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Nanosuspensions for the formulation of poorly soluble drugs I. Preparation by a size-reduction technique

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Abstract

A basic problem of poorly soluble drugs is often an insufficient bioavailability. To allow the i.v. injection of these drugs, they were formulated as nanosuspensions by high pressure homogenization. The effect of the production parameters pressure and cycle number on the mean particle size and on the polydispersity of the nanosuspension was investigated with special attention to contamination by microparticles — the limiting factor for i.v. injection. Properties of the nanosuspensions are increased saturation solubility C_s and dissolution rate dc/dt. These phenomena are explained using the Prandtl and the Ostwald–Freundlich equations. These properties promote the dissolution of the nanosuspensions in the blood after i.v. injection. The size distribution obtained and the use of an APV Gaulin homogenizer (FDA approved for parenterals) lead to a pharmaceutical product considered acceptable by the regulatory authorities. © 1998 Elsevier Science B.V.

Keywords: Poorly soluble drugs; Dissolution velocity; Nanoparticle; High pressure homogenization; Nanosuspension; Solubility

1. Introduction

An increasing number of newly developed drugs are poorly soluble in water and simultaneously in organic media. Consequently the bioavailability after oral or parenteral administration (e.g. i.m. or i.p.) is poor and very often below the therapeutic level. Due to the simultaneous insolubility in organic media, i.v. injection in a solvent mixture to achieve a sufficiently high bioavailability is not possible. Attempts to solubilize the drugs in micelles or with cyclodextrins are of limited success for many drugs.

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An alternative approach is the i.v. administration of poorly soluble drugs as a nanosuspension (Müller et al., 1995a,b). A prerequisite for the i.v. injection of suspensions is a small particle size, i.e. preferentially in the nanometer range with little content of microparticles. The microparticles lead to toxic effects and ultimately to emboli when they exceed a critical level in the administered dose (Davis and Traube, 1978; Schroeder et al., 1978; Slake et al., 1981). Additionally, the product should not contain toxicologically critical excipients or material from the production equipment which would prohibit registration by the regulatory authorities. The methods used in producing drug particles in the nanometer range are conventional milling techniques, such as the jet mill (Müller et al., 1995b) or pearl mill (Buchmann et al., 1996; Merisko-Liversidge et al., 1996). Problems associated are e.g. the polydispersity of the product requiring subsequent removal of microparticles or abrasion of pearls in a mill (Buchmann et al., 1996). In this paper the production of nanosuspensions by high pressure homogenization and the effect of production parameters on the quality of the product are presented. Special features of the nanosuspensions generally known for increasing the bioavailability are discussed. This includes the potential for the oral administration and their use not only for poorly soluble but also for slightly soluble drugs. Part II of this series will deal with sterilization and long-term stability of the nanosuspensions.

2. Materials and methods

2.1. Materials

The following model drugs were used: RMKP 22 (2-propanol, 1-[[2,7-bis(2,6-dimethyl-4-morpholinyl)-6-phenyl-4-pteridinyl](2-hydroxyethyl)-amino]-2-methyl-,[*cis*(*cis*)]) provided by Dr Karl Thomae (Biberach, Germany), RMKP 23 (a new antibacterial compound), Prednisolone provided by Ferring (Kiel, Germany) and Carbamazepine purchased from Sigma (Deisenhofen, Germany). Phospholipon 90 (soy lecithin, 93% phosphatidyl choline) was a gift from Nattermann Phospholipid (Cologne, Germany). Lipofundin 10% was obtained from Braun Melsungen (Melsungen, Germany), Intralipid 20% from Kabi Pharmacia (Erlangen, Germany). Mannitol and Glycerol 85% and all other chemicals were purchased from Sigma (Deisenhofen, Germany).

2.2. Methods

The drug powder was dispersed in an aqueous surfactant solution using an Ultra-Turrax stirrer T 25 (Janke und Kunkel, Staufen, Germany). The coarse pre-dispersion obtained was homogenized at pressures of 500 and 1500 bar with 5–10 cycles using an APV Gaulin Micron LaB 40 homogenizer (APV Homogenizer, Lübeck, Germany). The size reduction process resulted in a suspension in the nanometer range — a nanosuspension. All the data presented are the mean values of three different batches produced with identical production conditions.

Particle size analysis was performed by photon correlation spectroscopy (Malvern Zetasizer 4, Malvern Instr., UK), Coulter counter Multisizer II (Coulter Electronics, Krefeld, Germany) using a 30-μm capillary and laser diffraction particle size analysis (Mastersizer E, Malvern Instr., UK). Coulter counter calculations were performed using particle counts without further correction. From the laser diffractometry data the diameters 50%, 90% and 99% ($D(50)$, $D(90)$ and $D(99)$) were used to characterize the nanosuspension. The diameters were calculated using the volume distribution; diameter 50% is the mean of the volume distribution. Diameter 99% means that 99% of the particles are below the indicated size.

For the determination of the saturation solubility, the micro- and nanosuspensions were filtered using a drug-saturated 0.1-μm polyamide filter. The absorbance of the supernatant was measured using a spectrophotometer Uvikon 940 (Kontron Instruments, Hamburg, Germany) at a wavelength of 402 nm.

The mean values and standard deviations are shown in the figures.

The nanosuspensions were also determined by scanning electron microscopy (SEM). All samples

were air dried before coating in an Emitech K550 sputter coater using gold in an argon atmosphere. The electron microscopic photographs were taken using a Philips EM501B (Philips, The Netherlands).

3. Results and discussion

3.1. Particle size as a function of production parameters

The width of the gap of the Gaulin high pressure homogenizer is about 25 μm at a pressure of 1500 bar (pers. comm., APV Gaulin). The cavitation and shear forces in the gap were sufficiently high to break particles which were distinctly larger than the gap width (Müller et al., 1995a). Therefore it is possible to disrupt relatively large-sized powders (up to 200 μm, unpublished data). However, too large a fraction of big particles limits the solid content of the suspension to a few percent, otherwise the gap would be blocked. Therefore a jet milled powder was used in the case of RMKP 22 allowing the processing of a relatively highly concentrated suspension of 9%. Maximum concentrations used in our laboratory were 15% of the relatively hard crystalline RMKP 22. This maximum possible concentration depends not only on the particle size but also on the hardness of the drug crystals, i.e. it is higher for soft than for hard drugs.

The influence of homogenization pressure and cycle number on the reduction of the microparticles employing an aqueous drug suspension containing 9% of the jet milled powder RMKP 22 (diameter 90% = 8.9 μm) and 0.3% Tween 80 as a surfactant stabilizer is shown in Fig. 1. Applying a relatively low homogenization pressure of 500 bar leads to a decrease in the diameter 90% to 7.2 μm after the first cycle and to a diameter 90% of 5.7 μm after five cycles (Fig. 1, upper curve). Increasing the pressure to 1500 bar leads to smaller sizes after the first cycle (5.6 μm) and the fifth cycle (3.4 μm) (Fig. 1, lower curve).

A homogenisation process reduces the mean particle size (Fig. 2), and simultaneously narrows the width of the size distribution, i.e. reduces the

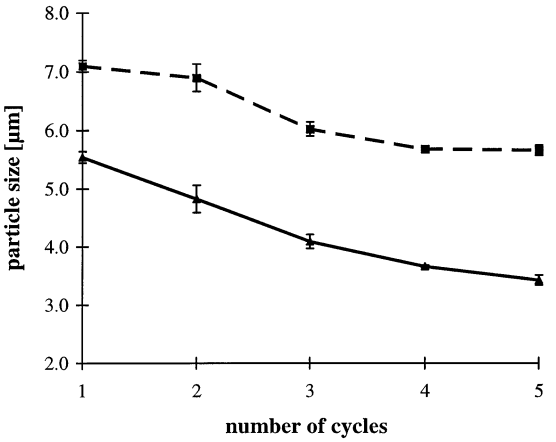

Fig. 1. The influence of homogenization pressure and cycle number on the reduction of microparticles. Diameter 90% (LD data) of RMKP 22 as a function of the pressures 500 bar (– ■ –) and 1500 bar (– ▲ –) and the cycle numbers 1 to 5 (9% drug dispersed in a surfactant solution containing 0.3% Tween 80).

number of microparticles and the polydispersity index (PI) of the bulk population (Fig. 2). Fig. 2 shows the mean particle size determined by photon correlation spectroscopy as a function of cycle numbers, employing an aqueous drug suspension containing 9% RMKP 22, 0.6% Phospholipon 90, 2.2% glycerol 85%, applying 1500 bar and 10 cycles. The mean particle size decreases clearly up

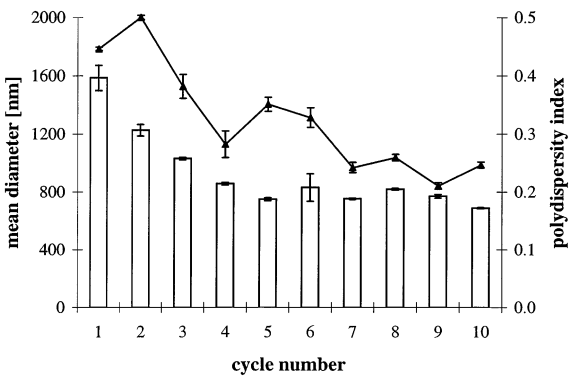

Fig. 2. The mean particle size and the polydispersity index determined by photon correlation spectroscopy as a function of cycle numbers. Mean particle size (□) and polydispersity index (– ▲ –) (PCS data) of RMKP 22 as a function of cycle numbers (9% drug dispersed in 0.6% Phospholipon 90, 2.2% glycerol 85%, pressure 1500 bar, $n = 3$).

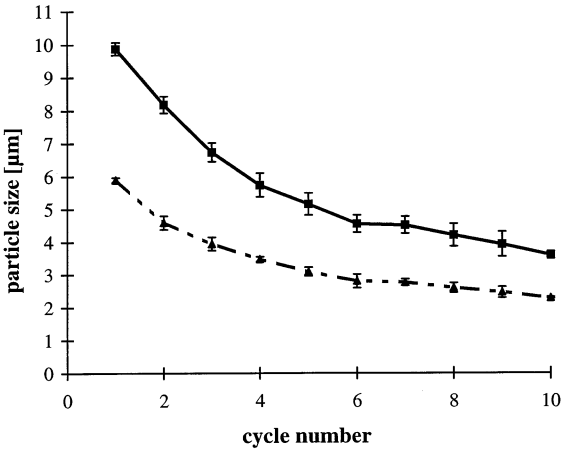

Fig. 3. The diameters 90% (– ▲ –) and 99% (– ■ –) (LD data) of RMKP 22 as a function of cycle numbers (9% drug dispersed in 0.6% Phospholipon 90, 2.2% glycerol 85%, pressure 1500 bar, $n = 3$).

to cycle number 5 and remains unchanged when increasing the cycle number to 10. The maximum degree of dispersion seems to be reached after cycle number 5. The polydispersity index (PI) is a measure of the width of the size distribution. In Fig. 2 the polydispersity index (PI) as a function of cycle numbers for this nanosuspension shows that the PI decreases until ca. cycle number 9. Microparticles above ca. 3 μm are outside the measuring range of the PCS and were therefore analyzed by laser diffractometry, using a lens with a measuring range from 0.1 μm to 80 μm. The diameters 90% and 99% of the volume distribution showed a decrease until ca. cycle number 9 (Fig. 3). At cycle 10 at 1500 bar, the volume diameter 50% was 1.19 μm (for comparison: PCS mean diameter 688 nm, cf. Fig. 2).

This indicates — despite reaching the maximum degree of dispersion at ca. 5 cycles — that higher cycle numbers are conducive to a more monodisperse product with a minimum content of microparticles, i.e. the lowest PI (Fig. 2) and the lowest content of microparticles (Fig. 3). The latter is a factor which limits the injectability of i.v. products.

3.2. Minimization of microparticulate content

Minimization of the content of micron-sized particles is important in case the nanosuspension should be administered intravenously. It is difficult to assess the maximum tolerable limit of microparticles (tolerable number as a function of microparticle size) because there are no official regulations. Nanosuspensions for disease treatment are not yet on the market. The pharmacopoeia avoid even limiting numbers for flexible particles such as oil droplets in emulsions for parenteral nutrition. The German pharmacopoeia requires only the 'measurement of particle size'; in the USP is only a monograph 'particulate matters' describing particle contamination from the packing material. Commercial particulate products for i.v. injection are diagnostics, e.g. albumin microspheres (Technescan) for gamma scintigraphy. However diagnostics are not administered chronically like drugs for treatment. Therefore they cannot be used for reference purposes. It appeared more sensible to compare the microparticulate content in nanosuspensions with a product being administered chronically in large volumes, i.e. emulsions for parenteral nutrition being applied for weeks or months in daily volumes of 0.5 l or more.

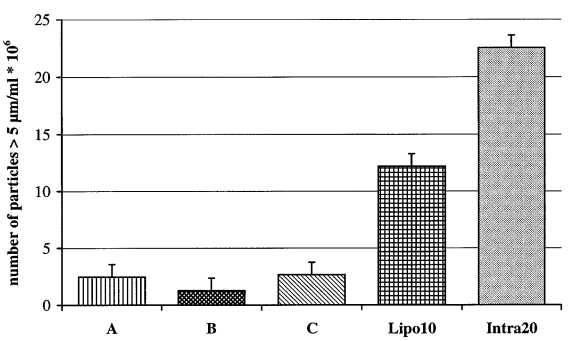

Fig. 4. The number of microparticles > 5 μm per μl original dispersion of various optimized nanosuspensions compared with Intralipid 20% and Lipofundin 10%. Content of particles > 5 μm/μl dispersion in Lipofundin 10% (Lipo10), Intralipid 20% (Intra20) and in the nanosuspensions A and B at the day of production and C after 2 years of storage. (A — 9% RMKP 22, 0.6% Phospholipon 90, 2.2% glycerol 85%; B — 3% RMKP 22, 0.3% Tween 80, 16.7% mannitol; C — nanosuspension A, measured 2 years after storage) (Coulter counter data, $n = 3$).

Fig. 4 shows the number of particles >5 μm per ml original dispersion of various optimized nanosuspensions compared with Intralipid 20% and Lipofundin 10%. The numbers in the nanosuspensions on the day of production (day 0) were distinctly lower than in the emulsion, and also after 2 years of storage (Fig. 4C). Of course, in contrast to solid nanosuspension particles, the oil droplets are flexible and might therefore pass smaller sized capillaries. In addition, the emulsion will be metabolised by lipase within 3–4 h thus reducing the risk of emboli. However when considering the relatively small administered volume of a nanosuspension (e.g. 10–20 ml) and its fast dissolution (cf. below), injection is considered to be well tolerated. This was confirmed by initial animal studies. Injections of 0.3 ml RMKP 23 nanosuspension (2.5% solid content) in C57Bl/6 mice were well tolerated without any signs of acute toxicity. When judging the tolerability one should bear in mind that the blood volume of the mice is ca. 2.0 ml, i.e. 15% of the blood volume was injected as a nanosuspension.

3.3. Particle size and shape as a function of drug

The achievable degree of fineness (minimal size) is a function of the given power density (Pv) in the homogenizer and of the nature of the drug to be disrupted. Fig. 5 gives selected examples for the mean particle size of model drugs after 2, 4, 6, and 10 cycles at constant power density. The increase in temperature during passage of the homogenizer will surely contribute to the efficient dispersion of soft, low melting drug crystals (e.g. tetracaine base). The product fed to the homogenizer at room temperature normally has an initial temperature of about 20°C. Inside the homogenizer the temperature will increase by ca. 20°C — in addition to temperature peaks — possibly leading to a softening of low melting drugs, close to the melting point, e.g. tetracaine at 45°C.

The effect of hardness and crystalline structure on the homogenization result is also demonstrated by the differences in the shape of the nanoparticles (Müller et al., 1996). To determine the influence of the drug on the nanoparticle shapes, batches with various drugs were produced using

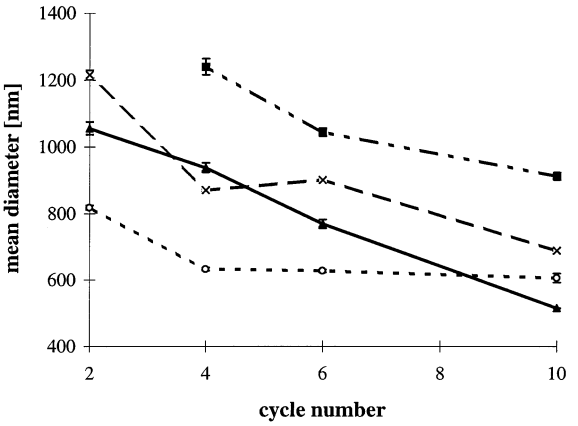

Fig. 5. The mean particle size of model drugs after 2, 4, 6 and 10 cycles at constant power density. Mean diameter (PCS data) of the model drugs Prednisolone (– ■ –), RMKP 22 (– × –), Clofazimine (– ▲ –), RMKP 23 (– ◦ –) after 2, 4, 6 and 10 homogenization cycles at a pressure of 1500 bar, $n = 3$.

the same surfactants and applying identical production parameters. The three drug suspensions containing 3% of the drug, 0.6% Phospholipon 90 and 0.5% sodium cholic acid were homogenized at 1500 bar and 7 cycles. The electron microscopic photographs of the nanosuspensions show that the drug RMKP 22 forms cube-shaped nanoparticles (Fig. 6), Prednisolone forms rod-shaped nanoparticles (Fig. 7), while the drug RMKP 23 forms needle-shaped nanoparticles (Fig. 8).

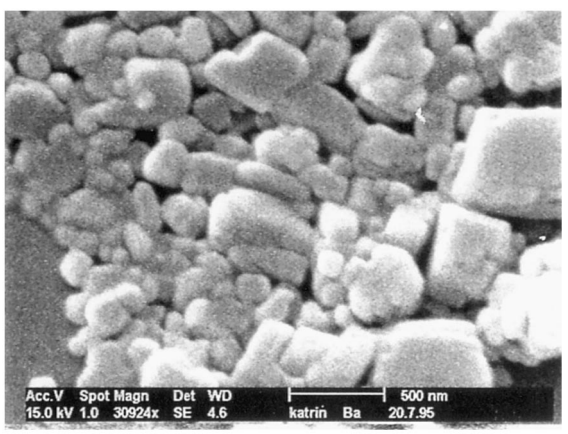

Fig. 6. Scanning electron micrographs of a nanosuspension containing 3% RMKP 22, 0.6% Phospholipon 90 and 0.5% sodium cholic acid, applying 1500 bar and 7 cycles.

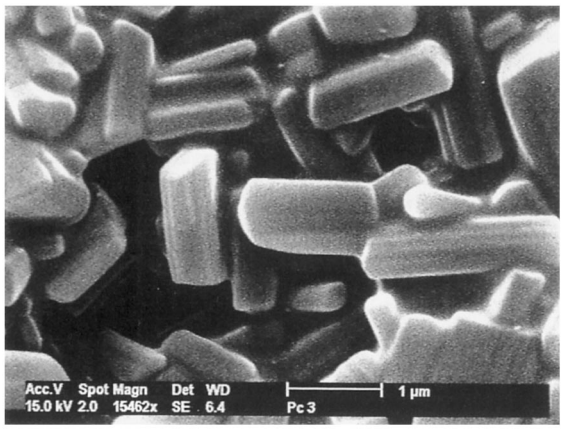

Fig. 7. Scanning electron micrographs of a nanosuspension containing 3% Prednisolone, 0.6% Phospholipon 90 and 0.5% sodium cholic acid, applying 1500 bar and 7 cycles.

3.4. Saturation solubility

To quantify the number of particles per volume unit, Coulter counter analysis was performed in a drug-saturated 0.9% sodium chloride solution. Despite the fact that the saturation was obtained using 2.4-μm drug particles — the nanosuspension particles dissolved during three subsequent measurements (Fig. 9).

The saturation solubility C_s increases with decreasing particle size according to the Ostwald–

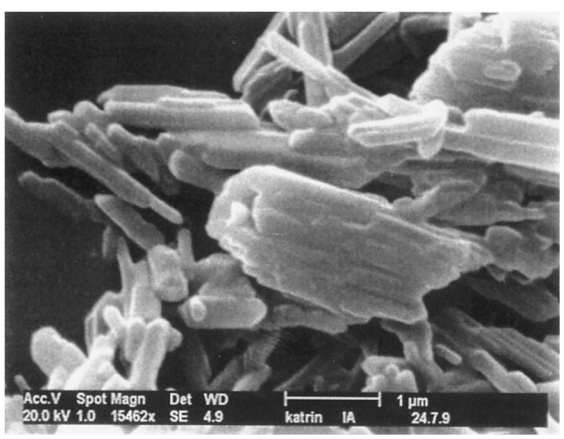

Fig. 8. Scanning electron micrographs of a nanosuspension containing 3% RMKP 23, 0.6% Phospholipon 90 and 0.5% sodium cholic acid, applying 1500 bar and 7 cycles.

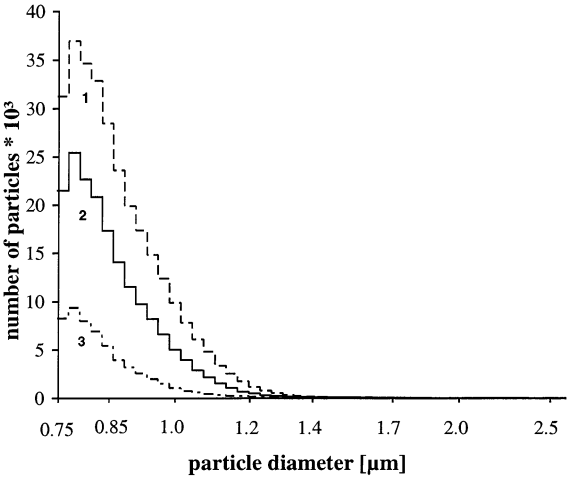

Fig. 9. Number of particles >5 μm per unit volume original dispersion determined by Coulter counter analysis. Volume distributions of a RMKP 22 nanosuspension obtained in a Coulter counter; three subsequent measurements (0, 5, 10 min). The particles dissolved to a great extend from measurement no. 1 (— — —) to no. 2 (——) and no. 3 (— – —); measuring range, 0.75–18 μm; measuring medium, drug-saturated 0.9% sodium chloride.

Freundlich equation (Eq. (1)) (Florence and Attwood, 1981):

$$\log \frac{C_s}{C_\infty} = \frac{2\sigma V}{2.303RT\rho r} \quad (1)$$

where C_s = solubility, C_∞ = solubility of the solid consisting of large particles, σ = interfacial tension substance, V = molar volume of the particle material, R = gas constant, T = absolute temperature, ρ = density of the solid, r = radius.

The increase in C_s can be seen in the range below ca. 1 μm (Florence and Attwood, 1981).

Another possible explanation for the increased saturation solubility is the creation of high energy surfaces when disrupting the more or less ideal drug microcrystals to nanoparticles. Lyophobic surfaces from the inner of the crystal will be exposed to the aqueous dispersion medium. According to Ostwald–Freundlich, C_s is also a function of the interfacial tension σ, that means the interfacial energy G ($G = \sigma \cdot A$). Such differences in interfacial energy are the reason for the differences in C_s of polymorphic forms; the same might be valid for the nanosuspension (high energy

form = polymorph II = higher C_s) compared to microparticulate suspensions (low energy form = stable polymorph I = lower C_s).

Dissolution experiments were performed to quantify the increase in C_s in a nanosuspension compared to the C_s in a microparticle suspension. The jet milled RMKP 22 powder was dispersed in an aqueous surfactant solution containing 0.3% Tween 80 and 2.2% glycerol 85%, then stirred using an Ultra-Turrax. The resulting microparticle suspension with a mean diameter of 2.4 μm was homogenized applying different production parameters resulting in nanosuspensions with a mean diameter of 800 nm and of 300 nm. Fig. 10 shows that C_s approximately doubled when entering the nanometer range. The increase in C_s appeared rather high. However the dissolution experiments with the microparticulate suspensions were carried out under the same conditions, 0.3% Tween 80 and 2.2% Glycerol 85% were added to the RMKP 22 powder and with addition of water the blends were suspended using an Ultra Turrax.

According to Noyes–Whitney (Mosharraf and Nyström, 1995), an increase in C_s and decrease in particle size (larger surface area) lead to an increased dissolution velocity dc/dt. In addition, the intrinsic dissolution velocity dc/dtA increases. The

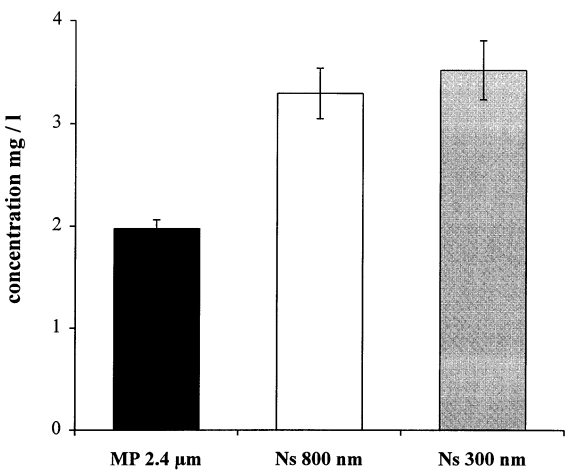

Fig. 10. The influence of particle size on saturation solubility. Saturation solubility of RMKP 22 microparticle suspension with a mean diameter of 2.4 μm (MP 2.4 μm) and of two RMKP 22 nanosuspensions with mean diameters of 800 nm (Ns 800 nm) and 300 nm (Ns 300 nm).

Prandtl equation Eq. (2) (Mosharraf and Nyström, 1995) describes the hydrodynamic boundary layer thickness (h_H) for flow passing a flat surface:

$$h_H = k \times (L^{1/2}/V^{1/2}) \quad (2)$$

where L = length of the surface in the direction of flow, k = denotes a constant, V = relative velocity of the flowing liquid against a flat surface, h_H = the hydrodynamic boundary layer thickness.

Corresponding to the Prandtl equation, Nyström and Bisrat (1988) have shown that, for solids dispersed in a liquid medium under agitation, a decrease in particle size results in a thinner hydrodynamic layer around particles and an increase of the surface specific dissolution rate. Anderberg et al. (1988) have found a hyperbolic relation between the particle size and the surface specific dissolution rate dc/dtA corrected for solubility. This phenomenon is especially pronounced for materials which have a mean particle size less than 2 μm. At a particle size of 1 μm the intrinsic dissolution rate is very fast, and further decrease in size will lead to no practical advantage in the case of e.g. oral adsorption. In most cases, the dissolution process is much faster than the absorption limiting step of penetration through the gut wall. This is different in the case of i.v. injected nanosuspensions. The dissolution in the blood directly increases the bioavailability of the drug. In addition, it might be desirable that a portion of the drug nanoparticles dissolves before they reach the liver and are taken up by the Kupffer cells (Müller, 1991).

3.5. Possible application areas for nanosuspensions

An immediate use for nanosuspensions is the screening of poorly soluble drugs for pharmacological activity. Newly developed drugs can be tested by simply injecting them intravenously as a nanosuspension. Further formulation development is not necessary because a long-term stability for weeks is not required for this purpose. Most of the surfactants accepted for i.v. use yield a nanosuspension stable for at least one or a few days without any formulation optimization, especially when using binary or ternary surfactant combinations.

Poorly soluble drugs can be formulated as i.v. injectable nanosuspensions acceptable to the regulatory authorities. The way of production — high pressure homogenisation — reduces the content of micron-sized particles. The equipment is acceptable for producing parenteral products.

In addition, the nanosuspensions can be used to reformulate existing drugs to remove toxicologically less favorable excipients. An example is Paclitaxel which is on the market as a solution containing Cremophor EL (anaphylactoid reactions). The alternative is a Paclitaxel nanosuspension. Furthermore slightly soluble drugs can be formulated as nanosuspensions to reduce the infusion volume. An example is again the Paclitaxel solution on the market (Taxol®). To administer the daily dose (110–175 mg/m^2) ca. 50 ml need to be infused. Formulating Paclitaxel as a 3% nanosuspension reduces the volume to only ca. 10 ml.

Nanosuspensions also appear attractive for absorption of poorly soluble drugs from the GIT. As a general feature nanoparticles tend to stick to the intestinal wall. This adhesiveness might reduce the variability of absorption — a problem for drugs such as Cyclosporin. Drug dissolution near the wall in combination with increased solubility C_s might enhance the absorption and consequently increase bioavailability.

4. Conclusions

It has been shown that high pressure homogenization can be used to formulate suspensions with particles in the nanometer range — nanosuspensions. The degree of particle fineness in the nanosuspensions was found to increase with production pressure and number of cycles. Furthermore, the achievable degree of particle fineness is a function of the nature of the drug, as are the particle shapes of the investigated drugs.

The number of particles larger than 5 μm in the nanosuspensions was below the number in the investigated fat emulsions for parenteral nutrition. As far as the particle distribution is concerned i.v. administration is possible.

The Ostwald–Freundlich and the Prandtl equations explain why the saturation solubility of a nanosuspension is distinctly increased compared to a suspension containing microparticles. The increases in saturation solubility, dissolution rate and intrinsic dissolution rate will accelerate drug dissolution after i.v. injection and might also enhance absorption from the GIT — especially when considering the adhesive properties of nanoparticles to the gut wall.

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