REVIEW

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Preparation, characterization and physico-chemical properties of Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC): Their benefits as colloidal drug carrier systems

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Solid lipid nanoparticles (SLN) have attracted increasing attention by various research groups and companies since the early 1990s. Their advantages over existing traditional carriers have been clearly documented. In addition, modified SLN have been described which are nanostructured lipid carriers (NLC) composed of liquid lipid blended with a solid lipid to form a nanostructured solid particle matrix. NLC combine controlled release characteristics with some advantages over SLN. This paper reviews the production techniques, characterization and physical stability of these systems including destabilizing factors and principles of drug loading, then considers aspects and benefits of SLN and NLC as colloidal drug carriers.

1. Introduction

Solid lipid nanoparticles (SLN) constitute an attractive colloidal drug carrier system. They are an alternative system to emulsions, liposomes, microparticles and nanoparticles based on synthetic polymers for various application routes because of their numerous advantages (Cavalli et al. 2002; Gualbert et al. 2003; Illing and Unruh 2004; Müller et al. 1995). Problems associated with the use of other systems include:

- Possible cytotoxic effects after phagocytosis of the polymeric material by macrophages (Smith and Hunneyball 1986) and human granulocytes (Müller et al. 1996b),
- Possible impairment of the reticuloendothelial system due to relatively slow degradation of the polymeric material (up to 4 weeks) (Ogawa 1988; Schwarz et al. 1994),
- Residual contamination from the production process, for example by organic solvents, polymerization initiators, large polymer aggregates, toxic monomers and toxic degradation products (Kante et al. 1982; Limayem et al. 2004),
- Limited physical stability and drug leakage during storage (Diederichs and Müller 1994; Lasic 1998)
- Lack of a method for production on a large industrial scale (e.g. no satisfactory and accepted scaling up method exists for polymeric nanocapsules) (Gohla and Dingler 2001),
- Expensive production methods and products unacceptable for registration by the regulatory authorities due to quality problems (Müller et al. 2000),
- Stability problems associated with autoclaving. Sterilization needs to be performed by gamma irradiation possibly leading to the formation of radicals and subsequently toxic reaction products (Schwarz et al. 1994).

On the other hand, there are many reports about the successful incorporation of active compounds into lipid matrix carriers and their related benefits. Speiser described microparticles produced by spray congealing in the area of solid lipid particles in the 1980s (Kecht-Wyrsch 1987), and further focused on reducing the particle size. Lipid nanopellets produced by high-speed stirring or ultrasonic treatment for oral delivery were later reported by the same working group (Speiser 1990). Lipid nanoparticles have also been studied by employing a process to similar Speiser's (Bergelson and Domb 1997) and a microemulsion technique (Cavalli et al. 1995; Morel et al. 1996). A high pressure homogenization technique was introduced as a different approach to the production of lipid nanoparticles (Müller et al. 1993). The superiority of this technique over others has been reported in the literature several times. Physicochemical characterization, physical and chemical short-term and long-term stability, drug incorporation and release patterns of drugs from SLN have been extensively studied. The trade name 'SLN^{TM}' has been registered world-wide in the pharmaceutical area, as has 'Lipopearls^{TM}' for cosmetics. Subsequently, a SLN modified by incorporation of liquid lipid into the solid structure has been proposed as NLC to overcome some limitations related to SLN, although SLN have many superiorities over the traditional carrier systems (Müller et al. 2002a, 2002b). The benefits of SLN and NLC as colloidal drug carriers are still being demonstrated and new approaches are introduced.

This review describes the characterization of SLN and NLC including their production methods, physicochemical properties, short-term and long-term stability including factors affecting it, drug loading, drug release pattern, relationship between drug release and drug loading mechanism, and their application routes. It highlights the potential perspectives for drug delivery using SLN and NLC.

2. Solid Lipid Nanoparticles (SLN)

The liquid lipid used in emulsions is replaced by a solid lipid including high-melting point glycerides or waxes to produce SLN for pharmaceuticals and cosmetics. The aims of SLN production include:

- 80–1000 nm size range with low content of microparticles (> 5 μm as limiting factor for i.v. injectability) (Müller et al. 1995; Zur Mühlen 1996),
- Controlled release of incorporated drugs over several weeks (Zur Mühlen and Mehnert 1998),
- Modification of dissolution rate of incorporated drugs (Demirel et al. 2001), and thus enhancement of bioavailability in oral administration (Yang et al. 1999b) or parenteral administration (Lim and Kim 2002),
- Enhancement of bioavailability of entrapped drugs via improvement of tissue distribution (Bargoni et al. 2001; Cavalli et al. 2000),
- Targeting of drugs (Yang et al. 1999a),
- Chemical protection of labile incorporated compounds (Jenning and Gohla 2000; Lim et al. 2004; Üner et al. 2005a).

Advantages of SLN:

- SLN are 10–100 fold less cytotoxic than their polymeric counterparts (Müller and Olbrich 1999; Müller et al. 1996a, 1996b).
- Most of the lipids and surfactants used in SLN and NLC production have approved status, e.g. GRAS status (Generally Recognized As Save) due to their low toxicity or they are already used as excipients in cosmetics or pharmaceuticals (Code of Federal Regulations, Food and Drugs 2001).
- SLN formulations stable for three years can be developed. This is of paramount importance with respect to colloidal drug carriers (Freitas and Müller 1998b, 1999).
- Excellent reproducibility with a cost-effective high pressure homogenization method has been reported (Gohla and Dingler 2001; Tabatt et al. 2004).
- Production by high pressure homogenization is suitable for scaling-up (Gohla and Dingler 2001; Tabatt et al. 2004).
- SLN have a wide potential application spectrum such as intravenous, dermal, per oral, topical.

3. Nanostructured Lipid Carriers (NLC)

In spite of all the advantages of SLN, some limitations have been reported such as limited drug loading capacity depending on the solubility of the drug in the solid lipid, drug loss during storage due to lipid crystallization to the stable β-modification and relatively high water content (70–99.9%) (Müller et al. 2002a, 2002b). At the end of 1990s, NLC with a special structure were developed for better drug accommodation in order to increase the payload and prevent drug expulsion. Three types of NLC have been described: (I) imperfect type, (II) amorphous type and (III) multiple type (Fig. 1).

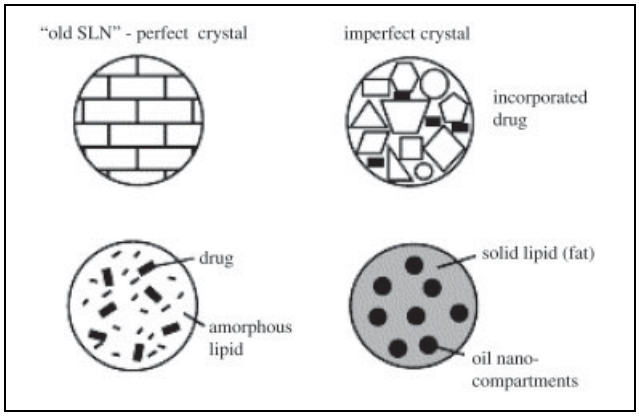


Fig. 1: The three types of NLC compared to the relatively ordered matrix of old SLN (upper left), NLC types: imperfect type (upper right), amorphous type (lower left), multiple type (lower right) (reprinted from Ref. Müller et al. 2002a with permission from Elsevier, 2005)

(I) Imperfect type NLC (imperfectly structured solid matrix): Imperfections in the crystal order are provided by mixing spatially different lipids, e.g. glycerides composed of different fatty acids are mixed. Thus, large distances between fatty acid chains can be increased by using glycerides composed of very different fatty acids. The matrix contains imperfections to accommodate the drug in molecular form and amorphous clusters (Fig. 1, upper right). Mixing small amounts of chemically very different liquid lipids (oils) with solid lipids in order to achieve highest incompatibility leads the highest drug payload.

(II) Amorphous type (structureless solid amorphous matrix): This kind of NLC can be achieved by mixing solid lipids with special lipids, e.g. hydroxyoctacosanylhydroxystearate, isopropylmyristate or medium chain triglycerides such as Miglyol® 812. Therefore, drug expulsion caused by the process of crystallization to β forms during storage is prevented by the special structure of the lipid matrix since NLC are solids in an amorphous but not crystalline state (Fig. 1, lower left) (Radtke and Müller 2001).

(III) Multiple type (multiple oil in fat in water (O/F/W) carrier): The solubility of the drug in the lipophilic phase decreases during the cooling process after homogenization and the crystallization process during storage. Continuously reducing drug solubility leads to drug expulsion from the lipid nanoparticles especially when the drug concentration in the formulation is too high. Solubility of many drugs in a liquid lipid is higher than in a solid lipid. It has been reported that in case of lipids which lack appropriate drug solubilities, addition of a higher amount of liquid lipid to the lipophilic phase combined the advantages of the solid matrix which prevented drug leakage, and of the liquid regions (oily nanocompartments) which showed comparatively high solubility for lipophilic drugs (Fig. 1, lower right) (Jenning et al. 2000d).

A reduction and retardation of lipid recrystallization in the particles is observed compared to SLN, because the crystal order in NLC is greatly disturbed due to the oil inside the particle remaining in a liquid state (Jenning et al. 2000d). Liquid lipid can be loaded in the solid matrix up to 75%. For example, incorporation of up to 38% Miglyol® 812 (caprylic/capric triglycerides) as liquid lipid for cetylpalmitate has been described (Jenning et al. 2000d) and then up to 75% for Compritol 888 ATO (glyceryl behenate) (wt.% related to lipid phase) (Jores et al. 2004).

All the types of NLC allow the production of highly concentrated particle dispersions (>30–95%) and increased drug contents (Müller et al. 2002a, 2002b).

NLC have so far been studied for topical use, but they offer all the advantages and production aims of SLN. Therefore, they present a promising colloidal drug carrier system for various application routes like SLN.

4. Production methods of lipid nanoparticles

4.1. High pressure homogenization

High pressure homogenization is performed by two basic production techniques for lipid nanoparticles: homogenization of a molten lipid phase at elevated temperature (hot homogenization technique) and homogenization of a solid lipid suspension in an aqueous phase at room temperature or below (cold homogenization technique). For both techniques, the drug is dissolved or solubilized in molten lipid at approximately 5–10 °C above its melting point.

Hot homogenization technique: The drug is dissolved in melted lipid. The drug-containing lipid melt is dispersed in the molten state in a hot aqueous surfactant solution. Dispersion is performed using a high-speed stirrer (e.g. Ultra Turrax). The coarse pre-emulsion obtained is then homogenized using a high pressure homogenizer (e.g. APV Gaulin LAB 40) at a pressure ranging from 100 to 1500 bar. Typically one to three homogenization cycles are sufficient. Cooling the oil-in-water nanoemulsion obtained to room temperature or below leads to lipid crystallization and formation of lipid nanoparticles. This technique can be successfully applied to lipophilic and insoluble drugs, but it is not entirely suitable for hydrophilic drugs. During homogenization, the hydrophilic drug partitions to the aqueous phase resulting in low entrapment efficiency.

Alternatively, the homogenization can be performed at temperatures slightly below the melting point of the lipid (e.g. 5 to 10 °C below) which seems to lead to a softening of the lipid during the homogenization process for hydrophilic drugs. The homogenization temperature needs to be carefully selected because the loss of hydrophilic drugs to the water phase can be too high (Müller and Runge 1998).

Cold homogenization technique: This technique is much more suitable for hydrophilic drugs. The drug is incorporated in the melted lipid. If the solubility of the hydrophilic drug in the lipid is too low, surfactants can be used for solubilization of the drug. The drug-containing lipid melt is solidified in dry ice or liquid nitrogen to increase the brittleness of the lipid and to ease the milling process. After milling (e.g. in a mortar mill), the microparticles obtained (approx. 50 to 100 μm) are dispersed in a cold aqueous surfactant solution. This lipid suspension is homogenized at room temperature or below (0 °C). The solid state of the matrix minimizes partitioning of the drug to the water phase. Many heat-sensitive drugs can be incorporated into lipid nanoparticles by this technique since the thermal exposure of the drug is relatively short (Müller et al. 1995; Müller and Runge 1998).

Most lipid nanoparticles produced by hot homogenization are characterized by an average particle size below 500 nm and low microparticle content. In general, larger particle size and broader size distribution are observed in SLN produced by cold homogenization compared to hot homogenization (Müller and Runge 1998).

In general, the advantages of the high pressure homogenization technique over others are narrow particle size distribution of the product with a low content of microparticles, higher lipid particle content in the dispersions, avoidance of organic solvents, acceptability of the homogenization equipment by the regulatory authorities (even for parenteral products), scale-up feasibility and the availability of homogenization production lines in industry. Depending on the size of production-scale homogenizers, a wide production range (500–60000 l/h) is possible (Jahnke 1998).

4.2. Preparation via o/w microemulsion

Production of lipid nanoparticles via microemulsions is based on precipitation of fine lipid droplets by breaking of the microemulsion. This method has been developed and introduced by Gasco. Formation of the lipid nanoparticles occurs by dispersing the o/w warm microemulsion in an cold aqueous medium under mechanical stirring (Cavalli et al. 1997; Gasco 1993). The lipid is melted at a certain temperature. An aqueous phase containing the co-surfactant(s) and surfactant heated to the same temperature is added to the lipid melt under stirring. This warm microemulsion is then dispersed in cold water (2–3 °C) under mechanical stirring, thus maintaining a small particle size due to precipitation. Typical volume ratios of the hot microemulsion to cold water are in the range of 1 : 25 to 1 : 50 (Gasco 1997). Low melting fatty acids (50–70 °C) (e.g. stearic acid, Imwitor 900) are more suitable as the solid lipid for this technique (Cavalli et al. 1999; Igartua et al. 2002).

Disadvantages of this method are relatively high water content, difficulties in removal of excess water and use of surfactants and co-surfactants at high concentrations.

4.3. Preparation by solvent emulsification-evaporation or -diffusion

In the method of solvent evaporation by precipitation in o/w emulsions, lipid dissolved in an organic solvent is emulsified in a water bath under mechanical stirring in an aqueous phase containing surfactant. Stirring of the resulting oil-in-water emulsion is maintained under ambient conditions to allow evaporation of the solvent. The solvent evaporation process can be performed under reduced pressure. Upon evaporation of the solvent, a lipid nanoparticle dispersion is formed by precipitation of the lipid material in the aqueous medium (Cortesi et al. 2002; Sjostrom and Bergenståhl 1992; Shahgaldian et al. 2003a). Depending on the solid lipid and surfactant used, particles with average diameters of 30–100 nm can be obtained. A particle size of around 30 nm could be obtained for cholesteryl acetate nanoparticles stabilized with a blend of phosphatidylcholine and sodium glycocholate according to photon correlation spectroscopy (PCS) number distribution (Siekman and Westesen 1996). Avoidance of heat during the preparation is the most important advantage of this method.

Solvent emulsification-diffusion in an aqueous system is based on emulsion solvent diffusion in water similar to evaporation by precipitation in o/w emulsions. Lipid is dissolved in the organic phase in a water bath since it could not be completely dissolved in the organic phase at room temperature. Addition of water to the resultant organic solution under mechanical agitation results in coacervation and formation of lipid nanoparticles. The lipid nanoparticle dispersion obtained is then separated by centrifugation, by evaporation of the solvent under reduced pressure or by cross-flow filtration (Hu et al. 2002; Quintanar-Guerrero et al. 2005)

Alternatively, injection moulding can be employed for lipid nanoparticle production. After the lipid is dissolved in

a water-miscible solvent and heated to its melting point, it is rapidly injected through an injection needle into a stirred aqueous phase with or without surfactant. The resulting dispersion is then filtered through a paper filter in order to remove any excess lipid. Typical volume ratios of the o/w emulsion to water in order to precipitate lipid nanoparticles are in the range of 1 : 5 to 1 : 10 (Schubert and Müller-Goyman 2003; Speiser 1998).

In the solvent emulsification-evaporation or -diffusion methods, the suspensions obtained are fairly dilute due to the limited solubility of the lipids in the organic solvents used. The particle concentration (up to 15%) obtained using this technique is lower than by high pressure homogenization. Particle dispersions with a solid content up to approximately 80% can be produced by high pressure homogenization. Additionally, toxicological problems may arise from solvent residues from the product obtained by this technique (Trotta et al. 2003).

4.4. Water-in-oil-in-water double emulsion (w/o/w) method

This method is based on the solubilization of the drug to be entrapped in the internal phase of a w/o/w double emulsion, along with a stabilizer able to prevent loss of drug to the external phase during solvent evaporation. An aqueous drug solution containing stabilizers is emulsified in a lipid melt by a high speed stirrer (e.g. Ultra Turrax) at an elevated temperature. The warm w/o nanoemulsion is then dispersed in the aqueous phase containing a stabilizer as the external phase of a w/o/w emulsion at 2–3 °C under mechanical stirring to obtain lipid nanoparticles. The lipid nanoparticles are then purified by diafiltration (Cortesi et al. 2002; Morel et al. 1998).

4.5. High shear homogenization and/or ultrasonication

High shear homogenization and ultrasonication are dispersing techniques which do not involve organic solvents, large amount of surfactants or additives. These techniques are easy and require familiar tools which are available in almost every laboratory. Melted lipid is added and dispersed in an aqueous surfactant solution under high shear homogenization or ultrasonication. Then the emulsion is cooled down to room temperature. While a homogenizer such as a rotor-stator homogenizer is required for high shear homogenization (Kržič et al. 2001), ultrasonication can be performed using a probe (Mei et al. 2003).

Low dispersion quality is a disadvantage of high shear homogenization and ultrasonication. Dispersion quality of the lipid nanoparticles produced by these techniques is often affected by the presence of microparticles leading to physical instability upon storage. Lipid concentration is low (<1%) and the surfactant concentration is comparatively high (Wissing et al. 2004). Metal contamination is the other important problem with ultrasonication.

5. Physical stability

5.1. Particle size

Particle size measurements are a good indicator of instability and are used to characterize the product. The mean size and the size distribution are often one of the most important quality response parameters that determine other macroscopic properties of the material and thus characterize it. Well-formulated systems should display a narrow particle size distribution in the submicron size range such as liposomes, nanospheres and nanoparticles according to the definition of colloidal particles as having a size below 1 μm (Benoit et al. 1986; Heurtault et al. 2003). Particles greater than 1 μm and an increase in their number over time can be indicators of physical instability.

The particle size of lipid nanoparticles is affected by various parameters such as the composition of the formulation (such as surfactant/surfactant mixture, structural properties of the lipid and drug incorporated), production methods and conditions (such as time, temperature, pressure, cycle number, equipment, sterilization and lyophilization). These parameters are modified to achieve the highest product quality by evaluating the data obtained from the particle size measurements. Therefore, particle growth should be followed over time during storage, especially for parenteral administration. A size > 5 μm is regarded as the limit for i.v. injectability by the regulatory authorities (Müller et al. 1995).

The concentration of the surfactant/surfactant mixture strongly affects the particle size of lipid nanoparticles. In general, smaller particle sizes are observed when a higher surfactant/lipid ratio is chosen (Bunjes et al. 1996, 2003). Decreasing surfactant concentrations result in an increase in particle size during storage (Lippacher et al. 2002).

Large particle size is obtained at lower processing temperature. The hot homogenization technique gives a smaller particle size, generally below 500 nm, and a narrower particle size distribution compared with cold homogenization. Additionally, when the homogenization is performed below the melting point of the lipid to give a softening of the lipid during the homogenization process with hydrophilic drugs, the softened lipid can be more easily dispersed leading to a more uniform product of smaller particle size compared to cold homogenization (Müller and Runge 1998).

Homogenization conditions have a critical effect on particle size distribution. Homogenization efficiency increases with higher homogenization pressure. Mean particle size as well as polydispersity index (PI) values are reported to be reduced at increasing homogenization pressure up to 1500 bar and number of cycles (3–7 cycles). The required particle size distribution and mean particle size can be obtained by a combination of homogenization pressure and number of homogenization cycles (Patravale and Ambarkhane 2003; Schwarz et al. 1994). Low processing temperature is reported to lead to an increase in particle size resulting in high viscosity of the tristearin melt (Bunjes et al. 1996).

Spray-drying and lyophilization may be required to achieve long-term stability of a product containing hydrolyzable drugs or a suitable product for per oral administration. However, spray-drying as an alternative method to lyophilization can destabilize the system due to the elevated temperature and shear forces. Both increase the kinetic energy, leading to frequent particle collisions (Freitas and Müller 1998a). Additionally, partial melting of the lipid phase during spraying is one of the major reasons for particle growth.

In case of freeze-drying of the product, all the lipid matrices used form larger SLN with a wider size distribution after freeze-drying due to the presence of aggregates between nanoparticles. The conditions of the freeze-drying process and removal of the water probably promote aggregation among SLN. An adequate amount of the most suitable cryoprotectant can protect the SLN during the freeze-drying process (Cavalli et al. 1997; Schwarz and Mehnert 1997; Shahgaldian et al. 2003b; Zimmermann et al. 2000).

Sterilization of the nanoparticles is desirable for parenteral administration and autoclaving is applicable to formulations containing heat resistant drugs. Effects of sterilization on particle size have been investigated, and it was found to cause a distinct increase in particle size of trilaurin nanoparticles stabilized with Poloxamer 188, because the Poloxamer layer could not provide sufficient steric stabilization against coalescence. In contrast to Poloxamer, Lipoid S75 – stabilized systems showed only a minor increase in particle size after autoclaving (Schwarz et al. 1994).
Influence of entrapped drug is also important. Higher drug concentrations tend to increase the particle size of lipid nanoparticles, while lower drug concentrations up to 1% do not lead to change in particle size according to both PCS and laser diffractometry (LD) measurements (Dingler et al. 1999).

5.2. Particle charge and zeta potential

Measurement of zeta potential (ZP) allows predictions to be made about the storage stability of colloidal dispersions. In general, particle aggregation is less likely to occur with charged particles (high zeta potential) due to electrical repulsion. It has been reported that reduction of ZP agreed well with the reduction in physical stability. In general, a ZP greater than −60 mV was required for excellent, and one greater than −30 mV for good electrostatic stabilization, thus indicating good physical stability (Freitas and Müller 1998b; Riddick 1968). However, this rule cannot be applied strictly for systems which contain steric stabilizers, because the adsorption of steric stabilizers will decrease ZP due to the shift in the shear plane of the particle. Müller (1996) discussed the relationship between ZP and surfactants in detail.
The ZP decreases with increasing energy input (light and temperature). Higher temperatures and light increase the kinetic energy of a system. This leads to SLN aggregation and gelation in combination with a reduced ZP. This energy input can lead to changes in the crystalline structure of the lipid (Freitas and Müller 1998b; Siekmann 1994). Crystalline re-orientation can result in changes in the charges on the particle surface (Nernst potential) and hence the measured ZP. In addition, different sides of a crystal can possess a different charge density (e.g. aluminium silicates like Bentone™). During one-dimensional growth of a crystal (e.g. formation of long β crystals (Sato 1988)), the surface ratio of differently charged crystal sides changes and consequently the measured ZP also changes. Reduction in ZP and hence in electrostatic repulsion facilitates aggregation of lipid crystals to build up the network (Freitas and Müller 1998b). ZP of SLN dispersions has been reported to decrease after autoclaving, particularly for nanoparticles composed of fatty acids (Cavalli et al. 1997).

5.3. Crystallization

Polymorphic transition and crystallization temperature of the lipid are important parameters. Special attention must be paid to characterize lipid crystallinity degree and modification of the lipid since they help to determine product quality. The emulsified dispersion must be cooled down below the critical crystallization temperature of the lipid to crystallize the nanoparticles during production. If this critical temperature is not reached, the particles remain in the liquid state and an emulsion of supercooled, liquid particles is obtained rather than the desired product. Polymorphic transitions are important during the formation of solid nanoparticles. Transition to a more stable lipid polymorph is accompanied by a rearrangement of the lipid molecules and an increase in lattice density (Bunjes et al. 1996). In a less ordered crystal or amorphous state, melting the substance requires less energy than with the perfect crystalline substance to overcome lattice forces. Therefore, higher melting enthalpy values would suggest a higher ordered lattice arrangement. The lipid within the lipid nanoparticle should be in a less ordered arrangement compared to the bulk materials (Hou et al. 2003).
Although the lipid nanoparticles were produced from crystalline raw materials, crystallization behaviour is affected by some factors. The production method, the presence of surfactant, melting point of the lipid, lipid concentration and the high dispersity as well as the small particle size of the resulting systems may account for changes in crystallization behaviour, degree of crystallinity and crystal modifications of the matrix constituents compared to the bulk materials (Westesen et al. 1993).
The type of homogenization affects the polymorphic transition of the system. The lipid is guaranteed to be in the solid state when the cold homogenization technique is employed, because the lipid melt is solidified in liquid nitrogen and homogenization is performed at room temperature or below (Almeida et al. 1997).
The type of surfactant/surfactant mixture is critical for the kinetics of polymorphic transition of lipids and the crystallization temperature of the dispersed phase. The stabilizers not only influence the colloidal state of the dispersion (e.g., with respect to particle size and stability), but may also have pronounced effects on the internal structure of the particles, which is also an important parameter for the development of drug carriers based on lipid nanosuspensions (Bunjes et al. 2003).
The high surface-to-volume ratio of lipid nanoparticles affects the recrystallization behaviour (Siekmann and Westesen 1992). In general, distortion of crystalline structures due to small particle size induces depression of the melting point and recrystallization point of the SLN, which can prevent recrystallization at room temperature. This may lead to liquid, amorphous or only partially crystallized metastable systems (Eldem et al. 1991; Siekmann and Westesen 1994; Westesen et al. 1993).
Recrystallization behaviour is strongly affected by the melting point of the lipid. For instance, Witepsol® E85 and Softisan® 142 which have a melting point of 42–44 °C are amorphous at room temperature (i.e. solid or lipid) or partially recrystallized (Siekmann and Westesen 1992; Üner et al. 2005a).
Lipid concentration is also important in polymorphic transition. A low lipid concentration such as 2.5% w/w has been reported to disturb the formation of crystals (Müller et al. 1995).
Incorporation of drug has been reported to prevent formation of unstable modifications. Thus drug incorporation can accelerate the transformation to the stable polymorph (Yoshino et al. 1982; Üner et al. 2004, 2005a; Zur Mühlen et al. 1998). Transition from a metastable to a stable form might occur slowly on storage given a small particle size and the presence of surfactant. In some cases, this may lead to drug expulsion from solid lipid nanoparticles during lipid modification on storage (Bunjes and Westesen 1995; Venkateswarlu and Manjunath 2004).
Crystallinity and modification of the lipid are strongly correlated with drug incorporation and release rates (Venkateswarlu and Manjunath 2004). The crystal order of the lipid and differences in supramolecular structures of the

polymorphs influence the loading capacity of nanoparticles. A less ordered arrangement of the lipid crystals appears to favour increasing the drug loading capacity (Bunjes et al. 1996; Hou et al. 2003). The type of crystal polymorph obtained and the kinetics of the transitions may thus have important consequences for dispersion stability as well as drug loading (Bunjes et al. 1996).
Thermodynamic stability increases, while drug incorporation rate decreases as seen in the polymorph order (Scheme, Mehnert and Mäder 2001).
Differential scanning calorimetry (DSC), X-ray diffraction (X-ray scattering) and high-resolution proton nuclear magnetic resonance (^{1}H NMR) are widely used to investigate the state and thermodynamic behaviour of the lipid.
DSC gives information about the melting and crystallization behaviour of crystalline materials like lipid nanoparticles. The breakdown or fusion of the crystal lattice by heating or cooling the sample yields information about internal polymorphism, crystal ordering, eutectic mixtures or glass transition processes (Ford and Timmins 1989). DSC is useful to understand solid dispersions like solid solutions, simple eutectic mixtures, drug-lipid interactions and the mixing behaviour of solid lipids and liquid lipids (Jenning et al. 2000b). DSC gives information on the melting and crystallization behaviour of the solid and liquid constituents of the particles. DSC uses the fact that different lipid modifications possess different melting points and enthalpies (Müller et al. 2000).
X-ray diffraction is used as a method allowing to distinguish between the various lipid polymorphs. These different polymorphic forms can be unambigously characterized by their spacings (Hoerr and Paulicka 1968; Westesen et al. 1993). It is possible to assess the length of the long and short spacings of the lipid lattice (Müller et al. 2000). X-ray diffraction only allows to differentiate between crystalline and amorphous materials and to assess the influence of the oily constituent on the subcell parameters and long spacings of the solid lipid nanocrystals, but DSC can be used to differentiate between amorphous solids and liquids. X-ray diffraction measurements confirm the polymorphism behaviour established by DSC measurements (Jenning et al. 2000d).
^{1}H NMR spectroscopy is especially suitable for characterization of the liquid lipid domains inside the SLN. When liquid lipid is incorporated in the lipophilic phase of SLN with the aim of improving the loading capacity of the particles, including solid lipid with high crystallinity, the resulting particles are solid, but the oil inside the particle may remain in a liquid state. Information about the mobility, arrangement and environment of the oil molecules is derived from ^{1}H NMR measurements (Jenning et al. 2000b). ^{1}H NMR spectroscopy is also employed to determine drug distribution within the compositions and to obtain information on the mobility of the drug molecules incorporated in the lipid matrix. This measurement is important, because sustained drug release from the lipid particles is difficult to obtain, although high drug loading can be achieved with high drug mobility (Westesen and Siekmann 1997).

6. Principles of drug incorporation into SLN and NLC

Factors affecting the loading capacity of a drug in the lipid are (Müller et al. 2000):
– solubility of the drug in the melted lipid,
– miscibility of drug melt and lipid melt,
– chemical and physical structure of solid matrix lipid,
– polymorphic state of lipid material.

Drug incorporation models of SLN are (Fig. 2):
– the solid solution model,
– the core-shell model; – drug-enriched shell,
– drug-enriched core.

In the case of the solid solution model, the drug is molecularly dispersed in the lipid matrix when the particles are produced by the cold homogenization technique and no surfactant or no drug-solubilizing surfactant is used. The

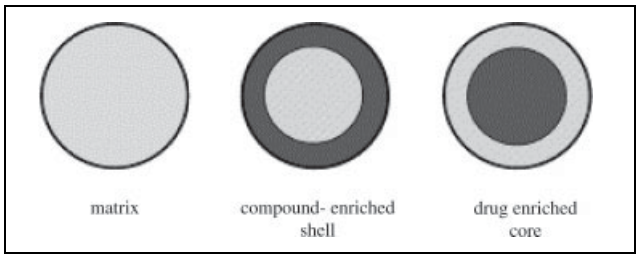
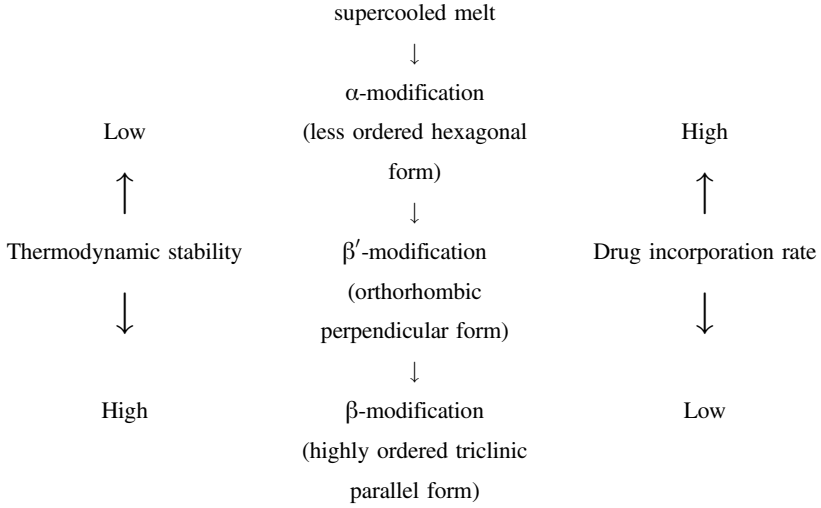


Fig. 2: Models of incorporation of active compounds into SLN: homogeneous matrix of solid solution (left), drug-free core with drug-enriched shell (middle), drug-enriched core with lipid shell (right) (reprinted from Ref. Müller et al. 2002a with permission from Elsevier, 2005)

Scheme



drug has strongly pronounced interactions with the lipid, which can be determined by the melting behaviour of the lipid matrix (Müller et al. 1994). Molecular dispersion of the drug in the particles can be substantiated by X-ray diffraction. Prolonged drug release over several weeks can be achieved with solid lipid particles (Schwarz 1995).

According to the drug-enriched shell model of drug incorporation, the drug partitions from the liquid oil phase to the water phase during production by hot homogenization. Partitioning into the external phase is facilitated by the liquid state of the particles. The amount of drug partitioning increases with the solubility of the drug in the water phase and with increasing temperature. The saturation solubility of the drug in the water phase is greater at a higher temperature. During the cooling of the o/w nanoemulsion produced, the solubility of the drug in the water phase decreases continuously with decreasing temperature. Re-partitioning of the drug into the lipid phase occurs. On reaching the recrystallization temperature of the lipid, a solid lipid core forms. Reducing the temperature of the dispersion still further increases the pressure on the drug to re-partition further into the lipid phase because of its reduced solubility in water. The already crystallized core is no longer accessible for the drug, and consequently the drug concentrates in the still liquid outer shell of the SLN and/or on the surface of the particles (Müller et al. 2002a; Zur Mühlen and Mehnert 1998).

According to the drug-enriched core model of drug incorporation, a drug-enriched core usually occurs with a drug which is dissolved in the lipid melt at or close to its saturation solubility. Then cooling the nanoemulsion leads to a supersaturation of the drug in the melted lipid and the drug precipitates prior to lipid recrystallization. Further cooling finally leads to the recrystallization of the lipid surrounding the drug as a membrane. This lipid membrane contains the drug only at a concentration corresponding to the saturation solubility of the drug at the recrystallization temperature of the lipid (Müller et al. 2000). In this entrapment model, the amount of SLN associated drug is also related to the nature and amount of surfactant surrounding the SLN core. Heiati et al. (1997) reported that amphiphilic drugs such as dexamethasone, dexamethasone palmitate and azidothymidine palmitate did not appear to be incorporated within the triglyceride core of SLN such as trilaurin (drug-enriched shell model). Drug incorporation was depended on increase in phospholipid concentration, and could be controlled by changing the phospholipid content.

7. Controlled drug delivery

The general principles listed below help to understand drug release from lipid nanoparticles (Venkateswarlu and Manjunath 2004).

- There is an inverse relationship between drug release and the partition coefficient of the drug.
- Higher surface area due to smaller particle size gives higher drug release.
- Slow release of drug can be achieved when the drug is homogenously dispersed in the lipid matrix.
- Poor crystallinity of the lipid carrier and high mobility of the drug lead to fast drug release.

These principles explain the importance of the production parameters in controlled drug delivery. When the cold homogenization technique is used, the drug loaded lipid phase remains mainly in the solid state during the production step (solid solution model). The mobility of the drug is reduced and a higher amount of drug compared to the hot homogenization technique is entrapped, i.e. partitioning to the water phase is minimized. Drug release is prolonged compared with the hot homogenization technique. The entrapment efficiency varies with the production temperature used in the hot homogenization process and with the surfactant concentration.

The release profile of SLN produced by the hot homogenization technique is characterized by a pronounced fast initial drug release (burst effect) in the first five minutes which is followed by prolonged drug release for the drug-enriched shell model (i.e. 100% within <5 min). Thus Müller and his research group have focused on investigating the drug release mechanism and factors affecting drug release. With this aim, SLN were produced by incorporating various model drugs which have different physicochemical properties. Tetracain base and etomidate base as lipophilic drugs, and iotrolan (Schering AG, Berlin) as a hydrophilic drug were studied in various triglyceride SLN formulations. Etomidate – and tetracain – loaded nanoparticles were reported to show nearly complete initial drug release over the first few minutes, independent of the production method (Müller et al. 1995). Investigations of the release behaviour of microparticles revealed that this burst release is due to the surface area and the location of the drug in the SLN (Müller et al. 1994). As a result, the burst release reduced with increasing particle size and prolonged release could be obtained when the particles were sufficiently large, i.e. lipid microparticles. Because the drug was entrapped in SLN according to the drug enriched shell model, this led to a relatively short distance of diffusion and hence burst release of the drug. The importance of crystallization phenomena in the drug entrapment model is explained in Section 6. The choice of the most suitable surfactant which will interact with the outer shell and affect its structure, is the other important factor. A low surfactant concentration leads to a minimal burst and prolonged drug release.

In the same study, importance of the homogenization process was reported. The prolonged release could be explained by the molecular distribution of the drug in the lipid according to the solid solution model. However, the drug release profile could be altered by using the hot or cold homogenization technique and by the lipid used. In general, slower release without a distinct burst was obtained with cold homogenization. This may be attributed to the homogenous molecular distribution of the drug in the solid lipid matrix leading to the formation of a solid dispersion before homogenization and to particle formation in cold homogenization retaining it as a solid dispersion. As a result, the release of prednisolone over a period up to six weeks was obtained.

The solid state of SLN offers more possibilities of modifying the release profile of the drugs incorporated. The localization of the drugs is critical for their release behaviour (Lukowski et al. 1998). The mobility of drugs incorporated is reduced due to the solid state of the particles at room temperature and this phenomenon leads to controlled drug release. The crystalline state of the particles controls the release behaviour of the drug incorporated. These results were also supported by another study (Zur Mühlen et al. 1998). Drug enriched core model of drug incorporation leads to a membrane controlled release governed by the Fick law of diffusion (Zur Mühlen and Mehnert 1998).

With NLC, the oil content of the particles solubilizes the drug and combines controlled release characteristics with high drug loading capacity. The imperfect type and amor-

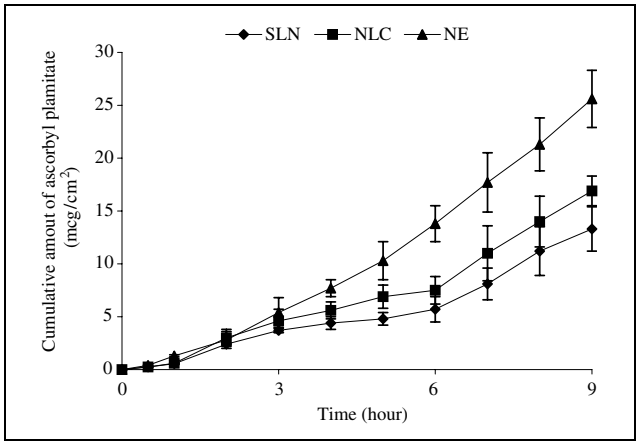


Fig. 3: Ascorbyl palmitate penetration through human skin from SLN, NLC and nanoemulsion (NE) incorporated into a hydrogel (reprinted from Ref. Üner et al. 2005b with permission from Govi-Verlag, 2005)

phous type of NLC, in particular, provide much more flexibility to achieve the desired prolonged release (Müller et al. 2002b). If a highly lipophilic drug such as clotrimazole was considered, NLC showed faster drug release compared to SLN. The entrapment efficiency and drug release profile depended on the drug and lipid concentration. This situation can be attributed to higher lipid concentrations with higher crystallization of SLN (Souto et al. 2004). In a recent study, ascorbyl palmitate which has an amphiphilic structure was entrapped in SLN and NLC. The rate of drug which penetrated through human skin from SLN and NLC was statistically identical. This similarity can be attributed to the drug entrapment model of SLN (drug-enriched shell model) and the crystallization behaviour of NLC (Fig. 3) (Üner et al. 2005b).

8. Chemical stability of incorporated drugs

Chemical problems with polymers, such as catalyst residues, and molecular non-homogeneity, are reasons for the lack of polymeric nanoparticles in pharmaceutics and cosmetics. Incorporation of drug into SLN protects against chemical degradation by the surrounding medium in addition to many advantages over polymeric nanoparticles (see Introduction and Section 2).

With this aim, SLN have been studied with labile compounds including coenzyme Q10 (Müller and Dingler 1998), tocopherol (Dingler 1998; Jenning and Gohla 2001), tocopherol acetate (Dingler et al. 1999), retinol (Dingler 1998; Jenning and Gohla 2001), all-trans retinoic acid (Lim et al. 2004) and ascorbyl palmitate (Kristl et al. 2003; Üner et al. 2005a). It was concluded that the solid state of lipid nanoparticles protected active molecules incorporated against chemical degradation.

The surfactant mixture was found to have a significant influence on the chemical stabilization of a drug which accumulates in the outer shell of SLN particles (Jenning 1999; Jenning and Gohla 2001). The choice of a suitable surfactant/surfactant mixture at a suitable concentration contributes to drug stability in SLN, since the surfactant enhances the water solubility of drug and affects the surface properties of SLN.

Storage temperature can influence the chemical stability of the drug. Storage at 40 °C has been shown to promote degradation of labile compounds compared to 4 °C and room temperature in various studies (Dingler et al. 1999; Üner et al. 2005a), because 40 °C retards crystallization of SLN and then improves the mobility of the drug in the lipid matrix. If the partition coefficient of a drug is not high, partition of drug to the aqueous phase becomes rapid and then degradation rate increases (Üner et al. 2005a).

The chemical stability of ascorbyl palmitate entrapped in NLC has also been studied and the protective effect of NLC was found to be similar to that of SLN during storage at 4 °C for 90 days (Üner et al. 2005a). Presence of liquid lipid in the structure of NLC increased drug solubility in the lipid nanoparticles leading to homogenous drug distribution in the carrier structure. Additionally, the solid lipid content also prevented the mobilization of the drug inside the nanoparticles.

9. Applications of SLN and NLC as delivery systems

Dermatological and cosmetic application routes have been reported for NLC since they were originally described, but other routes related to SLN can also be used with this carrier system. Potential applications are discussed below.

9.1. Parenteral administration

SLN are generally injected intravenously, intramuscularly or subcutaneously. Because of their particle size below 1 μm, SLN formulations can be used for systemic body distribution with a minimal risk of blood clotting and aggregation leading to embolism. The particle size of an intravenously administered drug must be below 5 μm to avoid blocking of fine capillaries leading to embolism. Because of the physical and chemical instability of some molecules such as proteins, oligonucleotides and DNA in the gastrointestinal tract, they must be administered parenterally (Almeida et al. 1997; Dass 2002; Hu et al. 2004).

Also SLN provide a sustained release depot of the drug when administered subcutaneously or accumulated in the mononuclear phagocytic system (Wissing et al. 2004). The drug is gradually released by erosion (e.g. degradation by enzymes) or by diffusion from the particles. Targeting a drug to the disease location is possible with SLN. For instance, targeting an anticancer drug is a distinctive feature of most studies, the aim being to convey a sufficient dose of drug to the tumor. SLN increase the tumour accumulation (Chen et al. 2001), antibacterial activity (Bargoni et al. 2001) of antiparasitic and antifungal drugs, and allow brain delivery of anticancer drugs not capable of crossing the blood brain barrier (Fundaro et al. 2000; Wissing et al. 2004). Improvement of the cytotoxic activity of anticancer drugs in cancer cells can be achieved (Serpe et al. 2004). Examples of many different drugs incorporated in SLN for parenteral application have been listed in a paper which extensively describes the use of SLN for parenteral drug delivery (Wissing et al. 2004).

9.2. Oral administration

The oral route continues to be a challenge as well as the most attractive way to administer drugs because of its unquestionable commercial potential. Incorporation of drugs into lipid nanoparticles opens the perspective of enhanced and/or less variable bioavailability and prolonged plasma levels (Runge 1998; Demirel et al. 2001; Penkler et al. 1999). While these systems may provide the greatest flexibility in the modulation of the drug release profile within the gastrointestinal tract, they may also provide protection against chemical degradation for labile drug molecules, e.g. peptide drugs (Yang et al. 1999b). It has been shown

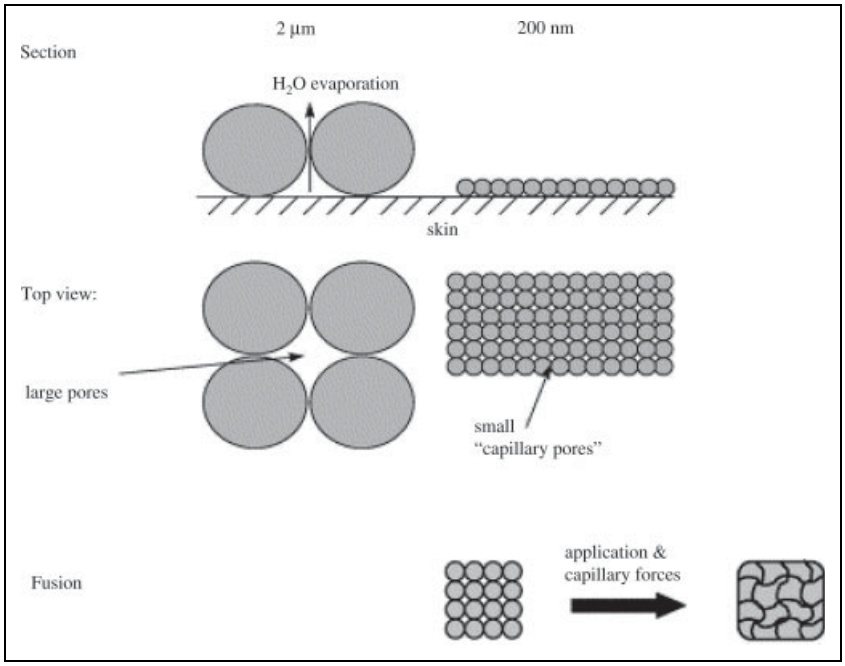


Fig. 4:
Model of film formation indicating occlusive effect on the skin for lipid 2 µm particles and lipid 200 nm particles shown as section (upper) and from the top (middle), and a new model of fusion of the nanoparticles to a pore-less film (lower) (reprinted from Ref. Müller et al. 2002a with permission from Elsevier, 2005)

that *in vitro* drug release could be varied from minutes (burst release) (Zur Mühlen et al. 1998) to up to 7 weeks (Mehnert et al. 1997). Drug release takes place by diffusion from the solid matrix and by enzymatic degradation of the matrix. The rate of enzymatic degradation can be controlled by the composition of the lipid matrix i.e. selection of lipid and stabilizing surfactant. In general, degradation rate decreases with increasing length of the fatty acid chain length when using glycerides. The degradation of SLN based on waxes (e.g. cetylpalmitate) was found to be slower compared with glyceride matrices (Olbrich and Müller 1999; Olbrich et al. 2002). A tablet, a capsule or a sachet for reconstitution as an oral suspension are possible formulations for oral administration. Production of SLN containing pellets is also possible (Runge et al. 1996). SLN were found to improve the bioavailability of drugs e.g. cyclosporin A (Olbrich et al. 2002), clozapine (Venkateswarlu and Manjunath 2004), camptothecin (Yang et al. 1999b), idarubicin (Zara et al. 2002) and piribedil (Demirel et al. 2001).

Macromolecular drugs such as peptides and proteins are unable to overcome the mucosal barriers and/or are degraded before reaching the blood stream. The use of nanoparticulate carriers represents a challenging but promising strategy to optimize the transport of these macromolecules across mucosal barriers in combination with the general adhesive properties of small particles (Prego et al. 2005; Ponchel et al. 1997). In this context, salmon calcitonin (Prego et al. 2005) and gonadorelin (Hu et al. 2004) have been used as polypeptide model drugs.

9.3. Dermatologic and cosmetic applications

SLN and NLC are very attractive colloidal carrier systems for skin applications due to their various desirable effects on skin besides the characteristics required of a colloidal carrier system as explained in detail above. They are well suited for use on damaged or in flamed skin since they are based on non-irritant and non-toxic lipids (Jenning et al. 2000a).

SLN and NLC have an occlusive effect leading to an increase in skin hydration, and thus to wrinkle smoothing and enhanced penetration into, or specific localization of compounds in, specific skin layers (Fig. 4). Enhancement of skin penetration of entrapped drugs is one of the benefits of these systems due to the close contact with the stratum corneum caused by the small particle size of the lipid particles in the nanometer range (De Vringer and De Ronde 1995; Wissing et al. 2001). A white pigment effect on the skin through covering unwanted colors of compounds or their degradation products is provided with topical application (Dingler et al. 1999; Krzic et al. 2001). The release rate of the drug can be controlled due to the solid matrix of SLN and the special structure of NLC which provide flexibility to achieve the desired prolonged release. Sustained release becomes important with active compounds that are irritating at high concentrations, in order to feed the skin with a drug over a prolonged period of time and to reduce systemic absorption (Jenning et al. 2000c; Mei et al. 2003). During the last few years, SLN and NLC have been studied with active compounds such as vitamin E (Dingler et al. 1999), tocopherol acetate (Wissing and Müller 2001), retinol (Jenning and Gohla 2001), ascorbyl palmitate (Kristl et al. 2003; Üner et al. 2005a, 2005b), clotrimazole (Souto et al. 2004) and triptolide (Mei et al. 2003) for topical application.

9.4. Ophthalmic administration

SLN prolong the duration of drug actions in the eye due to their adhesive properties. Moreover, patient compliance can be improved. SLN has been reported to improve the ocular bioavailability of drugs such as timolol (Ellis and Riegel 1988), pilocarpine (Cavalli et al. 1995) and tobramycin (Cavalli et al. 2002).

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