REVIEW

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Nanosuspensions as the most promising approach in nanoparticulate drug delivery systems

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Over the years, controlled drug delivery as well as site-specific delivery have made considerable advances. One area that contributed significantly to this progress is the rapidly developing field of colloidal drug delivery systems. Nanoparticles, one of the colloidal drug delivery systems, may enable new possibilities for therapy that presently have not been investigated. Recent advances in nanoparticle research are discussed here. The present review highlights new and upcoming developments such as nanosuspensions and solid lipid nanoparticles.

1. Introduction

Nanoparticles represent interesting alternatives to liposomes as drug delivery systems. Due to their similar size they may be used for similar purposes, while liposomes have some advantages because they consist of materials that are present as natural materials in the human body. The higher stability of nanoparticles yields longer shelf storage time as well as a better persistence of the particles in the body after administration. In addition it enables the administration by routes that are not practicable for liposomes, such as the peroral route. Compared to the efforts put into the discovery of new chemical entities, relatively little efforts have been put into the investigation of introducing nanoparticulate drug carriers into medical practice.
The Greek word 'nanos' means 'dwarf'. Nano means it is the factor of 10^{-9} or one billionth. For example, some comparisons of nano-scale are given here;

- 0.1 nm = Diameter of one hydrogen atom.
- 2.5 nm = Width of a DNA molecule.
- 800 nm = Diameter of a human red blood corpuscle.

Nanoparticles are solid colloidal particles ranging in size from 10 nm to 1000 nm. They consist of macromolecular materials in which the active principle is dissolved, entrapped or encapsulated, and/or to which the active principle is absorbed or attached (Kreuter 1983). Nanoparticles have been studied extensively as particulate carriers in several pharmaceutical and medical fields. The group of Speiser initiated the research in the 1970s. They were initially devised as carriers for vaccines and anticancer drugs (Couvreur et al. 1982). Nanoparticulate technology is progressing through the development of new approaches in the field of drug delivery.

2. Applications of nanoparticles

Nanoparticles, in general, can be used to provide targeted (cellular/tissue) delivery of drugs, to sustain drug effect in target tissue (Ravi Kumar and Majeti 2000), to improve oral bioavailability (Jia et al. 2003) to solubilize drugs for intra-vascular delivery and to improve the stability of therapeutic agents against enzymatic degradation. Nanoparticles formulated as amorphous spheres show higher solubility than standard crystalline formulations, thus improving the poor aqueous solubility of the drug and hence its bioavailability. Nanoparticles can be formulated as injections consisting of spherical amorphous particles which do not aggregate, hence they can be safely administered by the intravenous route. Since no co-solvent is used to solubilize the drug, the overall toxicity of the formulation is reduced.
Peptides, virus subunits and antigens produced by genetic engineering procedures, are weak antigens and produce little or no protection. Hence, suitable adjuvants are needed to render these antigens potent enough to be useful for vaccines. Poly (methyl methacrylate) nanoparticles are biodegraded at a very slow rate. For this reason, they are suitable as adjuvants for vaccines when the achievement of a very prolonged immune response is desired. Polystyrene nanoparticles are non-biodegradable. For this reason, they are mainly used as immunosorbents for basic biodistribution and vaccination studies (Hiremath and Hota 1999).
Nanoparticles represent a very promising carrier system for the targeting of anti-cancer agents to tumors as they exhibit a significant tendency to accumulate in a number of tumors after intravenous injection. The first anticancer drug bound to nanoparticles was actinomycin D. Over the last 20 years, considerable progress has been made in the preparation of well-characterized nanoparticle formulations

loaded with a variety of anticancer agents. It seems possible that nanoparticles will have interesting applications in limited tumors such as those of the mononuclear phagocyte system (e.g., monocytic leukemia, hairy cell leukemia) or for activating macrophages tumoricidal properties (Leroux et al. 1996). Among the mononuclear phagocyte system cells (MPS), the macrophages of the liver, called kupffer cells, are normally the most efficient cells for the phagocytosis of injected particles, polymethacrylic nanoparticles have thus been designed for passively targeting the anticancer drug doxorubicin to the liver to reduce toxicity and increase the therapeutic activity.

The development of appropriate vehicles to deliver new macromolecules coming out of the biotech industry is a challenge for pharmaceutical scientists. In many cases peptides are quite efficiently bound to nanoparticles. Oral insulin nanoparticles produced significant prolongation of the blood glucose level reduction. Chitosan based nanoparticles have been shown to enhance the transport of peptides and proteins through various mucosal barriers including intestinal, nasal and ocular mucosa (Janes et al. 2001). Chitosan coated poly(lactic-co-glycolic acid) [PLGA] mucoadhesive nanoparticles, were found to be useful peptide carriers for improving oral mucosal delivery due to their prolonged retention in the gastro-intestinal tract and excellent penetration into the mucus layer (Takeuchi et al. 2001). Nanoparticles composed of novel graft copolymers having a hydrophobic backbone and hydrophilic branches can improve the absorption of salmon calcitonin in rats (Sakuma et al. 1997a and 1997b). Nanoparticles were also studied for the delivery of antisense oligonucleotides. Oligonucleotides associated to nanoparticles were shown to be protected against degradation and to penetrate more easily into different types of cells.

The first experiments with the peroral administration of a nanoparticle bound drug, vincamine, were carried out by Maincent et al. They administered poly(hexyl cyanoacrylate) nanoparticles about 230 nm in size with bound vincamine to rabbits and determined the bioavailability (Maincent et al. 1986).

Nanoparticles are also used for ophthalmic drug delivery. The first nanoparticulate system with pilocarpine was a cellulose acetate hydrogen phthalate (CAP) pseudolatex formulation developed by Gurny et al. (Gurny 1981a), Piloplex® was one of the first commercial exploration of nanoparticle formulations in ocular drug delivery. The formulation consists of pilocarpine loaded nanospheres of poly(methyl methacrylate acrylic acid) copolymer. Since then many nanoparticles systems have been investigated for the prolongation of contact time in order to increase the ocular absorption (Wilson et al. 2001).

Nanoparticles are useful delivery systems for the treatment of a number of intra-cellular infections. Cells of the reticuloendothelial system are frequently infected by both strains of the human immuno-deficiency virus, HIV-1 and HIV-2. Nanoparticles hold great promise for targeting antiviral drugs to infected cells of the reticuloendothelial system.

Nanoparticles represent a tool to transport essential drugs across the blood-brain barrier (BBB) that normally are unable to cross this barrier. Drugs that have successfully been transported into the brain using this carrier include the hexapeptide dalargin, loperamide, tubocurarine, and doxorubicin. Nanoparticle mediated drug transport to the brain depends on the overcoating of the particles with polysorbates, especially polysorbate 80. Poly(butyl cyanoacrylate) nanoparticles were so far successfully used for the *in vivo* delivery of drugs to the brain. This polymer has the advantage that it is very rapidly biodegradable (Grislain et al. 1983).

Nanoparticles hold promise for the targeted delivery of drugs to inflammed areas of the body after administration by a number of possible routes. Nanoparticles have also been investigated for lymphatic targeting. Nanocapsules may have the potential to deliver drugs to the lymph node through tissue spaces by local administration.

The cosmetic applications of nanoparticles are currently under investigation. A cosmetic product containing nanocapsules of vitamin E, Primordiale® has recently been launched.

3. Polymeric nanoparticles

Polymeric nanoparticles invented by Speiser et al. as drug delivery systems usually exhibit a long shelf life and a good stability on storage. Nanoparticles are superior to liposomes in targeting them to specific organs or tissues by adsorbing and coating their surface with different substances (Kreuter 1994).

Polymeric nanoparticles are solid colloidal particles consisting of non-biodegradable synthetic polymers or biodegradable macromolecular materials of synthetic, semisynthetic or natural origin. According to the process used for the preparation of nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are vesicular systems in which the drug is confined to a cavity surrounded by a unique polymeric membrane. Nanospheres are matrix systems in which the drug is dispersed throughout the particles. Although both types of active ingredients may be incorporated, most often they are hydrophilic in the case of nanospheres and lipophilic in the case of nanocapsules. Polymeric nanoparticles including nanospheres and nanocapsules can be prepared according to numerous methods. The development of these methods occurred in several steps. Historically, the first nanoparticles proposed as carriers for therapeutic applications were made of gelatin and cross-linked albumin (Scheffel et al. 1972; Marty et al. 1978). Then, to avoid the use of proteins which may stimulate the immune system and to limit the toxicity of the cross-linking agents, nanoparticles made from synthetic polymers were developed. At first, the nanoparticles were made by emulsion polymerization of acrylamide and by dispersion polymerization of methyl methacrylate. However, nanoparticles were rapidly substituted by particles made of biodegradable synthetic polymers.

Nanoparticles can be prepared either from preformed polymers, such as polyesters (i.e. polylactic acid), or from a monomer during its polymerization, as in the case of alkyl-cyanoacrylates. Most of the methods based on the polymerization of monomers consists in adding a monomer into the dispersed phase of an emulsion (Couvreur et al. 1979), an inverse microemulsion (Birrenbach and Speiser 1976), or dissolved in a non-solvent of the polymer. In the preformed category, nanoparticles are formed by the precipitation of synthetic polymers or by denaturation or gelification of natural macromolecules. Two main approaches have been proposed for the preparation of nanoparticles of synthetic polymers. The first one is based on the emulsification of the water-immiscible organic solution of the polymer by an aqueous phase containing the surfactant, followed by solvent evaporation (Gurny et al. 1981b). The second approach is based on the precipitation of a polymer after addition of a non-solvent of the polymer (Ammoury et al. 1990). Concerning nanoparticles formed of natural macromolecules, nanoparticles can be

obtained by thermal denaturation of proteins (such as albumin) (Kramer 1974) or by a gelification process, as in the case of alginates.
The choice of appropriate polymer, particle size, and manufacturing process will primarily depend on the bio-acceptability of the polymer, followed by physicochemical properties of the drug and the therapeutic objective. Drug or any biologically active compound can be dissolved, entrapped or encapsulated into the nanoparticle or simply adsorbed onto its surface. Drug release from these carriers is dependent on both the type of carrier and the loading mechanism involved.
The major disadvantages of polymeric nanoparticles are their relatively slow biodegradability, which might cause systemic toxicity. Apart from polymer accumulation on repeated administration, toxic metabolites may be formed during the biotransformation of polymeric carriers, for example, formaldehyde as a metabolite of polycyanoacrylates (Kante et al. 1982). The presence of residual toxic agents (organic solvents) employed during preparation and lack of reproducibility of preparation method are some problems. Polymeric nanoparticles cannot be sterilised by autoclaving. They need to be sterilised by γ radiation. However, this treatment causes the formation of unacceptable toxic reaction products (Utreja and Jain 2001). The formation of larger polymer particles and lumps cannot be avoided totally in large scale production of nanoparticles (Kreuter 1990). The system also suffers from the lack of a cost-effective large scale production method yielding a product of a quality being acceptable by the regulatory authorities. Also polymeric nanoparticles possess limited drug loading capacity.

4. Solid lipid nanoparticles (SLN)

Solid lipid nanoparticles have been developed as alternative delivery system to conventional polymeric nanoparticles (Müller et al. 2000). The solid lipid nanoparticles (SLN) are sub-micron colloidal carriers (50–1000 nm) which are composed of a physiological lipid, dispersed in water or in an aqueous surfactant solution. Solid lipid nanoparticles combine advantages of polymeric nanoparticles, fat emulsions and liposomes, and avoid some of their disadvantages. They are biodegradable, biocompatible and non-toxic. The possibility of large scale production of SLN is an important feature (Müller 1999 and 2000). Advantages of SLN are:

- Avoidance of coalescence leads to enhanced physical stability.
- Reduced mobility of incorporated drug molecules leads to reduction of drug leakage.
- Static interface solid/liquid facilitates surface modification.

Speiser et al. developed lipid nanopellets for persorption by melt-emulsification. The lipid melt is dispersed in the aqueous phase by high-speed stirring and sonication. High shear homogenization (Lippacher et al. 2002; Yang and Zhu 2002) and ultrasound are dispersing techniques which were initially used for the production of solid lipid nanodispersions. The high-pressure homogenization technique is superior to sonication. Hot homogenization is carried out at temperatures above the melting point of the lipid and can therefore be regarded as the homogenization of an emulsion. Solid particles are expected to be formed by cooling the sample. Cold homogenization has been developed to overcome the problems with the hot homogenization technique.
Sjostrom and Bergenstahl described a method to prepare nanoparticle dispersions by precipitation in o/w emulsions (Sjostrom and Bergenstahl 1992; Sjostrom et al. 1993). Gasco developed a SLN preparation technique based on the dilution of micro-emulsions (Gasco 1993). Different drugs like timolol (Gasco et al. 1992), idarubicin (Zara et al. 2002), clobetasol propionate (Hu et al. 2002), have been studied regarding their incorporation into solid lipid nanoparticles.
The drug release can be controlled by varying the carrier matrices as well as by the choice of emulsifier. Besides parenteral administration, solid lipid nanoparticles are also suitable for other routes of administration and might be an interesting carrier system for the peroral administration of poorly water-soluble drugs with low peroral bioavailability. An advantage of the emulsified lipid particles might be their improved wettability in gastrointestinal fluids.
Potential problems associated with solid lipid nanoparticles are limited drug loading capacity, adjustment of drug release profile and potential drug expulsion during storage (Müller et al. 2002a; Olbrich et al. 2000. Low drug loading capacities due to the crystallinity of the lipids was found, especially when monoacid triglycerides were used. Knowledge regarding the recrystallization behaviour of the dispersed lipid melt, the time course of polymorphic transitions and the degree of crystallinity are very important to evaluate the stability of solid lipid nanoparticles (Westesen et al. 1996). Because of limited stability of SLN as aqueous dispersion, it is highly desirable to have a freeze dried product with a good reconstitution behaviour.
As mentioned above, a disadvantage of SLN is drug expulsion during storage by formation of more perfect lipid crystals (formation of an increased percentage of β-modification in the particle). This problem has been overcome by the new generation of solid lipid nanoparticles, the so called nanostructured lipid carriers (NLC). These particles are characterized as forming on purpose an imperfect lipid particle matrix. This matrix gives much more room to incorporate drugs, the drug loading is increased. To achieve this, spatially very different lipid molecules are used for particle production. The "old" SLN are made from a solid lipid and subsequent melting and homogenization. The NLC are made by mixing a solid lipid with a liquid lipid (oil), these lipid molecules are spatially so different that they form imperfect matrices. Of course, the blend needs to be chosen in a way that after homogenization and cooling the blend solidifies and is solid at body temperature (Müller 2002b).

5. Nanosuspensions (drug nanoparticles)

For many decades drug carriers have represented the only group of colloidal drug administration systems. Nowadays a fundamentally different group of dispersions i.e. nanosuspensions (drug nanoparticles) are also under investigation (Westesen 2000). Pharmaceutical nanosuspensions are usually very finely dispersed solid drug particles in an aqueous vehicle for both oral and topical use or for parenteral and pulmonary administration. The key difference from conventional suspensions is that the particle size distribution of the solid particles in nanosuspensions is usually less than 1 μm, with an average particle size range between 200–600 nm.
Micronization of poorly soluble drugs increases the dissolution rate of the drug due to the increase in surface area,

Table: DissoCubes versus NanoCrystals

Feature	DissoCubes	NanoCrystals
Equipment	High pressure homogenizer	Pearl mill
Principle	Impact, shear and severe pressure drop	Milling process
Crystalline status	Amorphous transitions, if this can be preserved further increase in dissolution is possible	Crystalline product
Product contamination	Low; iron < 10 ppm	Abrasion of pearls, contamination insoluble glass/zirconium micro and nanoparticles
Microbial quality	Parts of machine are autoclavable, homogenization itself disrupts micro organisms, easy to maintain.	Cumbersome, pearls have to be sterilised individually, difficult to maintain.
Product output	High	Losses due to sticking to pearls
Cost	Low	High
Intellectual property rights	SkyePharma PLC, London	Elan, USA

but does not change the saturation solubility. At very low saturation solubility, the achieved increase in dissolution rate does not lead to a sufficiently high bioavailability. The next development was transformation of a micronized drug powder into drug nanoparticles. In a nanosuspension, the overall bioavailability is improved by an increase in surface area and saturation solubility via particle size reduction. This system cannot be achieved by the conventional milling techniques. Patented technologies have come up based on the principle of pearl mill (called NanoCrystals®) and high-pressure homogenization (called DissoCubes®) (Vyas and Khar 2002; Grau et al. 2000).

NanoCrystals production by nanosystems involves filling an aqueous suspension into a pearl mill containing glass or zirconium oxide pearls as milling media. The drug microparticles are ground to nanoparticles in between the moving milling pearls.

Preparation of DissoCubes involves dispersion of drug powder in a surfactant solution by a high-speed stirrer. First, the particle size is reduced in a jet mill. The obtained macro-suspension is passed through a high-speed homogenizer leading to the formation of a nanosuspension. The cavitation forces experienced are sufficient to disintegrate drug microparticles to nanoparticles. The Table compares features of NanoCrystals and DissoCubes (Puri and Bansal 2001).

6. New developments in drug NanoCrystals

DissoCubes are prepared by homogenizing drug powder dispersed in pure water. This is based on the fact that it was believed that cavitation is the major force to disintegrate large particles to drug nanocrystals. To obtain cavitation one needs to have a liquid with a high vapour pressure, i.e. water. Cavitation should not be present or only present at a very limited extent when homogenizing in liquids with a low vapour pressure, e.g. liquid oils (MCT) or liquid PEG. Recently, a new patent application was filed describing the production of drug NanoCrystals in non-aqueous media. In addition, it is also claimed to produce drug NanoCrystals in mixtures of water with water-miscible liquids (e.g. production in isotonic dispersions of glycerol-water). The technology is owned by the German company PharmaSol GmbH Berlin/Germany (PhamaSol 2001). The registered trade name is Nanopure®. This technology is especially suited to produce drug NanoCrystals for oral administration, commercially the most promising area. Drug NanoCrystals are produced in melted PEG, the obtained nanosuspension is then filled straight away as liquid at 70 °C in hard gealtin or HPMC capsules. Cooling forms a solid matrix in the capsule which contains the drug nanocrystals in a finely dispersed form. This allows fast release in the gut by dissolution of the PEG releasing the drug nanocrystals as single particles and minimizing aggregation. The PEG nanosuspensions can also be solidified, milled or granulated and then filled into capsules or alternatively mixed with other excipients to produce tablets. This is a very straightforward way. In addition, stock dispersions of water-sensitive drugs can be prepared. Such a stock suspension in e.g. glycerol can then be diluted prior to parenteral administration using sterile water to yield on isotonic suspension (Müller 2002c; Krause et al. 2002).

The major advantages of nanosuspension technology are its general applicability to most drugs and its simplicity. Interesting special features of nanosuspensions are (Müller et al. 2001):

- Increase in saturation solubility and consequently an increase in the dissolution rate of the drug.
- Increase in adhesive nature, thus resulting in enhanced bioavailability.
- Increasing the amorphous fraction in the particles, leading to a potential change in the crystalline structure and higher solubility.
- Absence of ostwald ripening, producing physical long-term stability as an aqueous suspension.
- Possibility of surface-modification of nanosuspensions for site specific delivery.
- Possibility of large-scale production, the pre-requisite for the introduction of a delivery system to the market.

The use of nanosuspensions opens new perspectives in the formulation of poorly soluble drugs like clofazimine (Peters et al. 2000), budesonide (Jacobs and Müller 2002). Contamination by erosion products of the processing equipment, (abrasion from the pearls) is a typical problem faced by NanoCrystals.

7. Conclusion

Polymeric nanoparticles, able to deliver drugs to specific sites of action for a prolonged time, represent a potential therapeutic approach for several diseases. For more than 30 years, intensive research has been performed in poly-

meric nanoparticles, but this system does not really exist in the market due to the reasons mentioned earlier. Some alternatives are under investigation to overcome the drawbacks of polymeric nanoparticles. New and promising approaches evolving in this area are SLN, NLC and nanosuspensions. The ability to produce nanosuspensions in large scale provides the key for future success.

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