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Review Article

Nanosuspensions: a promising drug delivery strategy

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Abstract

Nanosuspensions have emerged as a promising strategy for the efficient delivery of hydrophobic drugs because of their versatile features and unique advantages. Techniques such as media milling and high-pressure homogenization have been used commercially for producing nanosuspensions. Recently, the engineering of nanosuspensions employing emulsions and microemulsions as templates has been addressed in the literature. The unique features of nanosuspensions have enabled their use in various dosage forms, including specialized delivery systems such as mucoadhesive hydrogels. Rapid strides have been made in the delivery of nanosuspensions by parenteral, peroral, ocular and pulmonary routes. Currently, efforts are being directed to extending their applications in site-specific drug delivery.

Introduction

The formulation of poorly water-soluble drugs has always been a challenging problem faced by pharmaceutical scientists and it is expected to increase because approximately 40% or more of the new chemical entities being generated through drug discovery programmes are poorly water-soluble (Lipinski 2002).

The problem is even more intense for drugs such as itraconazole and carbamazepine (belonging to class III as classified by Washington 1996), as they are poorly soluble in both aqueous and organic media, and for drugs having a log P value of 2 (Pouton 2000). Such drugs often have an erratic absorption profile and highly variable bioavailability because their performance is dissolution-rate limited and is affected by the fed/fasted state of the patient.

Traditional strategies, such as micronization, solubilization using co-solvents, the use of permeation enhancers (Aungst 1993, 2000), oily solutions (Aungst 1993) and surfactant dispersions (Aungst et al 1994), which evolved earlier to tackle the formulation challenges, have limited use. Although reasonable success has been achieved in formulating water-insoluble drugs using liposomes (Schwendener & Schott 1996), emulsions (Floyd 1999; Nakano 2000), microemulsions (Lawrence & Rees 2000), solid dispersion technology (Serajuddin 1999; Leuner & Dressman 2000; Breitenbach 2002) and inclusion complexes employing cyclodextrins (Loftsson & Brewster 1996; Stella & Rajewski 1997; Akers 2002), there is no universal approach applicable to all drugs. Hence, there is a growing need for a unique strategy that can tackle the formulation-related problems associated with the delivery of hydrophobic drugs in order to improve their clinical efficacy and optimize their therapy with respect to pharmacoeconomics.

Nanosuspensions have revealed their potential to tackle the problems associated with the delivery of poorly water-soluble and poorly water- and lipid-soluble drugs, and are unique because of their simplicity and the advantages they confer over other strategies. This review focuses on the various aspects of nanosuspensions and their potential as a promising strategy in drug delivery.

Nanosuspensions can be defined as colloidal dispersions of nano-sized drug particles that are produced by a suitable method and stabilized by a suitable stabilizer.

Methods of production

Media milling (NanoCrystals)

This patent-protected technology was developed by Liversidge et al (1992). Formerly, the technology was owned by the company NanoSystems but recently it has been acquired by Elan Drug Delivery. In this method the nanosuspensions are produced

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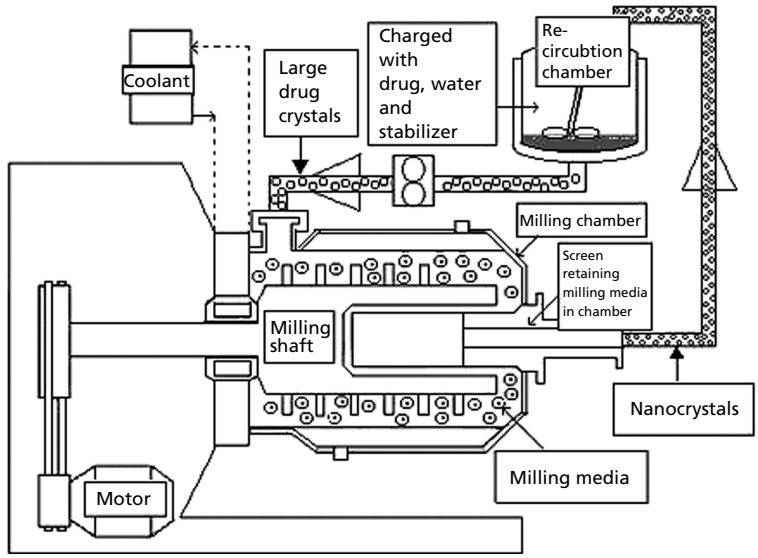

Figure 1 Schematic representation of the media milling process. The milling chamber charged with polymeric media is the active component of the mill. The mill can be operated in a batch or recirculation mode. A crude slurry consisting of drug, water and stabilizer is fed into the milling chamber and processed into a nanocrystalline dispersion. The typical residence time generated for a nanometer-sized dispersion with a mean diameter of <200 nm is 30–60 min. Reprinted from Merisko-Liversidge et al 2003 with permission from Elsevier Publications.

using high-shear media mills or pearl mills. The media mill consists of a milling chamber, a milling shaft and a recirculation chamber (Figure 1). The milling chamber is charged with the milling media, water, drug and stabilizer, as depicted in Figure 1, and the milling media or pearls are then rotated at a very high shear rate. The milling process is performed under controlled temperatures.

Principle The high energy and shear forces generated as a result of the impaction of the milling media with the drug provide the energy input to break the microparticulate drug into nano-sized particles. The milling medium is composed of glass, zirconium oxide or highly cross-linked polystyrene resin. The process can be performed in either batch or recirculation mode. In batch mode, the time required to obtain dispersions with unimodal distribution profiles and mean diameters <200 nm is 30–60 min. The media milling process can successfully process micronized and non-micronized drug crystals. Once the formulation and the process are optimized, very little batch-to-batch variation is observed in the quality of the dispersion.

Advantages

- Drugs that are poorly soluble in both aqueous and organic media can be easily formulated into nanosuspensions.
- Ease of scale-up and little batch-to-batch variation.
- Narrow size distribution of the final nano-sized product. A comparison of the size of naproxen crystals before and after media milling is given in Figure 2.
- Flexibility in handling the drug quantity, ranging from 1 to 400 mg mL^{-1}, enabling formulation of very dilute as well as highly concentrated nanosuspensions.

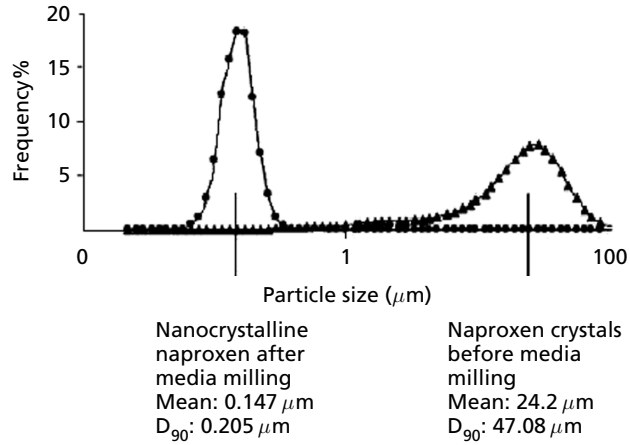

Figure 2 The particle size distribution of naproxen crystals before (▲) and after (●) milling. Before milling the drug crystals had a mean particle size of 24.2 μm. After being processed for 30 min in a media mill, the mean particle size of the nanocrystalline dispersion was 0.147 μm with $D_{90} = 0.205$ μm. The particle size measurements were generated using laser light diffraction in a Horiba LA-910 using polystyrene nanospheres ranging from 0.1 to 10 μm as standards. Reprinted from Merisko-Liversidge et al 2003 with permission from Elsevier Publications.

Disadvantages

- The major concern is the generation of residues of milling media, which may be introduced in the final product as a result of erosion (Buchmann et al 1996; Müller & Böhm 1998). This could be problematic when nanosuspensions are intended to be administered

for a chronic therapy. The severity of this problem has been reduced to a great extent with the advent of polystyrene resin-based milling medium. For this medium, residual monomers are typically 50 ppb and the residuals generated during the milling processing are not more than 0.005% w/w of the final product or the resulting solid dosage form.

High-pressure homogenizers (Disso Cubes)
Disso Cubes technology was developed by R. H. Müller (Müller et al 1998). The patent rights of Disso Cubes were initially owned by DDS (Drug Delivery Services) GmbH but currently they are owned by SkyePharma plc. Disso Cubes are engineered using piston-gap-type high-pressure homogenizers. A commonly used homogenizer is the APV Micron LAB 40 (APV Deutschland GmbH, Lubeck, Germany). However, other piston-gap homogenizers from Avestin (Avestin Inc., Ottawa, Canada) and Stansted (Stansted Fluid Power Ltd, Stansted, UK) can also be used. A high-pressure homogenizer (Figure 3) consists of a high-pressure plunger pump with a subsequent relief valve (homogenizing valve). The task of the plunger pump is to provide the energy level required for the relief. The relief valve consists of a fixed valve seat and an adjustable valve. These parts form an adjustable radial precision gap. The gap conditions, the resistance and thus the homogenizing pressure vary as a function of the force acting on the valve. An external impact ring forms a defined outlet cross-section and prevents the valve casing from being damaged due to the flow (Jahnke 1998). The instrument is available in discontinuous and continuous versions. The continuous version is suitable for optimizing the various parameters of the homogenization process. Use of the discontinuous version is sensible if the drug is very costly or of limited availability. The instrument can be operated at pressures varying from 100 to 1500 bars. In some instruments, a maximum pressure of 2000 bars can be reached. High-pressure homogenizers are available with different capacities ranging from 40 mL (for laboratory purposes) to a few thousand litres (for large-scale production). It is advisable to start with the micronized drug (particle size $< 25\,\mu$m) for production of nanosuspensions in order to prevent blocking of the homogenization gap. Hence, generally a jet-milled drug is employed as the starting material for producing Disso Cubes. Before subjecting the drug to the homogenization process, it is essential to form a presuspension of the micronized drug in a surfactant solution using high-speed stirrers. During the homogenization process, the drug suspension is pressed through the homogenization gap in order to achieve nano-sizing of the drug.

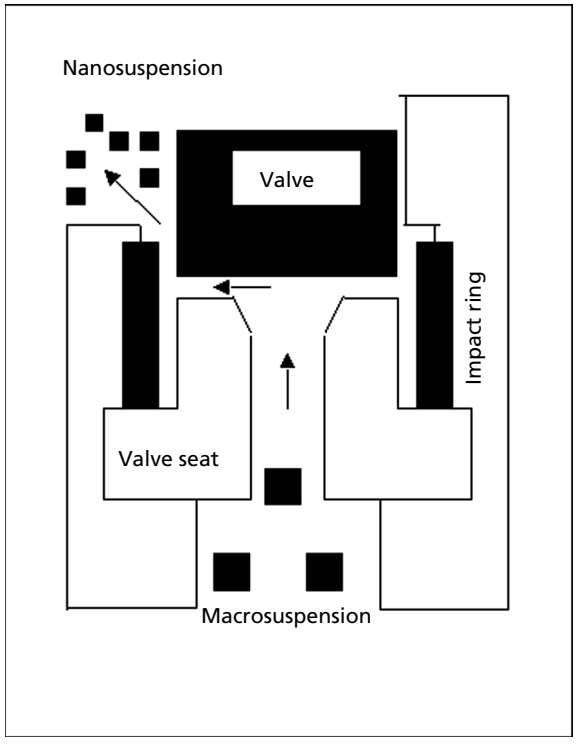

Figure 3 Schematic representation of the high-pressure homogenization process.

Principle During homogenization, the fracture of drug particles is brought about by cavitation, high-shear forces and the collision of the particles against each other. The drug suspension, contained in a cylinder of diameter about 3 mm, passes suddenly through a very narrow homogenization gap of 25 μm, which leads to a high streaming velocity.

In the homogenization gap, according to Bernoulli's equation, the dynamic pressure of the fluid increases with the simultaneous decrease in static pressure below the boiling point of water at room temperature. In consequence, water starts boiling at room temperature, leading to the formation of gas bubbles, which implode when the suspension leaves the gap (called cavitation) and normal air pressure is reached again. The implosion forces are sufficiently high to break down the drug microparticles into nanoparticles. Additionally, the collision of the particles at high speed helps to achieve the nano-sizing of the drug. To improve the efficiency of nano-sizing, the addition of viscosity enhancers is advantageous in certain cases as increasing the viscosity increases the powder density within the dispersion zone (homogenization gap).

In order to obtain an optimized formulation, the effect of the following process variables should be investigated.

- *Effect of homogenization pressure*. As the homogenizer can handle varying pressures, ranging from 100 to 1500 bars, the effect of the homogenization pressure on the particle size should be investigated in each case in order to optimize the process parameters. It is expected that the higher the homogenization pressure, the lower the particle size obtained. The studies carried out on RMKP 22, 4-[N-(2-hydroxy-2-methyl-propyl)-ethanolamino]-2,7-bis (*cis*-2,6-dimethylmorpholin-4-yl)-6-phenyl-pteridine, revealed that an inverse relationship exists between the homogenization pressure and the particle size (Müller & Böhm 1998; Müller & Peters 1998; Grau et al 2000).

- *Number of homogenization cycles*. For many drugs it is not possible to obtain the desired particle size in a single homogenization cycle. Typically, multiple cycles are required. Hence, depending on the hardness of the drug, the desired mean particle size and the required homogeneity of the product, homogenization can be carried out in three, five or 10 cycles. It is anticipated that the higher the number of homogenization cycles, the smaller the particle size obtained. The optimum number of homogenization cycles can be arrived at by analysing the particle size and polydispersity index of the drug after each cycle.

Advantages
- Drugs that are poorly soluble in both aqueous and organic media can be easily formulated into nanosuspensions.
- Ease of scale-up and little batch-to-batch variation (Grau et al 2000).
- Narrow size distribution of the nanoparticulate drug present in the final product (Müller & Böhm 1998).
- Allows aseptic production of nanosuspensions for parenteral administration.
- Flexibility in handling the drug quantity, ranging from 1 to 400 mg mL^{-1}, thus enabling formulation of very dilute as well as highly concentrated nanosuspensions (Krause & Müller 2001).

Disadvantages
- Prerequisite of micronized drug particles.
- Prerequisite of suspension formation using high-speed mixers before subjecting it to homogenization.

Emulsions as templates
Apart from the use of emulsions as a drug delivery vehicle, they can also be used as templates to produce nanosuspensions. The use of emulsions as templates is applicable for those drugs that are soluble in either volatile organic solvent or partially water-miscible solvent. Such solvents can be used as the dispersed phase of the emulsion. There are two ways of fabricating drug nanosuspensions by the emulsification method.

In the first method, an organic solvent or mixture of solvents loaded with the drug is dispersed in the aqueous phase containing suitable surfactants to form an emulsion. The organic phase is then evaporated under reduced pressure so that the drug particles precipitate instantaneously to form a nanosuspension stabilized by surfactants. Since one particle is formed in each emulsion droplet, it is possible to control the particle size of the nanosuspension by controlling the size of the emulsion. Optimizing the surfactant composition increases the intake of organic phase and ultimately the drug loading in the emulsion. Originally, organic solvents such as methylene chloride and chloroform were used (Bodmeier & McGinity 1998). However, environmental hazards and human safety concerns about residual solvents have limited their use in routine manufacturing processes. Relatively safer solvents such as ethyl acetate and ethyl formate can still be considered for use (Sah 1997, 2000).

Another method makes use of partially water-miscible solvents such as butyl lactate, benzyl alcohol and triacetin as the dispersed phase instead of hazardous solvents (Trotta et al 2001). The emulsion is formed by the conventional method and the drug nanosuspension is obtained by just diluting the emulsion. Dilution of the emulsion with water causes complete diffusion of the internal phase into the external phase, leading to instantaneous formation of a nanosuspension. The nanosuspension thus formed has to be made free of the internal phase and surfactants by means of diultrafiltration in order to make it suitable for administration. However, if all the ingredients that are used for the production of the nanosuspension are present in a concentration acceptable for the desired route of administration, then simple centrifugation or ultracentrifugation is sufficient to separate the nanosuspension.

Advantages
- Use of specialized equipment is not necessary.
- Particle size can easily be controlled by controlling the size of the emulsion droplet.
- Ease of scale-up if formulation is optimized properly.

Disadvantages
- Drugs that are poorly soluble in both aqueous and organic media cannot be formulated by this technique.
- Safety concerns because of the use of hazardous solvents in the process.
- Need for diultrafiltration for purification of the drug nanosuspension, which may render the process costly.
- High amount of surfactant/stabilizer is required as compared to the production techniques described earlier.

The production of drug nanosuspensions from emulsion templates has been successfully applied to the poorly water-soluble and poorly bioavailable anti-cancer drug mitotane, where a significant improvement in the dissolution rate of the drug (five-fold increase) as compared to the commercial product was observed (Trotta et al 2001).

Microemulsions as templates
Microemulsions are thermodynamically stable and isotropically clear dispersions of two immiscible liquids, such as oil and water, stabilized by an interfacial film of surfactant and co-surfactant (Eccleston 1992). Their advantages, such as high drug solubilization, long shelf-life and ease of manufacture, make them an ideal drug delivery vehicle. There are several research papers available that describe the use of microemulsions as drug delivery vehicles (Constantinides et al 1994, 1995; Kim et al 1998; Park & Kim 1999; Kawakami & Yoshikawa 2002). Recently, the use of microemulsions as templates for the production of solid lipid nanoparticles (Gasco 1997) and polymeric nanoparticles (Rades et al 2002) has been described. Taking advantage of the microemulsion structure, one can use microemulsions even for the production of nanosuspensions (Trotta et al 2003). Oil-in-water microemulsions are preferred for this purpose. The internal phase of these microemulsions could be either a partially miscible liquid or a suitable organic solvent, as described earlier.

The drug can be either loaded in the internal phase or pre-formed microemulsions can be saturated with the drug by intimate mixing. The suitable dilution of the microemulsion yields the drug nanosuspension by the mechanism described earlier. The influence of the amount and ratio of surfactant to co-surfactant on the uptake of internal phase and on the globule size of the microemulsion should be investigated and optimized in order to achieve the desired drug loading. The nanosuspension thus formed has to be made free of the internal phase and surfactants by means of diultrafiltration in order to make it suitable for administration. However, if all the ingredients that are used for the production of the nanosuspension are present in a concentration acceptable for the desired route of administration, then simple centrifugation or ultracentrifugation is sufficient to separate the nanosuspension.

The advantages and disadvantages are the same as for emulsion templates. The only added advantage is the need for less energy input for the production of nanosuspensions by virtue of microemulsions.

The production of drug nanosuspensions using microemulsions as templates has been successfully applied to the poorly water-soluble and poorly bioavailable antifungal drug griseofulvin, where a significant improvement in the dissolution rate of the drug (three-fold increase) as compared to the commercial product was observed. It was found that the nature of the co-surfactant affected the dissolution rate of the drug nanosuspension, as anticipated (Trotta et al 2003). However, this technique is still in its infancy and needs more thorough investigation.

Formulation considerations

Stabilizer

Stabilizer plays an important role in the formulation of nanosuspensions. In the absence of an appropriate stabilizer, the high surface energy of nano-sized particles can induce agglomeration or aggregation of the drug crystals. The main functions of a stabilizer are to wet the drug particles thoroughly, and to prevent Ostwald's ripening (Rawlins 1982; Müller & Böhm 1998) and agglomeration of nanosuspensions in order to yield a physically stable formulation by providing steric or ionic barriers. The type and amount of stabilizer has a pronounced effect on the physical stability and in-vivo behaviour of nanosuspensions. In some cases, a mixture of stabilizers is required to obtain a stable nanosuspension.

The drug-to-stabilizer ratio in the formulation may vary from 1:20 to 20:1 and should be investigated for a specific case. Stabilizers that have been explored so far include cellulosics, poloxamers, polysorbates, lecithins and povidones (Liversidge et al 1992). Lecithin is the stabilizer of choice if one intends to develop a parenterally acceptable and autoclavable nanosuspension.

Organic solvents

Organic solvents may be required in the formulation of nanosuspensions if they are to be prepared using an emulsion or microemulsion as a template. As these techniques are still in their infancy, elaborate information on formulation considerations is not available. The acceptability of the organic solvents in the pharmaceutical arena, their toxicity potential and the ease of their removal from the formulation need to be considered when formulating nanosuspensions using emulsions or microemulsions as templates. The pharmaceutically acceptable and less hazardous water-miscible solvents, such as ethanol and isopropanol, and partially water-miscible solvents, such as ethyl acetate, ethyl formate, butyl lactate, triacetin, propylene carbonate and benzyl alcohol, are preferred in the formulation over the conventional hazardous solvents, such as dichloromethane. Additionally, partially water-miscible organic solvents can be used as the internal phase of the microemulsion when the nanosuspensions are to be produced using a microemulsion as a template.

Co-surfactants

The choice of co-surfactant is critical when using microemulsions to formulate nanosuspensions. Since co-surfactants can greatly influence phase behaviour, the effect of co-surfactant on uptake of the internal phase for selected microemulsion composition and on drug loading should be investigated. Although the literature describes the use of bile salts and dipotassium glycerrhizinate as co-surfactants, various solubilizers, such as Transcutol, glycofurol, ethanol and isopropanol, can be safely used as co-surfactants in the formulation of microemulsions.

Other additives

Nanosuspensions may contain additives such as buffers, salts, polyols, osmogent and cryoprotectant, depending on either the route of administration or the properties of the drug moiety.

Post-production processing

Post-production processing of nanosuspensions becomes essential when the drug candidate is highly susceptible to hydrolytic cleavage or chemical degradation. Processing may also be required when the best possible stabilizer is not able to stabilize the nanosuspension for a longer period of time or there are acceptability restrictions with respect to the desired route. Considering these aspects, techniques such as lyophillization or spray drying may be employed to produce a dry powder of nano-sized drug particles. Rational selection has to be made in these unit operations considering the drug properties and economic aspects. Generally, spray drying is more economical and convenient than lyophillization. The effect of post-production processing on the particle size of the nanosuspension and moisture content of dried nano-sized drug should be given due consideration.

Advantages of nanosuspensions

Increase in the dissolution velocity and saturation solubility of the drug

This is an important advantage that makes nanosuspensions amenable to numerous applications. The reason

behind the increase in the dissolution velocity and saturation solubility of the nanosuspensions can be given as follows. According to the Nernst–Brunner and Levich modification of the Noyes Whitney dissolution model equation (Dressman et al 1998; Horter & Dressman 2001), the dissolution velocity of the nanosuspension increases due to a dramatic increase in the surface area of the drug particles from microns to particles of nanometer size:

$$dX/dt = ((D \times A)/h)\ (C_s - X/V)$$

where dX/dt is the dissolution velocity, D is the diffusion coefficient, A is the surface area of the particle, h is the diffusional distance, C_s is the saturation solubility of the drug, X is the concentration in the surrounding liquid and V is the volume of the dissolution medium.

In addition, as described by the Prandtl equation, the decrease in the diffusional distance with increasing curvature of ultrafine nano-sized particles contributes to the increase in the dissolution velocity. The Prandtl equation (Mosharraf & Nyström 1995) describes the hydrodynamic boundary layer thickness or diffusional distance (h_H) for flow passing a flat surface:

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

where L is the length of the surface in the direction of flow, k denotes a constant, V is the relative velocity of the flowing liquid against a flat surface and h_H is 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. This phenomenon is especially pronounced for materials that have mean particle size of less than 2 μm.

The increase in the saturation solubility of the drug with a decrease in particle size can be explained by Ostwald–Freundlich's equation:

$$\log(C_s/C_\alpha) = 2\sigma V/2.303RT\rho r$$

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

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 inside of the crystal are exposed to the aqueous dispersion medium during nanosizing. According to Ostwald–Freundlich, C_s is dependent on the interfacial tension σ and subsequently on the interfacial energy G ($G = \sigma A$). Differences in interfacial energy have a profound effect on the saturation solubilities of polymorphic forms of the drug; the same explanation 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 can be performed to quantify the increase in the saturation solubility of the drug when formulated into a nanosuspension. In a study carried out by Müller and Peters (1998), an increase in the saturation solubility of RMKP 22 with decrease in particle size was observed.

Improved biological performance

An increase in the dissolution velocity and saturation solubility of a drug leads to an improvement in the in-vivo performance of the drug irrespective of the route used. The advantages related to various routes are discussed later in detail.

Ease of manufacture and scale-up

Unlike nanoparticulate carriers such as polymeric nanoparticles, which were investigated earlier, nanosuspensions are easy to manufacture. The production processes described earlier are easily scaled up for commercial production. The introduction of nanosuspension products such as Rapamune and the NanoCrystal colloidal ketoprofen is sufficient to substantiate this.

Long-term physical stability

Another special feature of nanosuspensions is the absence of Ostwald ripening, which is suggestive of their long-term physical stability (Peters & Müller 1996). Ostwald ripening (Rawlins 1982; Müller & Böhm 1998) has been described for ultrafine dispersed systems and is responsible for crystal growth and subsequently formation of microparticles. Ostwald ripening is caused by the differences in dissolution pressure/saturation solubility between small and large particles. It is in practice an effect based on the higher saturation solubility of very small particles as compared to larger ones. Molecules diffuse from the higher concentrated area around small particles (higher saturation solubility) to areas around larger particles possessing a lower drug concentration. This leads to the formation of a supersaturated solution around the large particles and consequently to drug crystallization and growth of the large particles. The diffusion process of the drug from the small particles to the large particles leaves an area around the small particles that is not saturated any more, consequently leading to dissolution of the drug from the small particles and finally complete disappearance of the small particles. The lack of Ostwald ripening in nanosuspensions is attributed to their uniform particle size, which is created by various manufacturing processes. The absence of particles with large differences in their size in nanosuspensions prevents the existence of the different saturation solubilities and concentration gradients in the vicinity of differently sized particles, which in turn prevents the Ostwald ripening effect.

Versatility

The flexibility offered in the modification of surface properties and particle size, and ease of post-production processing of nanosuspensions enables them to be incorporated in various dosage forms, such as tablets, pellets, suppositories and hydrogels, for various routes of administration, thus proving their versatility.

Characterization of nanosuspensions

The essential characterization parameters for nanosuspensions are as follows.

- *Mean particle size and particle size distribution*. The mean particle size and the width of particle size distribution are important characterization parameters as they govern the saturation solubility, dissolution velocity, physical stability and even biological performance of nanosuspensions. It has been indicated by Müller & Peters (1998) that saturation solubility and dissolution velocity show considerable variation with the changing particle size of the drug.

  Photon correlation spectroscopy (PCS) (Müller & Müller 1984) can be used for rapid and accurate determination of the mean particle diameter of nanosuspensions. Moreover, PCS can even be used for determining the width of the particle size distribution (polydispersity index, PI). The PI is an important parameter that governs the physical stability of nanosuspensions and should be as low as possible for the long-term stability of nanosuspensions. A PI value of 0.1–0.25 indicates a fairly narrow size distribution whereas a PI value greater than 0.5 indicates a very broad distribution. No logarithmic normal distribution can definitely be attributed to such a high PI value. Although PCS is a versatile technique, because of its low measuring range (3 nm to 3 μm) it becomes difficult to determine the possibility of contamination of the nanosuspension by microparticulate drugs (having particle size greater than 3 μm). Hence, in addition to PCS analysis, laser diffractometry (LD) analysis of nanosuspensions should be carried out in order to detect as well as quantify the drug microparticles that might have been generated during the production process. Laser diffractometry yields a volume size distribution and can be used to measure particles ranging from 0.05–80 μm and in certain instruments particle sizes up to 2000 μm can be measured. The typical LD characterization includes determination of diameter 50% LD (50) and diameter 99% LD (99) values, which indicate that either 50 or 99% of the particles are below the indicated size. The LD analysis becomes critical for nanosuspensions that are meant for parenteral and pulmonary delivery. Even if the nanosuspension contains a small number of particles greater than 5–6 μm, there could be a possibility of capillary blockade or emboli formation, as the size of the smallest blood capillary is 5–6 μm. It should be noted that the particle size data of a nanosuspension obtained by LD and PCS analysis are not identical as LD data are volume based and the PCS mean diameter is the light intensity weighted size. The PCS mean diameter and the 50 or 99% diameter from the LD analysis are likely to differ, with LD data generally exhibiting higher values. The nanosuspensions can be suitably diluted with deionized water before carrying out PCS or LD analysis.

  For nanosuspensions that are intended for intravenous administration, particle size analysis by the Coulter counter technique is essential in addition to PCS and LD analysis. Since the Coulter counter gives the absolute number of particles per volume unit for the different size classes, it is a more efficient and appropriate technique than LD analysis for quantifying the contamination of nanosuspensions by microparticulate drugs.
- *Crystalline state and particle morphology*. The assessment of the crystalline state and particle morphology together helps in understanding the polymorphic or morphological changes that a drug might undergo when subjected to nanosizing. Additionally, when nanosuspensions are prepared drug particles in an amorphous state are likely to be generated. Hence, it is essential to investigate the extent of amorphous drug nanoparticles generated during the production of nanosuspensions. The changes in the physical state of the drug particles as well as the extent of the amorphous fraction can be determined by X-ray diffraction analysis (Müller & Böhm 1998; Müller & Grau 1998) and can be supplemented by differential scanning calorimetry (Shanthakumar et al 2004). In order to get an actual idea of particle morphology, scanning electron microscopy is preferred (Müller & Böhm 1998).
- *Particle charge (zeta potential)*. The determination of the zeta potential of a nanosuspension is essential as it gives an idea about the physical stability of the nanosuspension. The zeta potential of a nanosuspension is governed by both the stabilizer and the drug itself. In order to obtain a nanosuspension exhibiting good stability, for an electrostatically stabilized nanosuspension a minimum zeta potential of $\pm$30 mV is required whereas in the case of a combined electrostatic and steric stabilization, a minimum zeta potential of $\pm$20 mV is desirable (Müller & Jacobs 2002b).
- *Saturation solubility and dissolution velocity*. The determination of the saturation solubility and dissolution velocity is very important as these two parameters together help to anticipate any change in the in-vivo performance (blood profiles, plasma peaks and bioavailability) of the drug. As nanosuspensions are known to improve the saturation solubility of the drug, the determination of the saturation solubility rather than an increase in saturation solubility remains an important investigational parameter. The saturation solubility of the drug in different physiological buffers as well as at different temperatures should be assessed using methods described in the literature. The investigation of the dissolution velocity of nanosuspensions reflects the advantages that can be achieved over conventional formulations, especially when designing the sustained-release dosage forms based on nanoparticulate drugs. The dissolution velocity of drug nanosuspensions in various physiological buffers should be determined according to methods reported in the pharmacopoeia.
- *In-vivo biological performance*. The establishment of an in-vitro/in-vivo correlation and the monitoring of the in-vivo performance of the drug is an essential part of the study, irrespective of the route and the delivery system employed. It is of the utmost importance in the case of intravenously injected nanosuspensions since the

in-vivo behaviour of the drug depends on the organ distribution, which in turn depends on its surface properties, such as surface hydrophobicity and interactions with plasma proteins (Blunk et al 1993, 1996; Lück et al 1997a,b). In fact, the qualitative and quantitative composition of the protein absorption pattern observed after the intravenous injection of nanoparticles is recognized as the essential factor for organ distribution (Müller 1989; Blunk et al 1993, 1996; Lück et al 1997a,b). Hence, suitable techniques have to be used in order to evaluate the surface properties and protein interactions to get an idea of in-vivo behaviour. Techniques such as hydrophobic interaction chromatography can be used to determine surface hydrophobicity (Wallis & Müller 1993), whereas 2-D PAGE (Blunk et al 1993) can be employed for the quantitative and qualitative measurement of protein adsorption after intravenous injection of drug nanosuspensions in animals.

Applications of nanosuspensions in drug delivery

Oral drug delivery

The oral route is the preferred route for drug delivery because of its numerous well-known advantages. The efficacy or performance of the orally administered drug generally depends on its solubility and absorption through the gastrointestinal tract. Hence, a drug candidate that exhibits poor aqueous solubility and/or dissolution-rate limited absorption is believed to possess low and/or highly variable oral bioavailability. Owing to low oral bioavailability, such a drug candidate would have to be administered in a larger excess than actually required if it were completely bioavailable in order to achieve a therapeutically active concentration, thus making the therapy costly. Orally administered antibiotics such as atovaquone and bupravaquone reflect this problem very well. Nanosizing of such drugs can lead to a dramatic increase in their oral absorption and subsequently bioavailability. The amelioration in oral bioavailability can be attributed to the adhesiveness of the drug nanosuspension, increased surface area (due to reduction in particle size by 10–50-fold), increased saturation solubility, leading to an increased concentration gradient between the gastrointestinal tract lumen and blood, and increased dissolution velocity. This enhancement in bioavailability will lead to a subsequent reduction in drug dose, rendering the therapy cost-effective and obliterating any undue drug dumping in the body.

Some milestones Atovaquone, an antibiotic indicated for treating opportunistic *Pneumocystis carinii* infections in HIV patients, non-complicated *P. falciparum* malaria and leishmanial infections (Looareesuwan et al 1999), shows poor bioavailability (10–15%) because of its dissolution-rate limited absorption and has to be administered in high doses (750 mg twice daily). Administration of atovaquone as a nanosuspension resulted in a 2.5-fold increase in oral bioavailability as compared to the commercial product Wellvone, which contains the micronized drug (Schöler et al 2001).

Danazol, a poorly bioavailable gonadotropin inhibitor, showed a drastic improvement in bioavailability when administered as a nanosuspension as compared to the commercial danazol macrosuspension Danocrine (Liversidge & Cundy 1995). Danazol nanosuspension led to an absolute bioavailability of 82.3%, whereas the marketed danazol suspension Danocrine was 5.2% bioavailable. In addition, danazol nanosuspension resulted in a reduction in the intersubject variability and fed/fasted ratio of danazol (Figure 4).

Amphotericin B, a highly effective polyene antibiotic used for systemic mycoses and leishmaniasis lacks oral bioavailability. However, oral administration of amphotericin B as a nanosuspension produced a substantial improvement in its oral absorption in comparison to orally administered conventional commercial formulations such as Fungizone, AmBisome and micronized amphotericin B (Kayser et al 2003). Orally administered amphotericin B nanosuspension brought about a high uptake of nanoparticulate drug through the gastrointestinal tract. This is reflected by the considerable reduction it brought about in the number of *L. donovani* parasites in the liver of infected female Balb/c mice as compared to other commercial formulations (Figure 5).

Nanosuspensions are also advantageous in achieving quick onset of action for drugs that are completely but slowly absorbed, i.e. those having high t_{max} values. This is illustrated by the study carried out for naproxen, a nonsteroidal anti-inflammatory drug. A dosage form with fast onset of action would be highly desirable for naproxen. A study involving a comparison of the pharmacokinetic profiles of naproxen in the form of nanosuspension, suspension (Naprosyn) and tablet (Anaprox) forms revealed that the time required to achieve C_{max} was reduced by approximately 50% for the nanosuspension compared to the suspension and tablet (Table 1). Additionally, naproxen nanosuspension resulted in a 2.5–4.5-fold increase in the

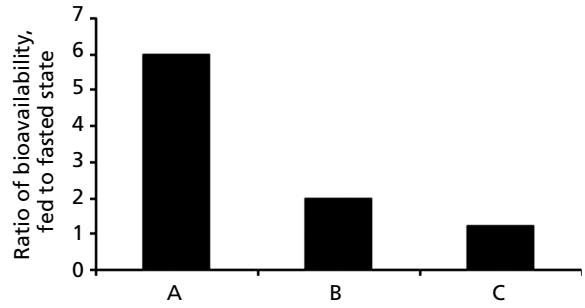

Figure 4 Nanocrystalline particles can reduce the absorption variability resulting from the presence or absence of food. The data compare the performance of various dosage forms of danazol administered to volunteers in the fed and fasted state at a 200 mg dose. The variability observed in the commercial product was significantly lowered when danazol was formulated using nanoparticles and administered as a liquid dispersion or a dry filled capsule. A = marketed product; B = nanocrystalline capsule; C = nanocrystalline dispersion. Reprinted from Merisko-Liversidge et al 2003 with permission from Elsevier Publications.

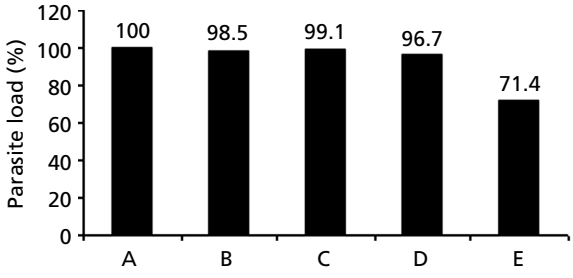

Figure 5 Percentage reduction of *Leishmania donovani* parasite load in livers of infected Balb/c mice. A = untreated control; B = Abisome; C = fungizone; D = amphotericin B (micronized); E = amphotericin B (nanosuspension). Reprinted from Kayser et al 2003 with permission from Elsevier Publications.

AUCs during the first hour of the study (Liversidge & Conzentino 1995; Merisko-Liversidge et al 2003).

Apart from improving oral absorption, nanosuspensions offer the following advantages:

- improved dose proportionality
- reduced fed/fasted state variability (Figure 5)
- reduced inter-subject variability.

Numerous drug candidates that are poorly water-soluble are required to be taken over a prolonged period of time for effective medication. However, many of them cannot be formulated into sustained-release dosage forms because of the risk of dose dumping and poor in-vivo performance. Although approaches such as a change in microenvironment and complexation with cyclodextrins have resulted in the successful incorporation of some poorly water-soluble drugs in sustained-release dosage forms (Chowdhary et al 2003), these solutions are not applicable to all poorly water-soluble drugs. Nanosuspensions, on the other hand, enable incorporation of all hydrophobic drugs in well-established sustained-release technologies. However, while doing so, the effect and the interaction of dosage form excipients with the nanocrystalline drug must be critically investigated. Drug nanosuspensions can also be incorporated into dosage forms such as tablets, capsules and fast melts by means of standard manufacturing techniques. Ketoprofen nanosuspension has been successfully incorporated into pellets to release the drug over a period of 24 h (Remon et al 2001).

In spite of the tremendous potential of nanosuspensions in oral delivery, formulating compounds as nanocrystalline dispersions is not of value when metabolic and/or permeation-related issues affect bioavailability. However, in future, it should be possible to engineer nanosuspensions by using the agents that enhance permeation (Aungst 2000) and/or minimize gut-related metabolic issues (Kusuhara et al 1998; Benet et al 1999). This amalgamated approach would facilitate delivery of the compounds belonging to BCS Class IV that exhibit poor water solubility and poor membrane permeability.

Parenteral drug delivery

The parenteral route is an invasive route. Parenteral administration of drugs is critical and often associated with the problems such as the limited number of acceptable excipients, restrictions on the quantities of excipients approved for parenteral use, the stringent requirements of the aseptic production process, safety issues, patient noncompliance and biological problems such as allergic reactions and thrombophlebitis. Despite all these limitations, the parenteral route still retains its value because of its special advantages, such as quick onset of action in case of emergency, reduction in dose of the drug and the ability to target the drug quickly to the desired site of action, especially in the case of severe infections. The parenteral route is often employed as an alternative when the drug is either not absorbed through the gastrointestinal tract or undergoes extensive first-pass metabolism.

For administration by the parenteral route, the drug either has to be solubilized or have particle/globule size below 5 μm to avoid the capillary blockade. The current approaches for parenteral delivery include salt formation, solubilization using co-solvents, micellar solutions (Kim et al 2001), complexation with cyclodextrins and recently liposomes (Dupont 2002). However, there are limitations on the use of these approaches because of limitations on their solubilization capacity and parenteral acceptability. In this regard, liposomes are much more tolerable and versatile in terms of parenteral delivery. However, they often suffer from problems such as physical instability, high manufacturing cost and difficulties in scale-up.

Nanosuspensions appear to be a unique approach to solving the problems mentioned above. From the formulation perspective, nanosuspensions meet almost all the requirements of an ideal drug delivery system for the

Table 1 Human pharmacokinetics of naproxen following oral administration of a nanocrystalline naproxen suspension, Naprosyn suspension and Anaprox tablets in the fed state

Pharmacokinetic parameter	Nanocrystalline naproxen	Naprosyn	Anaprox
t_{max} (h)	1.69 ± 1.05	3.33 ± 1.32	3.2 ± 1.68
AUC 0–1 h ($mg\,h^{-1}\,L^{-1}$)	33.49 ± 9.12 (27.3% CV)	13.38 ± 8.1 (61.1% CV)	7.41 ± 8.94 (120.7% CV)
C_{max} ($mg\,L^{-1}$)	50.89 ± 6.03	49.09 ± 6.44	53.45 ± 7.16
$t_{1/2}$ (h)	16.18 ± 4.42	16.16 ± 2.81	14.82 ± 3.55

Dose: 500 mg/volunteer; n = 23. Reprinted from Merisko-Liversidge et al 2003 with permission from Elsevier Publications.

parenteral route. Since the drug particles are directly nanosized, it becomes easy to process almost all drugs for parenteral administration. Moreover, the absence of any harsh solvents/co-solvents and/or any potentially toxic ingredient in nanosuspensions enables them to bypass the limitations of parenteral administration attributed to conventional formulations strategies. Hence, nanosuspensions enable significant improvement in the parenterally tolerable dose of the drug, leading to a reduction in the cost of the therapy and also improved therapeutic performance.

The maximum tolerable dose of paclitaxel nanosuspension was found to be three times higher than the currently marketed Taxol, which uses Cremophore EL and ethanol to solubilize the drug. In the MV-522 human lung xenograft murine tumour model, paclitaxel nanosuspensions at doses of 90 and 100 mg kg^{-1} showed no cases of death ($n = 9$) whereas Taxol at a concentration of 30 mg kg^{-1} showed a 22% death rate (Merisko-Liversidge et al 1996, 2003). Similarly, the nanosuspensions of other anti-cancer agents, such as etoposide and camptothecin, revealed an improvement in the tolerance level of the drug compared to the marketed preparations.

In addition, nanosuspensions have been found to increase the efficacy of the parenterally administered drug (Merisko-Liversidge et al 2003). A comparison of the efficacy of paclitaxel nanosuspension with Taxol using the mammary 16-C murine tumour model ($n = 5$ for each experimental group) revealed the superiority of paclitaxel nanosuspension over Taxol in reducing the median tumour burden (Merisko-Liversidge et al 2003). Similarly, aphidicolin, a poorly water-soluble new anti-parasitic lead molecule, when administered as nanosuspension revealed an improvement in EC_{50} from 200 to 40 ng mL^{-1} in comparison to DMSO-dissolved drug (Kayser 2000). Recently, clofazimine, a poorly water-soluble anti-leprotic drug, has been successfully formulated as a nanosuspension. Clofazimine nanosuspension revealed an improvement in stability and efficacy over the liposomal clofazimine in *M. avium*-infected female mice (Peters et al 2000).

It is noteworthy that after intravenous administration of a nanosuspension, the drug nanoparticles are sequestered by mononuclear phagocytic system (MPS) cells and Kupffer cells, as observed in the case of various other colloidal drug carriers (Illum et al 1982; Juliano 1988; Toster et al 1990; Stolnik et al 1995; Neal et al 1998; Liu et al 2000; Moghimi et al 2001).

The particles are recognized as being foreign bodies and are phagocytosed by the macrophages mainly in the liver (60–90%), spleen (approximately 1–5%) and to a very small extent in the lungs. Since this uptake by macrophage is a natural process, it is referred to as 'natural targeting' in the literature. Natural targeting does not affect the safety profile of the drug. In fact, it helps in increasing the drug tolerance as MPS cells or Kupffer cells can act as a controlled-release vehicle for the drug, enabling its prolonged action (Martin et al 1982; Adachi et al 1992; Soma et al 2000).

Sequestration by MPS is believed to be the reason for the increased efficacy of the antibiotics and anti-infectives in nanosuspension form as they are naturally targeted to the macrophages that are the mainstays of various bacterial and fungal infections. Moreover, in order to achieve macrophage targeting in a rapid manner, the surface properties of nanosuspensions could be modulated in a controlled way to alter the plasma protein adsorption pattern. A multitude of factors, such as the physical properties of the drug particles, the dose, the infusion time, the intrinsic solubility of the drug in the hemodynamic pool of the blood, the drug–plasma protein interaction, the plasma protein interaction pattern and the phenomenon of natural targeting influence the biodistribution and pharmacokinetic profile of the nanoparticulate drug after parenteral administration. Nanosuspensions can be administered via different parenteral routes, ranging from intra-articular to intraperitoneal to intravenous injection.

Currently, studies are in progress to identify strategies for manipulating the surface properties, size and shape of drug nanosuspensions in order to eliminate sequestration by the phagocytic cells of MPS-enriched organs whenever desired.

Ocular drug delivery

Nanosuspensions can prove to be a boon for drugs that exhibit poor solubility in lachrymal fluids. For delivery of such drugs, approaches such as suspensions and ointments have been recommended. Although suspensions offer advantages such as prolonged residence time in a cul-de-sac (which is desirable for most ocular diseases for effective treatment) and avoidance of the high tonicity created by water-soluble drugs, their actual performance depends on the intrinsic solubility of the drug in lachrymal fluids. Thus, the intrinsic dissolution rate of the drug in lachrymal fluid governs its release and ocular bioavailability. However, the intrinsic dissolution rate of the drug will vary because of the constant inflow and outflow of lachrymal fluids. Hence, suspensions may fail to give consistent performance. However, nanosuspensions, by their inherent ability to improve the saturation solubility of the drug, represent an ideal approach for ocular delivery of hydrophobic drugs. Moreover, the nanoparticulate nature of the drug allows its prolonged residence in the cul-de-sac, giving sustained release of the drug. To achieve sustained release of the drug for a stipulated time period, nanosuspensions can be incorporated in a suitable hydrogel base or mucoadhesive base or even in ocular inserts. An approach that has recently been investigated to achieve the desired duration of action of the drug is the formulation of polymeric nanosuspensions loaded with the drug. The bioerodible as well as water-soluble/permeable polymers possessing ocular tolerability (Pignatello et al 2002a) could be used to sustain the release of the medication. The nanosuspensions can be formulated using the quasi-emulsion and solvent diffusion method. The polymeric nanosuspensions of flurbiprofen and ibuprofen have been successfully formulated using acrylate polymers such as Eudragit RS 100 and Eudragit RL 100 (Bucolo et al 2002; Pignatello et al 2002b,c). The polymeric nanosuspensions have been characterized for drug loading, particle size, zeta potential, in-vitro drug release, ocular tolerability and in-vivo biological performance. The designed polymeric

nanosuspensions revealed superior in-vivo performance over the existing marketed formulations and could sustain drug release for 24 h. The scope of this strategy could be extended by using various polymers with ocular tolerability.

Pulmonary drug delivery

Nanosuspensions may prove to be an ideal approach for delivering drugs that exhibit poor solubility in pulmonary secretions. Currently such drugs are delivered as suspension aerosols or as dry powders by means of dry powder inhalers. The drugs used in suspension aerosols and dry powder inhalers are often jet milled and have particle sizes of microns. Because of the microparticulate nature and wide particle size distribution of the drug moiety present in suspension aerosols and dry powder inhalers, the following disadvantages are encountered:

- limited diffusion and dissolution of the drug at the site of action because of its poor solubility and microparticulate nature, which may affect the bioavailability of the drug
- rapid clearance of the drug from the lungs because of ciliary movements (Müller & Jacobs 2002b)
- less residence time for the drugs, leading to absence of prolonged effect
- unwanted deposition of the drug particles in pharynx and mouth.

Nanosuspensions can solve the problems associated with conventional systems because of their versatile nature. The nanoparticulate nature of the drug allows the rapid diffusion and dissolution of the drug at the site of action. At the same time, the increased adhesiveness of the drug to mucosal surfaces (Ponchel et al 1997) offers a prolonged residence time for the drug at the absorption site. This ability of nanosuspensions to offer quick onset of action initially and then controlled release of the active moiety is highly beneficial and is required by most pulmonary diseases. Moreover, as nanosuspensions generally contain a very low fraction of microparticulate drug, they prevent unwanted deposition of particles in the mouth and pharynx, leading to decreased local and systemic side-effects of the drug.

Additionally, because of the nanoparticulate nature and uniform size distribution of nanosuspensions, it is very likely that in each aerosol droplet at least one drug nanoparticle is contained, leading to even distribution of the drug in the lungs as compared to the microparticulate form of the drug. In conventional suspension aerosols many droplets are drug free and others are highly loaded with the drug, leading to uneven delivery and distribution of the drug in the lungs. Nanosuspensions could be used in all available types of nebulizer. However, the extent of influence exerted by the nebulizer type as well as the nebulization process on the particle size of nanosuspensions should be ascertained.

Budesonide, a poorly water-soluble corticosteroid, has been successfully formulated as a nanosuspension for pulmonary delivery (Müller & Jacobs 2002b). A good relationship was obtained between increasing the drug concentration in the formulation and the number of micrograms of drug delivered per 2 s actuation.

Targeted drug delivery

The need to target drugs to specific sites is increasing day by day as a result of therapeutic and economic factors. Nanoparticulate systems have shown tremendous potential in targeted drug delivery, especially to the brain (Schröeder et al 1998; Kreuter 2001). Successful targeting of the peptide dalargin to the brain by employing surface-modified polyisobutyl cyanoacrylate nanoparticles has been a major achievement in targeted delivery (Kreuter et al 1997).

Likewise, nanosuspensions can be used for targeted delivery as their surface properties and in-vivo behaviour can easily be altered by changing either the stabilizer or the milieu. Their versatility and ease of scale-up and commercial production enables the development of commercially viable nanosuspensions for targeted delivery. Natural targeting of MPS by nanosuspensions has already been described. However, the natural targeting process could pose an obstacle when macrophages are not the desired targets. Hence, in order to bypass the phagocytic uptake of the drug, its surface properties need to be altered, as in the case of stealth liposomes (Gregoriades 1995; Allen 1997; Lasic et al 1997; Papisov 1998; Woodle 1998; Vyas et al 2000). The engineering of stealth nanosuspensions (analogous to stealth liposomes) by using various surface coatings for active or passive targeting of the desired site is the future of targeted drug delivery systems.

Another good example is targeting of *Cryptosporidium parvum*, the organism responsible for cryptosporidiosis, by using surface-modified mucoadhesive nanosuspensions of bupravaquone (Kayser 2001; Müller & Jacobs 2002a). A considerable difference has been observed in the efficacy of bupravaquone nanosuspensions when delivered with and without mucoadhesive polymers (Kayser 2001; Müller & Jacobs 2002a). Mucoadhesive bupravaquone nanosuspensions, because of their prolonged residence at the infection site, revealed a 10-fold reduction in the infectivity score of *Cryptosporidium parvum* as compared to the bupravaquone nanosuspensions without mucoadhesive polymers (Table 2). Similarly, conditions such as pulmonary aspergillosis can easily be targeted by using suitable

Table 2 Anticryptosporidial activity of bupravaquone and its formulation as a nanosuspension in neonatal mice

Compound formulation	Infectivity score	
	Ileum	Caecum
Bupravaquone in DMSO	1.56 ± 0.18	0.46 ± 0.7
Bupravaquone nanosuspension	1.17 ± 0.15	0.67 ± 0.12
Bupravaquone mucoadhesive nanosuspension	0.17 ± 0.12	0.17 ± 0.14
PBS (control)	2.0 ± 0.2	2.0 ± 0.2

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drug candidates, such as amphotericin B, in the form of pulmonary nanosuspensions instead of using stealth liposomes (Kohno et al 1997). With the advent of polymeric nanosuspensions loaded with drug (as described in the section on ocular delivery), it should be possible to target sites, such as the colon, or bacteria, such as *H. pylori*, by suitable modifications in the formulation strategy. Overall, nanosuspensions have indicated a good potential in targeted drug delivery but this has yet to be fulfilled.

Conclusion

Nanosuspensions appear to be a unique and yet commercially viable approach to combating problems such as poor bioavailability that are associated with the delivery of hydrophobic drugs, including those that are poorly soluble in aqueous as well as organic media. Production techniques such as media milling and high-pressure homogenization have been successfully employed for large-scale production of nanosuspensions. The advances in production methodologies using emulsions or microemulsions as templates have provided still simpler approaches for production but with limitations. Further investigation in this regard is still essential. Attractive features, such as increased dissolution velocity, increased saturation solubility, improved bioadhesivity, versatility in surface modification and ease of post-production processing, have widened the applications of nanosuspensions for various routes. The applications of nanosuspensions in parenteral and oral routes have been very well investigated and applications in pulmonary and ocular delivery have been realized. However, their applications in buccal, nasal and topical delivery are still awaiting exploration. The development of stealth nanosuspensions laced with functionalized surface coatings capable of eliciting passive or active targeting as per the requirement can be regarded as the future step in the nanosuspension research.

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