mucosolvan retard capsules). The production of lipid microparticles by spray congealing was described by Speiser at the beginning of the eighties [4] followed by lipid nanopellets for peroral administration. Basically, lipids can be used which are well tolerated by the body (e.g. glycerides composed of fatty acids which are present in the emulsions for parenteral nutrition). Large scale produc-tion can be performed in a cost-effective and relatively simple way using high pressure homogenization leading to SLN. An alternative approach is the production of SLN via microemulsions [3]. This paper reviews the present state of the art in drug delivery using solid lipid nanoparticles and highlights the potential future perspectives.

2. PREPARATION TECHNIQUES FOR LIPID PARTICLES

2.1. Preparation of SLN by high pressure homogenization

SLN are particles made from solid lipids with a mean photon correlation spectroscopy (PCS) diameter between approximately 50 and 1000 nm. One can derive them from the emulsions for parenteral nutrition just by replacing the liquid lipid (oil) of the emulsion droplets by a solid lipid. In contrast to emulsions for parenteral nutrition which are normally stabilized by lecithin, the SLN can be stabilized by other surfactants or polymers and their mixtures. However as a distinct advantage of SLN compared to polymeric nanoparticles, they can be produced by high pressure homogenization identical to parenteral O/W emulsions. This is a technique well established on the large scale since the fifties and already available in the pharmaceutical industry. The production lines for parenteral emulsions are in most cases equipped with temperature control units because an increased temperature facilitates emulsion production, this means that existing production lines can be used for producing SLN by the hot homogenization technique.

The two basic production methods for SLN are the hot homogenization technique and the cold homogenization technique [5]. For both techniques the drug is dissolved or solubilized in the lipid being melted at approximately 5-10°C above its melting point. For the hot homogenization technique the drug containing melt is dispersed under stirring in a hot aqueous surfactant solution of identical temperature. Then the obtained pre-emulsion is homogenized using a piston-gap homogenizer, the produced hot O/W nanoemulsion is cooled down to room temperature, the lipid recrystallizes and leads to solid lipid nanoparticles (Figure 1). Of course, care needs to be taken that recrystallization of the lipid occurs. For glycerides being composed of short chain fatty acids e.g. Dynasan 112 and glycerides with a low melting point (too close to room temperature) it might be necessary to cool the nanoemulsions to even lower temperatures to initiate recrystallization. Recrystallization can also be initiated, e.g. by lyophilization.

The hot homogenization technique is also suitable for drugs showing some temperature sensitivity because the exposure to an increased temperature is relatively short. In case of highly temperature-sensitive compounds the cold homogenization technique can be applied. It is also necessary to use this technique when formulating hydrophilic drugs because they would partition between the melted lipid and the water phase during the hot homogenization process. For the cold homogenization technique the drug-containing lipid melt is

cooled, the solid lipid ground to lipid microparticles (approximately 50-100 mm) and these lipid microparticles are dispersed in a cold surfactant solution yielding a pre-suspension. Then this pre-suspension is homogenized at or below room temperature, the cavitation forces are strong enough to break the lipid microparticles directly to solid lipid nanoparticles. This process avoids, or minimizes, the melting of the lipid and therefore minimizing loss of hydrophilic drugs to the water phase. Of course, the difference between the melting point of the lipid and the homogenization temperature needs to be large enough to avoid melting of the lipid in the homogenizer. The homogenization process itself increases the product temperature (e.g. 10-20°C per homogenization cycle). There are also temperature peaks in the homogenizer. To further minimize the loss of hydrophilic compounds to the aqueous phase of the SLN dispersion, water can be replaced by liquids with low solubility for the drug, e.g. oils or PEG 600. Production of SLN in oil or PEG 600 is advantageous for oral drug delivery because this dispersion could be directly filled into soft gelatin capsules.

2.2. SLN produced by microemulsion technique

Microemulsions are clear or slightly bluish solutions being composed of a lipophilic phase (e.g. lipid), a surfactant and in most cases also a co-surfactant and water. The microemulsions show properties of real macroemulsions (e.g. small particle sizes can be measured by laser light scatter-ing) and simultaneously properties of a real solution (e.g. drugs possess saturation solubility in a microemulsion and do not show a distribution coefficient as in macroemulsions). Addition of a microemulsion to water leads to precipitation of the lipid phase forming fine particles. This effect is exploited in the preparation method for SLN developed by Gasco [4].

To form a microemulsion 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 (fatty acids and/or glycerides) are melted, a mixture of water, co-surfactant(s) and the surfactant is 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 ratio for microemulsion formation. This microemulsion is then dispersed 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 [6,7]. Surfactants and co-surfactants include lecithin, biliary salts, but also alcohols such as butanol [8]. Excipients such as butanol are less favorable with respect to regulatory aspects. From the technical point of view precipitation of the lipid particles in water is a dilution of the system, which leads to a reduction of solid content of the SLN dispersion. For some technological operations it is highly desirable to have a high lipid solid content, e.g. 30%. An example is the transfer of the SLN dispersion to a dry product (e.g. tablet, pellet) by a granulation process. The SLN dispersion can be used as granulation fluid, but in the case of low particle content too much water needs to be removed.

Large scale production of SLN by the microemulsion technique also appears feasible and is at present under development at Vector pharma (Trieste, Italy). The micro-emulsion is prepared in a large, temperature controlled tank and then pumped from this tank into a cold water tank for the precipitation step [9]. Important process parameters during the scaling up are e.g. the temperatures of the microemulsion and the water, but also temperature flows in the water medium and the hydrodynamics of mixing which should change as little as possible during scaling up to maintain the same product characteristics.

2.3. Lipid nanopellets and lipospheres

The lipid nanopellets for oral delivery developed by Speiser are produced by dispersing a melted lipid in a surfactant solution by stirring or sonication. The obtained particle size is determined by the power density of the stirrer. In general, a mixture of nanoparticles and microparticles is obtained [10]. To preferentially obtain nanoparticles, relatively high surfactant concentrations are employed (i.e. one moves towards solubilization of the lipid). 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, the route that nanopellets were intended for according to the patent [9], but might cause some problems for other administration routes such as intravenous.

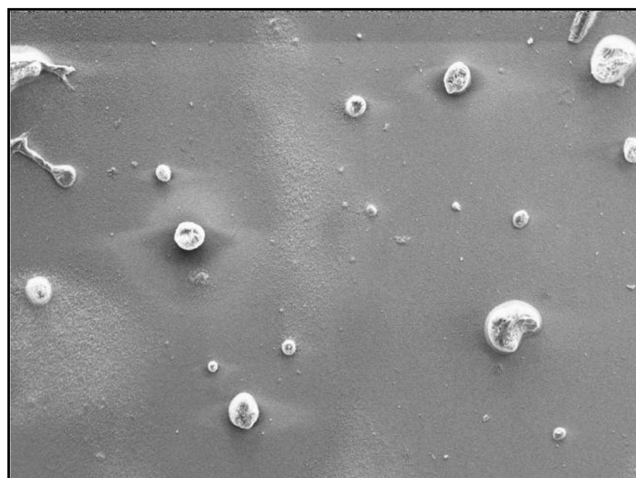

Fig.1. SEM picture of SLN made from Compritol stabilized with Poloxamer 188

The lipospheres developed by Domb are `solid, water-insoluble microparticles that have a layer of a phospholipid embedded on their surface' [11-13]. According to the patent claims, lipospheres comprise a core formed of a hydrophobic material solid at room temperature and a phospholipid coating surrounding the core. The average particle diameter is between 0.3 and 250 mm. The particles are prepared by melting the core material, adding phospholipid along with an aqueous medium and dispersing the melted material at increased temperature by mixing techniques, such as mechanical stirring or sonication. Cooling leads to solid lipospheres. The liposphere is restricted to one stabilizing material, which means the phospholipid layer. For SLN it has been reported that suspensions stabilized only with phospholipid can tend to form semi-solid ointment-like gels [14]. Gel formation can be prevented by adding a co-emulsifier which is not covered by the liposphere patent. The SLN produced by our group are in most cases stabilized by binary or ternary surfactant mixtures providing optimal physical long-term stability.

2.4. Precipitated lipid particles

Solid lipid particles can also be produced by a precipitation method comparable to the production of polymeric nanoparticles by solvent evaporation. In contrast to SLN this method is characterized by the need for solvents. The glyceride is dissolved in an organic solvent (e.g. chloroform) and this solution is emulsified in an aqueous phase. After evaporation of the solvent the lipid precipitates forming nanoparticles [15]. A clear disadvantage is the need to use organic solvents. In addition, other problems arise similar to when scaling up production of polymeric nanoparticles on the basis of solvent evaporation. In contrast, SLN produced by high pressure homogenization have the advantage of avoiding the use of solvents.

3. DRUG INCORPORATION AND LOADING CAPACITY

Many different drugs have been incorporated in SLN. 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. Westesen et al. studied the incorporation of drugs using loading capacities of typically 1-5%, for Ubidecarenone loading capacities of up to 50% were reported [16]. For Tetracaine and etomidate capacities of 10-20% are reported [17], for retinol up to 5% [18, 19], for coenzyme Q_{10} 20% and for cyclosporin 20-25% [20].

Factors determining the loading capacity of drug in the lipid are, for example:

1. solubility of drug in melted lipid;
2. miscibility of drug melt and lipid melt;
3. chemical and physical structure of solid lipid matrix;
4. 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 required because it decreases 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 mono- and di-glycerides in the lipid used as matrix material promotes drug solubilization.

The chemical nature of the lipid is also important because lipids which form highly crystalline particles with a perfect lattice (e.g. monoacid triglycerides) lead to drug expulsion [16]. More complex lipids are being mixtures of mono-, di- and triglycerides and also containing fatty acids of different chain length form less perfect crystals with many imperfections offering space to accommodate the drugs. Chemically polydisperse lipids such those used in cosmetics showed very good drug incorporation capacities.

Crystalline structure of course related to the chemical nature of the lipid is a key factor to decide in determining whether a drug will be expelled or firmly incorporated in the long-term. Therefore, for a controlled optimization of drug incorporation and drug loading, intensive characterization of the physical state of the lipid particles by NMR, X-ray and other new techniques in this area such as ESR is highly essential.

The polymorphic 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 *a*-form, whereas bulk lipids tend to recrystallize preferentially in the β'-modification and transforming rapidly into the β-form [21]. With increasing formation of the more stable modifications the lattice is getting more perfect and the number of imperfections decreases, that means formation of β'/ β_i -modifications promotes drug expulsion. In general the transformation is slower for long-chain than for short-chain triglycerides [22]. An optimal SLN carrier can be produced in a controlled way when a certain fraction of *a*-form can be created and preserved during the storage time. By doing this the normal SLN carrier transforms to an intelligent drug delivery system by having a built-in trigger mechanism to initiate transformation from *a*- to β-forms and consequently controlled drug release [23,24]. Triggering factors for the lipid transformation are, e.g. temperature and water loss of the SLN dispersion, e.g. after topical administration.

4. DRUG RELEASE FROM SLN

As can be seen from Table 1 there are many studies dealing with drug incorporation, however, there are distinctly less data available about drug release [25] especially information

about the release mechanisms. Most of the data about in vitro drug release mechanisms were generated by Mehnert et al. studying the model drugs tetracaine, etomidate and prednisolone [26].

A major problem during the work with lipid nanopellets was the burst release observed with these systems. A similar burst release was obtained when incorporating tetracaine and etomidate into SLN independent on the production method (hot vs. cold homogenization). A prolonged drug release was first obtained when studying the incorporation of prednisolone. This demonstrated the principle suit-ability of the SLN system for prolonged drug release [4]. Even more important it was possible to modify the release profiles as a function of lipid matrix, surfactant concentration and production parameters e.g. temperature. In vitro drug release could be achieved for up to 5-7 weeks. The profiles could be modulated showing prolonged release without any burst at all, but also generating systems with different percentages of burst followed by prolonged release (scheme.1). The burst can be exploited to deliver an initial dose when desired.

It is highly important that it could be shown 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. The profiles obtained in scheme1 could be explained by partitioning effects of the drug between the melted lipid phase and the aqueous surfactant phase during particle production. During particle production by the hot homogenization technique, drug partitions from the liquid oil phase to the aqueous water phase. The amount of drug partitioning to the water phase will increase with the solubility of the drug in the water phase, which means with increasing temperature of the aqueous phase and increasing surfactant concentration. The higher the temperature and surfactant concentration, the greater is the saturation solubility of the drug in the water phase. During the cooling of the produced O/W nanoemulsion the solubility of the drug in the water phase decreases continuously with decreasing temperature of the water phase that means a re-partitioning of the drug into the lipid phase occurs. When reaching the recrystallization temperature of the lipid, a solid lipid core starts forming including the drug which is present at this temperature in this lipid phase. Reducing the temperature of the dispersion further increases the pressure on the drug because of its reduced solubility in water to further re-partition into the lipid phase. The already crystallized core is not accessible anymore for the drug, consequently the drug concentrates in the still liquid outer shell of the SLN and/or on the surface of the particles.

The amount of drug in the outer shell and on the particle surface is released in the form of a burst, the drug incorporated into the particle core is released in a prolonged way. Therefore, the extent of burst release can be controlled via the solubility of the drug in the water phase during production that means via the temperature employed and the surfactant concentration used. Higher temperatures and higher surfactant concentrations increase the burst; production at room temperature avoids partitioning of drug into the water phase and subsequent re-partitioning to the oil phase, thus showing no burst at all. To avoid or minimize the burst, SLN can be produced surfactant free or using surfactants which are not able to solubilize the drug. Extent of burst is a function of production temperature and surfactant concentration. No burst occurs at room temperature and 0% surfactant, the burst increases with increasing temperature and also with increasing surfactant concentration at a given temperature.

Mehnert group [26] published three drug incorporation models in SLN

1. solid solution model;
2. core-shell model, drug-enriched shell;
3. core-shell model, drug-enriched core.

The SLN matrix is a solid solution (i.e. drug molecularly dispersed in the lipid matrix) when the particles are produced by the cold homogenization technique and using no surfactant or no drug-solubilizing surfactant. The core-shell model with a drug-enriched shell will be obtained when performing the production as described in scheme1, which involves re-partitioning of the drug during cooling. 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 (e.g. prednisolone) in the lipid melt at or close to its saturation solubility [26]. Cooling of the nanoemulsion 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 recrystallization of the lipid surrounding the drug core as a membrane (Figure 2).

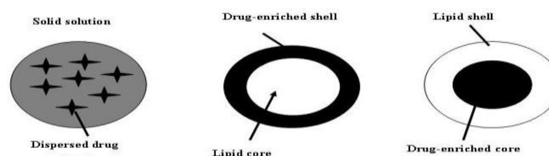

Fig. 2. Three drug incorporation models, (a) solid solution model, (b) core-shell model with drug-enriched shell, (c) core-shell model with drug-enriched core.

5. ANALYTICAL CHARACTERIZATION OF SLN

Characterization of SLN is a serious challenge due to the small size of the particles and the complexity of the system, which includes also dynamic phenomena. Several parameters have to be considered which have direct impact on the stability and release kinetics:

1. particle size and zeta potential;
2. degree of crystallinity and lipid modification;
3. co-existence of additional colloidal structures (micelles, liposomes, supercooled melts, drug-nanoparticles) and dynamic phenomena.

Photon correlation spectroscopy (PCS) and laser diffraction (LD) are the most powerful techniques for routine measurements of particle size. PCS (also known as dynamic light scattering) measures the fluctuation of the intensity of the scattered light which is caused by particle movement. This method covers a size range from a few nanometers to about 3 mm. This means PCS is a good tool to characterize nanoparticles, but it is not able for the detection of larger microparticles. They can be visualized by means of LD measurements. This method is based on the dependency of the diffraction angle on the particle radius. Smaller particles cause more intense scattering at high angles compared to the larger ones. A clear advantage of LD is the coverage of a broad size range from the nanometer to the lower millimeter range. The development of phase-sensitive-intensity-difference-scattering (PIDS) technology greatly enhanced the sensitivity of LD to smaller particles. Electron microscopy provides, in contrast to PCS and LD, direct information on the particle shape. However, the investigator should pay special attention to possible artifacts which may be caused by the sample preparation. For example, solvent removal may cause modification changes which will influence the particle shape [27]. The same cautionary note applies for atomic force microscopy (AFM), because an immobilization of the SLN is required to assess their shape by the very tiny AFM tip.

Rapid progress in the development of field-flow-fractionation (FFF) has been observed during the last years. The separation principle of FFF is based on the different effect of a perpendicular applied field on particles in a laminar flow [28-31]. The separation principle corresponds to the nature of the perpendicular field and may for example be based on different mass (sedimentation-FFF), size (cross-flow-FFF), charge (electric-field-FFF). A combination of different FFF-separation principles may give unique resolution. A certain advantage of FFF over PCS is the high resolution of small particle size differences. Pilot studies with lattices of different size demonstrate that particles with a size difference of 30 nm are well resolved. Furthermore, FFF leads to a separation of the particles which means that the separated particles may be subjected to further characterization [32]. The high dilution of the sample by FFF may cause potential problems because it may disturb the sample characteriztics (e.g. dilution with pure water may cause removal of the surfactant from the particle surface). Current studies investigate the influence of the dilution media on the particle characteristics [33]. Due to the advantages of FFF and the development of commercial FFF-product lines it can be anticipated that FFF will be a key tool for the characterization of colloidal dispersions like SLN in the future.

The measurement of the zeta potential allows predictions about the storage stability of colloidal dispersion [34]. In general, particle aggregation is less likely to occur for charged particles (high zeta potential) due to electric repulsion. However, this rule cannot not strictly applied for systems which contain steric stabilizers, because the adsorption of steric stabilizer will decrease the zeta potential due the shift in the shear plane of the particle.

Degree of crystallinity and lipid modification: Particle size analyzis is a necessary, but not a sufficient step to characterize SLN quality. 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. Thermodynamic stability and lipid packing density increase, and drug incorporation rates decrease in the following order:

Supercooled melt < ά-modification < β-modification < β’-modification

Differential scanning calorimetry (DSC) and X-ray scattering are widely used to investigate the status of the lipid. DSC uses the fact that different lipid modifications possess different melting points and melting enthalpies [35]. By means of X-ray scattering it is possible to assess the length of the long and short spacings of the lipid lattice. It is highly recommended to measure the SLN dispersion themselves because solvent removal will lead to modification changes. Sensitivity problems and long measurement times of conventional X-ray sources might be overcome by synchrotron irradiation [21]. However, this source has limited accessibility for most investigators. Infrared and Raman spectroscopy are useful tools to investigate structural properties of lipids [27]. However, their potential to characterize SLN dispersions remains to be investigated.

The magnetic resonance techniques, NMR and ESR, are powerful tools to investigate dynamic phenomena and the characteriztics of nanocompartments in colloidal lipid dispersions. Due to the non-invasiveness of both methods, repeated measurements of the same sample are possible.

NMR active nuclei of interest are ^{1}H, ^{13}C, ^{19}F and ^{35}P. Due to the different chemical shifts it is possible to attribute the NMR signals to particular molecules or their segments. For example, lipid methyl protons give signals at 0.9 ppm while protons of the polyethyleneglycol chains give signals at 3.7 ppm. Simple ^{1}H-spectroscopy permits an easy and rapid detection of supercooled melts. It permits also the characterization of liquid nanocompartments in recently developed lipid particles, which are made of blends from solid

and liquid lipids [24]. This method is based on the different proton relaxation times in the liquid and semisolid/ solid state. Protons in the liquid state give sharp signals with high signal amplitudes, while semisolid/solid protons give weak and broad NMR signals under these circumstances. The great potential of NMR with its variety of different approaches (solid-state-NMR, determination of self-diffusion coefficients etc.) has scarcely been used in the SLN field, although it will provide unique insights into the structure and dynamics of SLN dispersions.

ESR requires the addition of paramagnetic spin probes to investigate SLN dispersions. A large variety of spin probes is commercially available. The corresponding ESR spectra give information about the microviscosity and micropolarity. ESR permits the direct, repeatable and non-invasive characterization of the distribution of the spin probe between the aqueous and the lipid phase. Experimental results demonstrate that storage induced crystallization of SLN leads to an expulsion of the probe out of the lipid into the aqueous phase [35]. Furthermore, using an ascorbic acid reduction assay it is possible to monitor the time scale of the exchange between the aqueous and the lipid phase. The development of low-frequency ESR permits non-invasive measurements on small mammals. ESR spectroscopy and imaging will give new insights about the fate of SLN in vivo.

6. STERILIZATION OF SLN

Sterilization of SLN is an issue in the case of pulmonary or parenteral administration. For lecithin-stabilized SLN it could be shown that autoclaving is possible [17]. The SLN melt during the autoclaving and recrystallize during the cooling down. However, autoclaving is not possible when a certain structure has been given to the SLN in a controlled way by adjusting the production parameters. This special structure leading to the desired modulated release profile would be lost when the particles melt again during the autoclaving and recrystallize in a non-controlled way.

Autoclaving at 121°C cannot be performed when using sterically stabilizing polymers, e.g. poloxamer series [17-23]. The autoclaving temperature seems to be too close to the critical flocculation temperature (CFT) of the polymers, at least the polymer adsorption layer seems partially to collapse leading to insufficient stabilization and particle aggregation. This can be avoided by reducing the autoclaving temperature (e.g. 121°C to 110°C, but simultaneously prolonging the autoclaving time).

The physical stability during autoclaving cannot be stated in a general manner, it depends very much on the composition of the SLN formulation. Therefore, the above two statements can only be seen as a rough guideline. SLN dispersions can also be sterilized by filtration similar to emulsions for parenteral nutrition. It is highly important to filter them in the liquid state; this allows even particles with a size larger than the pores in the filter to be filtered [17]. This technology is well known from parenteral emulsions and easy to apply to SLN. Alternatively, the SLN can be produced aseptically, again identical to parenteral emulsions.

7. TOXICITY AND STATUS OF EXCIPIENTS

Toxicity and the status of excipients are a major issue for the use of a delivery system. One can have a very neat delivery system, but if there is a necessity to undertake toxicity studies this will be a major obstacle for its introduction into the clinic and the pharmaceutical market [36].

The status of excipients for SLN has to be discussed as a function of the administration routes. Topical and oral administration of SLN is absolutely non-problematic regarding the excipients. For topical SLN, all excipients can be used which are currently employed for the formulation of pharmaceutical and cosmetic ointments and creams. For oral SLN, all the lipids and surfactants used in traditional dosage forms such as tablets, pellets and capsules can be exploited. In addition all compounds of GRAS status or accepted GRAS status can be employed. There is also the option to use lipids and surfactants from the food industry [37]. Of course, use in the food industry does not allow directly its use in pharmaceutical products. However, the toxicity material available for the food area can be used for submission to the pharmaceutical regulatory authorities that means it is a relatively easy case.

The situation is slightly different for parenteral administration. Up to now there are no products on the market containing solid lipid particles for parenteral injection. Therefore, a toxicity study would be necessary. However, one can use glycerides composed of fatty acids which are contained in oils of parenteral fat emulsions. Therefore, no toxic effects are expected from the SLN degradation products [37]. In addition, one has to consider that a toxicity study with the parenteral new product has to be made anyway, that means the lipid itself might contribute very little to the total costs of the study required. To formulate parenteral SLN, surfactants accepted for parenteral administration can be used, that means, e.g. lecithin, Tween 80, Poloxamer 188, PVP, sodium glycocholate, Span 85 etc. For the intravenous route it is recommended to focus on the i.v. accepted surfactants (e.g. lecithin, Tween 80, Poloxamer 188, sodium glycocholate).

The good tolerability of SLN has been confirmed in both in vitro and in vivo studies. In cell cultures SLN were compared with polyester nanoparticles (PLA, PLA/GA). At 0.5% of PLA/GA nanoparticles 100% of the cells died, at 10% SLN in the cell suspension the viability remained at around 80% [36].

Good tolerability was also found when performing bolus injections into mice. The administered dose was 1.33 g lipid/kg body weight, 6 bolus injections were performed. There was no acute toxicity, for cetyl palmitate, no increase in liver and spleen weight was observed. Histopathology was also performed giving no critical evidence, for details compare [37]. SLN were also injected intravenously in studies performed by other research groups [38].

8. SLN FOR TOPICAL APPLICATION

An area of big potential for SLN and with a short time-to-market are topical products based on the SLN technology, that means pharmaceutical but also cosmetic formulations. SLN are considered as being the next generation of delivery system after liposomes [39]. Similar to liposomes they are composed of well tolerated excipients and due to their small particle size they possess similar adhesive properties leading to film formation on the skin. Distinct advantages of SLN are their solid state of the particle matrix, the ability to protect chemically labile ingredients against chemical decomposition and the possibility to modulate drug release.

Apart from technological benefits the solid state of SLN has also an advantage with regard to product registration for pharmaceuticals but also cosmetics. For example, in Japan even for cosmetic products it needs to be proven that liposomes are present not only qualitatively but also quantitatively. For liposomes a qualitative proof is easy by electron microscopy; however it is extremely difficult to quantify them and to show that they are still present in a sufficient amount (e.g. 90%) during the storage of the product. This is a major obstacle to the introduction of liposomal cosmetic products to the potentially lucrative Japanese market. In contrast to this, quantitative analysis of SLN in creams is very simple and straightforward. Many cream bases do not exhibit a melting peak below 100°C that means the content of SLN in a cream can be quantified by their melting peak determined by DSC [35]. The stability during storage can easily be monitored just by looking at the change in melting enthalpy. Analysis is even possible in cases where a cream contains a fraction which melts below100°C. It is no problem if the peaks are separated. If there is an overlapping, one can determine the total melting energy as a function of time. This special property of SLN opens new markets for topical products containing colloidal carriers for active ingredients.

A completely new, recently discovered area of application is the use of SLN in sun-protective creams. Due to the reduction of the protective ozone layer there is a steep increase in skin cancer; melanoma is the form of cancer showing the strongest increase world-wide, especially in countries like Australia [40]. Side effects of molecular sunscreens (UV blockers) are penetration into the skin and consequently irritation. Particulate sunscreens like titanium dioxide were also found to possibly penetrate into the skin. This can be avoided or minimized by entrapping molecular and particulate sunscreens into the SLN matrix. Surprisingly it was found that the SLN themselves have also a sun-protective effect. Due to their particulate character they are protective due to scattering of UV light (similar to titanium dioxide). In addition it was found that molecular sunscreens and SLN in combination show a synergistic effect. Molecular sunscreens are much more effective after incorporation into SLN and at the same time side effects are reduced. This opens the perspective to a new class of sun-protective creams.

9. SLN FOR ORAL ADMINISTRATION

Oral administration of SLN is possible as aqueous dispersion or alternatively after transform into a traditional dosage form, i.e. tablets, pellets, capsules or powders in sachets [41]. For the production of tablets the aqueous SLN dispersion can be used instead of a granulation fluid in the granulation process. Alternatively SLN can be transferred to a powder (e.g. by spray drying) and added to the tabletting powder mixture. For the production of pellets the SLN dispersion can be used as wetting agent in the extrusion process [42]. SLN powders can be used for the filling of hard gelatine capsules, alternatively, the SLN can be produced directly in liquid PEG 600 and filled into soft gelatine capsules. Sachets are also possible using spray-dried or lyophilized powders. In both cases it is beneficial to have a higher solid content to avoid the necessity of having to remove too much water. For cost reasons spray-drying might be the preferred method for transferring SLN dispersions into powders [43].

10. PULMONARY ADMINISTRATION OF SLN

SLN can be delivered as aqueous formulation or dry powder as inhalers. SLN could be spray-dried using, e.g. lactose as excipient in the spray-drying process. Basic advantages of drug release from SLN in the lung are control of the release profile, achievement of a prolonged release and having a faster degradation compared to particles made from some polymeric materials. In addition, SLN proved to possess a high tolerability, one might also consider drug targeting to lung macrophages. Particles in the lung are easily accessed by lung macrophages that means one could use the SLN system for treating infections of the MPS system. In particular parasites such as mycobacteria are difficult to reach with a normal treatment. Within the MPS macrophages, liver and spleen macrophages are more accessible than the parasites in the lung macrophages.

11. SLN FOR PARENTERAL ADMINISTRATION

Basically SLN can be used for all parenteral applications suitable for polymeric nanoparticles. This ranges

from intra-articular to intravenous administration. Studies using intravenously administered SLN have been performed by various groups [25]. Gasco et al. produced stealth and non-stealth solid lipid nanoparticles and studied them in cultures of macrophages [44] and also after loading them with Paclitaxel in vivo. The i.v. administered SLN led to higher and prolonged plasma levels of Paclitaxel. Interestingly both non-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 brain [38]. This study demonstrates nicely the potential of SLN to achieve prolonged drug plasma levels. The observed 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, an effect described previously by Kreuter [45].

12. SLN AS POTENTIAL NEW ADJUVANT FOR VACCINES

Adjuvants are used in vaccination to enhance the immune response. The more safer new subunit vaccines are less effective in immunization and therefore effective adjuvants are required [46]. Increase the amount of antigen delivered is not a solution because this also increases the costs. Especially with regard to the third world such a solution prohibits the desired broad vaccinations in these countries. The side effects of Freund's complete adjuvant (FCA) and Freund's incomplete adjuvant (FIA) are too strong to be employed, but Freund's complete adjuvant is still considered as a `gold standard' when developing new adjuvants [47]. The adjuvant frequently used for many years consist of aluminium hydro-xide particles, however they can also exhibit side effects. New developments in the adjuvant area are emulsion systems, for example SAF 1 and MF 59 [46]. They are oil-in-water emulsions that degrade rapidly in the body. Being in the solid state, the lipid components of SLN will be degraded more slowly providing a longer lasting exposure to the immune system. Degradation can be slowed down even more when using sterically stabilizing surfac-tants that hinder the anchoring of enzyme complexes. In a first study SLN have been tested as adjuvant in comparison to FIA in sheep. The two unoptimized SLN formulations exhibited 43 and 73% of the immune response (antibody titer) of FIA investigated as standard [46]. These data are promising and currently the SLN are being optimized regarding their surface properties to give a maximum immune response. Advantages compared to traditional adjuvants are the biodegradation of SLN and their good tolerability by the body.

13. CLINICAL BATCH PRODUCTION AND LARGE SCALE PRODUCTION OF SLN

An important step towards a pharmaceutical product are first human trials, a prerequisite for this is the availability of a GMP production unit to provide first clinical batches. A GMP production unit was developed to produce clinical batches between 2 kg up to a maximum of 10 kg SLN dispersion. Such a unit exists at the company Pharmatec (Milan, Italy) and also at SkyePharma (Muttenz/Basel, Switzerland).

For topical products, i.e. creams containing SLN, a batch size of approximately 50 kg to a few hundred kg is required. For this batch size a production line was developed having a capacity of 50 kg SLN dispersion/h. It consists of two homogenizers being placed in series, that means instead of running a dispersion twice through one homogenizer (two homogenization cycles), the product is run continuously through two homogenizers placed in series (APV LAB60, Gaulin 5.5). Such solutions are possible because it is low-cost equipment from the shelf. The size of the batch is given by the size of the feeding vessel and product container, respectively.

Meanwhile a production line has been designed running on a continuous basis and having a capacity of 150 kg/h. The melted lipid and the hot aqueous surfactant solution are mixed by static blenders instead of mixing them batch wise in a large feeding container. A basic advantage of the homogenizers employed is their ability to be cleaned in place (CIP) and sterilized in place (SIP). The homogenizers can be sterilized by streaming steam; the product containers (e.g. employed for cosmetic batches) can be autoclaved.

14. PERSPECTIVES OF THE DELIVERY SYSTEM SLN

During the last 15 years the number of research groups working with SLN has distinctly increased as well as the number of publications in this area. It reflects that more and more scientists in academia have realized the potential of the SLN system and started to develop it. Research groups are placed all over the world in countries like Germany, Canada, China, but also in India.

15. CONCLUSION

There is no break through for a delivery system if only academic research groups are developing it. Success can only be possible if also pharmaceutical industry takes up developments. To guarantee a broad application of a carrier system it is highly desirable that companies specialized in drug delivery systems engage them in the new technology. Drug delivery companies develop pharmaceutical solutions adapted to the needs of many different pharmaceutical companies, that means the technology will spread to many companies and not only be localized inside

one company using this new technology just limited to their own drugs. In 1999, the complete patent rights for production of SLN by high pressure homogenization have been acquired by SkyePharma AG (Muttenz, Switzerland), a drug delivery company specialized in oral delivery, but also having the potential for parenteral production. The company Vectorpharma (Trieste, Italy) is developing SLN produced by microemulsion technology. That means the SLN system has successfully found its way to pharmaceutical industry, a prerequisite for the introduction of new SLN-based formulations into clinic and the pharmaceutical market.

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