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Review

The role of solid nanoparticle technology in the parenteral delivery of poorly water-soluble drugs

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Abstract

Water insolubility has always been a key obstacle in pharmaceutical formulation, affecting formulation stability and drug bioavailability. Approaches for achieving complete dissolution often have disadvantages associated with the large quantities of required excipients. Small-particle suspensions (200 nm–2 μm), consisting essentially of pure drug, require only a minimum amount of surface-active agent for stabilization. Such suspensions may be formulated for rapid dissolution, thus achieving pharmacokinetic properties similar to those of a solution, or drug insolubility may be leveraged to afford prolonged in vivo release. In both situations, higher dosing may be possible than with a drug solution. This may afford enhanced efficacy at reduced excipient concentrations with potentially less toxicity. We present a brief introduction to the pharmaceutical technology of pure submicron drug particles in relationship to other dosage forms, and study examples are presented to underscore the potential benefits of this approach in parenteral delivery.

Keywords: Nanosuspension; Solubility; Stability; Parenteral; Drug delivery; Homogenization; Milling; Precipitation; Itraconazole; Clofazimine

Contents

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1. Introduction

An urgent need has always existed for safe and effective delivery of poorly soluble drugs. Insolubility is caused by either a limited ability to hydrogen bond with water (hydrophobicity) or by difficulty in breaking apart molecules in the solid state (high lattice energy). Water is unique in its capacity to form molecular clusters that are tightly interlinked through hydrogen bonding. To readily dissolve, a solute molecule must successfully compete with these strong intermolecular interactions. Dissolution therefore requires either modification of the solid phase to reduce lattice energy (lower melting point) or modification of the formulation vehicle to break up hydrogen bonding between water molecules.

It has been estimated that roughly 40% of all investigational compounds fail development because of poor bioavailability that is often associated with aqueous insolubility (Prentis et al., 1998). Receptor-based screening studies frequently lead to false acceptance of poorly soluble drugs. Many poorly water-soluble drugs show good activity in simple in vitro screening because of interaction with hydrophobic receptor domains. Furthermore, in order to enhance solubility for receptor binding studies, pharmaceutical agents are often dissolved in dimethylsulfoxide (DMSO). DMSO is fairly non-selective in its solubilization properties, dissolving a wide range of polar and nonpolar organic compounds. Screening thus tends to include compounds that although soluble in DMSO, fail solubility or bioavailability tests in aqueous environments. The recourse is to alter the drug environment, alter the chemical structure of the drug, or modify the lattice structure of the solid.

Modification of lattice structure, or crystallinity, may temporarily enhance solubility by affecting molecular cohesion within the solid. A metastable material of high thermodynamic activity exhibits a greater solubility than that of the most physically stable solid of the same molecule. Because the same solute is in equilibrium with solids of different activity at the same pressure and temperature, the system will eventually revert to the most stable solid phase, that which is the least soluble. It is thus critical to monitor stability of polymorphs, solvates, or anhydrous solids throughout product shelf life.

Altering chemical structure may drastically change pharmacological activity by modifying the affinity of the drug for its receptor. Development of water-soluble derivatives is costly however because of the need to demonstrate efficacy and safety of the new chemical species. In many cases formulation by any means may not be viable. Limited membrane transport, despite strong receptor interaction in vitro, is an obvious roadblock that is difficult to surmount without synthetic modification of the drug.

The use of solubilizing excipients in drug solubilization is primarily limited by either their toxicity, that of the drug at the elevated concentration, or a combination of both factors. Nanosuspensions consisting of essentially pure drug and minimal quantities of surface-stabilizing agents, may enable high dosing with reduced toxicity provided that toxic drug levels are not reached in vivo. This may be realized not only by minimizing surfactant, but also by providing sustained release at a level below the maximum tolerated dose, yet at a therapeutically effective concentration.

Alteration of the formulation vehicle is the most attractive option from the aspect of development cost and time. However, complete solubilization of a drug with very low intrinsic solubility may be very difficult or untenable. For example, extreme pH that is far removed from the pK_a of the drug, may be required in order to reach the desired levels of soluble ionic species. Phenytoin (pK_a = 8.3), which has an intrinsic solubility of approximately 10 μg/mL, cannot be formulated as an aqueous solution below pH 11 (Philip et al., 1984; Alvarez-Nunez and Yalkowsky, 1999). At such pH extremes, chemical instability (AHFS Drug Information, 1997) and local tissue irritation or necrosis near the injection site (Wheless, 1998) may also be important considerations.

Low aqueous solubility also affects the quantities of cosolvents or surfactants necessary for complete dissolution. For water miscibility the cosolvent must effectively hydrogen bond with water. Hydrophobic compounds cannot readily interact through hydrogen bonding, and therefore the miscibility requirement limits the capacity for the cosolvent to favorably interact with the hydrophobic solute. In such cases, solubility is often exponentially related to the mole fraction of the cosolvent (Yalkowsky, 1999). High osmolality associated with such large molar quantities becomes an issue, and limits the product volume, typically under 10 mL for parenteral use. High levels of surfactant can also lead to adverse pharmacology. CREMOPHOR EL[1] (polyethoxylated castor oil) is added to TAXOL[2] (paclitaxel), for example, to enhance drug solubility. Unfortunately, it may also cause hypersensitivity reactions in certain individuals (Volcheck and Van Dellen, 1998; Singla et al., 2002). Elevated levels of drug also contribute to the toxicity.

The ability to form inclusion complexes with cyclodextrins is also limited for those compounds having very low intrinsic solubility. Solubility of the complex and viscosity of the resulting formulation limit the feasible concentration of cyclodextrin, and only a fixed solubility enhancement can be realized at this critical level. Furthermore, a molar excess of cyclodextrin must usually be added to drive the equilibrium toward complexation. Because of their high molecular weight, large quantities of cyclodextrin may be required to reach the desired drug concentration. For example, the molecular weight of 2-hydroxypropyl-β-cyclodextrin (HPBCD), is approximately 1400 (degree of substitution is approximately 4). The commercial product SPORANOX IV[3] contains 400 mg of HPBCD per 10 mg of itraconazole (Janssen, SPORANOX package insert). High excipient levels may also limit the route of administration. Oral delivery, for example, may be impractical because the tablet or capsule would be too large to swallow.

[1] CREMOPHOR EL is a registered trademark of BASF Corporation.

[2] TAXOL is a registered trademark of Bristol-Myers Squibb Company.

[3] SPORANOX and SPORANOX IV are registered trademarks of Janssen Pharmaceutica Products, L.P.

Very low drug solubility in aqueous media may also hamper preparation of an emulsion. At fixed temperature and partitioning equilibrium, the drug concentration in the oil phase is limited by the intrinsic water solubility and the fractional oil volume. Furthermore, a number of water-insoluble drugs also have limited solubility in oils. For example, the antimalarial drug artemisinin has poor solubility in both water and oil (Ashton et al., 1999). This dual insolubility often applies to high-melting solids for which a substantial fraction of the free energy of solution is attributable to high lattice energy. The same reasoning applies to liposomes and other structured lipid dispersions. High drug loading may be limited by solubility in the aqueous voids, solubility within the hydrophobic lipid compartment, and respective volumes of these compartments.

Traditional methods have proven successful in commercializing many poorly soluble drugs, and should be encouraged as primary strategies. However, nonconventional methods may confer new hope for promising drugs that would otherwise be abandoned due to aqueous insolubility. Our group has worked on the development of nanosuspensions to handle such intractable cases. The purpose of this paper is to give a brief perspective of solid nanoparticle technology, with emphasis on carrier-free, pure drug suspensions for parenteral delivery. Thus matrices such as albumin or PLGA are not discussed.

2. Micro- and nanosuspensions

Nanotechnology has a long history. Heterogeneous catalysts were among the first examples, developed in the early 19th century (Kauffman, 1999; Robertson, 1983). This technology also includes photographic processes that rely on finely divided silver halide (Bergthaller, 1996). However, the most important scientific advancements have only taken place within the last two decades. In the photographic and semiconductor field, nanoparticles have been traditionally associated with structures less than 100 nm in diameter (Mendel et al., 1999), a limit at which the distinction between the electronic properties of bulk materials and individual atoms or molecules starts to fall apart. Metals and semiconductors having this size restriction begin to exhibit distinguishable quantum electronic properties, and these properties can be tailored by altering

particle size and form. In the pharmaceutical field, the term "nanoparticle" has been rather loosely applied to structures less than 1 μm in diameter. They can be produced by either chemical or mechanical means, and characterized by conventional analytical methods such as microscopy or light scattering.

The use of nanosuspensions in parenteral drug delivery is a fairly new concept. For many decades, coarse solid suspensions (10–100 μm) have been produced for intramuscular or subcutaneous delivery of poorly water-soluble drugs. Examples include penicillin G benzathine (BICILLIN[4] L-A by Wyeth-Ayerst), prepared by the reaction of dibenzylethylene diamine with two molecules of penicillin G, dexamethasone acetate (DECADRON-LA,[5] by Merck), and methylprednisolone acetate (DEPO MEDROL,[6] Pfizer), which are administered intramuscularly. Insulin has long been formulated with zinc as a suspension for subcutaneous delivery (for example, HUMULIN, ILETIN, LENTE and NOVOLIN,[7] developed and manufactured by Lilly).

Conventional milling and precipitation processes generally result in particles much greater than 1 μm. Techniques were later refined by Liversidge and coworkers at NanoSystems (now part of Elan Corporation), to enable milling of solid drug particles (NANOCRYSTAL[8] technology) to well below 1 μm (Liversidge et al., 1992). NanoSystems' approach relies on the use of the ball mill or pearl mill, in which milling beads of sizes ranging from 0.4 to 3 mm are used. These beads may be composed of glass, zirconium salts, ceramics, or plastics (e.g., cross-linked polystyrene). Even with very hard grinding materials, however, erosion of the milling material may be an issue (Redzisqewski, 1997; Joost and Schwedes, 1996; Chandrasekaran, 1993; Kerr and Reed, 1992). After separation of the grinding medium from the suspension, fine particles may potentially remain. The time required for milling depends on the hardness and brittleness of the drug material in comparison to the milling material and the inertial forces set up within the mill. Milling processes often require grinding for hours to days in order to reach a desired size range (Liversidge, 1996). Another potential drawback is that prolonged milling may induce the formation of amorphous domains in crystalline starting material. Hydration of these regions may lead to instability, either during subsequent processing or upon storage of the final product. This effect was observed in the jet milling of albuterol sulfate (Ward and Schultz, 1995). Crystalline to amorphous transitions have also been observed in the ball milling of organic compounds (Willart et al., 2001; Font et al., 1997). Solid phase transitions that can occur upon grinding or milling may also be accompanied by generation of high-energy surfaces that affect wettability. For example, "activated" acetylsalicylic acid that was produced by grinding was monitored over 120 h by measurement of contact angle. The contact angle decreased in an apparent first-order fashion from 106 to 77°, indicating surface instability. However, repeated measurements did not reach the contact angle (73–75°) of the unmilled material (Huettenrauch, 1984). The half-life of the activated state was approximately 24 h. In another study (Huettenrauch and Moeller, 1983), mechanical activation of the solid surface through milling significantly altered the wettability of sulfathiazole and lactose in water and propanol, respectively. Interfacial tension was measured by suspending the powder in solvent, shaking for 30 min, and allowing settling over 6 days. Milling significantly increased the sedimentation velocity in all cases.

Nonetheless, particles smaller in median diameter than 400 nm may be milled. Naproxen nanoparticles, approximately 300 nm in median diameter, have been prepared (Setler, 1999). Danazol particles have been milled to a median diameter of 169 nm (Liversidge and Cundy, 1995). The danazol nanosuspension showed enhanced oral bioavailability (82.3 ± 10.1%) that was similar to that of a danazol-2-hydroxypropyl-β-cyclodextrin dispersion (106.7 ± 12.3%), and far superior to that of the conventional drug suspension (5.1 ± 1.9%). Two products using the NANOCRYSTAL technology are already on the market. The first approved drug is a tablet dosage form for the macrolide immunosuppressant, sirolimus (RAPAMUNE,[9] by Wyeth). Re-

[4] BICILLIN L-A is a registered trademark of Wyeth-Ayerst.

[5] DECADRON-LA is a registered trademark of Merck & Co., Inc.

[6] DEPO MEDROL is a registered trademark of Pfizer, Inc.

[7] HUMULIN, ILETIN, LENTE and NOVOLIN are registered trademarks of Eli Lilly & Co.

[8] NANOCRYSTAL is a registered trademark of NanoSystems, a business unit of the Elan Corporation.

[9] RAPAMUNE is a registered trademark of Wyeth Pharmaceuticals.

cently, Merck's antiemetic drug, EMEND,[10] was approved for marketing in the US.

Another early success in creating submicron drug particles was reported by Dearn (1995) and Westesen and Siekmann (1998). Dearn applied homogenization (microfluidization) to atovaquone to obtain particles in the 100–3000 nm range. Westesen was able to produce fine particles by dissolution of drug in a high-melting organic material, such as a lipid. When heated to above its melting point, the liquid could easily be comminuted into a fine emulsion by piston-gap homogenization. Cooling of the melt to room temperature afforded solid particles of the lipid entrained with drug.

Muller and coworkers have produced drug nanoparticles (DISSOCUBES[11]) by piston-gap homogenization (Muller et al., 2000). In some cases, micronization of the raw material was required before homogenization in order to obtain the desired final particle size. Muller and coauthors have suggested jet milling or ball milling as a size reduction step prior to piston-gap homogenization (Muller and Bohm, 1998). The need for processing prior to homogenization may be attributed to the hardness, density and particle size of the starting raw material.

As with liposomes, the ability of solid particles to reach specific tissue sites is severely limited by rapid clearance from the circulation by phagocytic cells of the reticuloendothelial system, or RES (Abra and Hunt, 1981), also often termed the mononuclear phagocyte system (MPS). The spleen and liver contain fixed macrophages (mainly Kupffer cells of the liver and marginal-zone, marginal metallophilic, and red pulp macrophages in the spleen) that phagocytize small particles that cannot be rapidly broken down in the bloodstream (Fattal et al., 1998; Demoy et al., 1998; Buiting et al., 1995). This uptake is clearly observed following intravenous injection of liposomes, where the majority are taken up by liver and spleen within a few minutes to hours, depending on particle size and composition (Senior, 1987). A second factor that limits tissue distribution results from the tight endothelial junctions in most blood vessels, which except for the smallest particles (less than 150 nm), do not permit extravasation (Poste et al., 1984). Organs that are particularly rich in capillary vasculature, such as the spleen, liver and lungs, can act as repositories for delivered particles. "Stealth" liposomes have been developed that evade the RES (Allen et al., 1991), circulate in the bloodstream for prolonged periods and avoid opsonization and sequestration by macrophages. Coating liposomal vesicles with a covalently-bound hydrophilic polymer such as PEG reduces uptake by the liver. This prolonged circulation may allow enough time for many particles to permeate through fenestrated vasculature at sites other than the "filtering organs" (e.g., liver and spleen), thereby facilitating "passive targeting" of sites (e.g., tumors) that have the largest degree of endothelial permeability. Regions of increased capillary permeability also include sites of infection and inflammation. Furthermore, by incorporating targeting ligands on the liposomal surface, it may be possible to direct them to certain tissues (Vingerhoeds et al., 1994). Folate-mediated targeting is especially promising for tumor cells, which generally overexpress the folate receptor (Sudimack and Lee, 2000).

In vivo, nanoparticles are surprisingly well tolerated. The inner diameter of the smallest blood vessels ranges from approximately 5 to 7 microns. Nonetheless, injection into dogs of large quantities of polystyrene–divinylbenzene 'particles (2.4×10^9) can be tolerated with no gross histopathology, even up to a diameter of 25 μm if administered slowly over 1 h (Schroeder et al., 1978). Size effects on regional sequestration of particles have also been noted. In work by Kanke and coworkers, polystyrene beads greater than 7 μm in diameter were deposited almost entirely in the lung, whereas administration of 3.4 μm particles resulted in the majority (83%) being sequestered in the spleen and liver (Kanke et al., 1980). Hemodynamic effects, such as hypotension, have also been reported when inert polystyrene beads (approximately 3 μm) were injected (Slack et al., 1981). This effect was also observed by De Garavilla et al. (1996). Infusion (1 mL/min) of a 5% suspension of polystyrene nanospheres into anesthetized dogs resulted in acute hypotension which peaked within 2.5 min and completely abated within one hour. Reducing the concentration and infusion rate, 5% and 0.5 mL/min, respectively, eliminated this response. The hypotensive effect decreased as particle diameter was reduced from 200 to 100 nm, and was absent at 50 nm. Hypotension appeared to be mediated by histamine release as evi-

[10] EMEND is a registered trademark of Merck & Co., Inc.

[11] DISSOCUBES is a registered trademark of DDS—Drug Delivery Services GmbH/Krohnshagen, Germany.

denced by highly elevated plasma levels. The detailed cause is still poorly understood and needs to be further evaluated.

3. Physical properties of small-particle suspensions

Important determinants of suspension stability encompass solid properties (such as density, hardness, number and type of lattice defects), surface properties (such as interfacial tension and structure of the solid–liquid interface), and properties of the suspending medium (e.g., viscosity, drug solubility, and micellization capacity of surfactants). Solid properties affect the ability to fragment the particle by impact forces that shatter the lattice along dislocation boundaries. The surface tension, surfactant structure, and zeta potential at the interface affect particle aggregation, whereas viscosity of the suspending medium and drug solubility affect the rate of drug diffusion away from the particle surface. Both sets of factors contribute to long-term physical stability; that is, the resistance to aggregation, secondary nucleation, and Ostwald ripening. For this reason, the most stable oral suspensions have fairly low aqueous solubility and are suspended in a medium that contains viscosity enhancers such as carboxymethylcellulose.

Successful material design relies upon a fundamental understanding of the interplay of physical and chemical factors. By adjusting particle morphology and size it may be possible to design a drug formulation with desired pharmacokinetics. The Ostwald–Freundlich equation:

$$\ln \frac{S}{S_0} = \frac{2v\gamma}{rRT} = \frac{2M\gamma}{\rho rRT} \quad (1)$$

which hypothetically pertains to spherical particles, defines the effects of particle radius (r), molar volume (v), density (ρ), and interfacial tension (γ) on solubility, S, at temperature T. S_0 is the solubility of a flat, solid sheet ($r \rightarrow \infty$). M is the molecular weight of the solid, and R the ideal gas constant. Reducing the particle size increases drug solubility, all other factors being constant. Theoretically, this effect is not substantial (S/S_0 greater than 2), until the particle radius is quite small, well under 200 nm. Fig. 1 illustrates the calculated effect of particle radius on S/S_0 for a hypothetical particle with a molecular weight of 708, an interfacial surface tension of 50, 75, or 100 dyn cm^{-1}, and a density of 1 g/mL. Eq. (1) also indicates that lattice packing as well as particle size may affect solubility at a given temperature, inasmuch as such structure affects molar volume and solid density. Generally for a given compound, the polymorph with the closest packing (and greater density) will have a higher heat of fusion (and melting point), and lower solubility. Kitaigorodkii (1961) proposed that increased packing density lowers solid enthalpy. Brock et al. (1991) later confirmed Wallach's rule, which states that racemic solids are generally denser than the individual enantiomeric crystals, and are thus more stable. There are, of course, exceptions due to influences such as hydrogen bonding and lattice symmetry (Byrn et al., 1999a).

Shefter (1981) reviewed the value of morphology manipulation to enhance solubility, and a list of metastable polymorphs was compiled. A classic example of how enhanced polymorph solubility affects pharmacokinetics and bioavailability was demonstrated in studying peak serum levels of chloramphenicol palmitate in humans. It was found that a linear correlation could be drawn between percent of Form B, in mixtures of Forms A and B, and peak serum levels in humans (Aguiar et al., 1967; Aguiar and Zelmer, 1969). Form B has approximately twice the molar solubility of A.

Increased solubility near the particle surface results in an enhancement in the concentration gradient between the surface and the bulk solution. This high gradient, by Fick's law, must lead to an increased mass flux away from the particle surface. As the particle diameter decreases, its surface area to volume ratio increases inversely, further leading to an increased dissolution rate. Under sink conditions in which the drug concentration in the surrounding medium approaches zero, rapid dissolution may theoretically occur. Amobarbital as the Form II polymorph shows a 1.6-fold increase in dissolution rate at 37 °C as compared with Form I (Kato and Kohetsu, 1981). Correspondingly, Form II is more rapidly absorbed in vivo.

Numerous other factors contribute to the dissolution rate. The solute may be rapidly protonated or deprotonated at physiologic pH, affording ionic species having increased solubility. Nanosuspensions may be formulated at a pH at which the drug is least soluble. If the drug is soluble in its in vivo environment (e.g., pH 7.4), the suspension will rapidly dissolve. Because organic solvents may obfuscate preclinical toxicity screening,

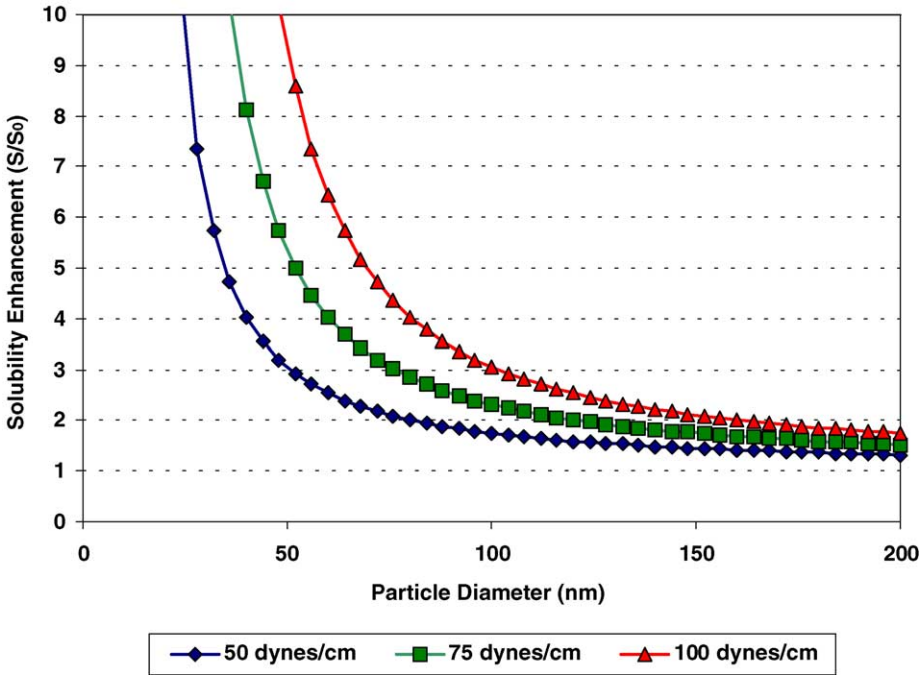

Fig. 1. Effect of solid particle diameter on solubility for hypothetical examples (S: solubility at the surface of the particle; S_0: intrinsic solubility).

this strategy may afford high drug loading without organic solvent. Flurbiprofen is a non-steroidal analgesic that is an aryl acetic acid having a pK_a of 4.2. At pH 10 in carbonate buffer the drug can be formulated as a 5 mg/mL solution. At pH 6.5, the drug has sufficiently reduced solubility to enable preparation as a nanosuspension. When intravenously injected into the tail vein of ether-anesthetized rats, it elicits the same pharmacokinetic profile in blood plasma and the same tissue distribution as the drug solution (Clement et al., 1992).

Extended depot release of a nanosuspension that would rapidly dissolve if intravenously delivered, may be achieved by delivery of the nanosuspension into a body compartment of limited fluid volume. Delayed local dissolution, and transport through cellular interstitia into blood circulation can result in a significantly prolonged effect. Bupivacaine is formulated between pH 4.0 and 6.5 as a solution (MARCAINE[12]). A nanosuspension (10%) may be prepared above the pK_a (8.2). Intradermal delivery of the nanosuspension as a bolus (injection into base of tail) prolongs local anesthesia in a rat for at least 40 h whereas intradermal delivery of a 1% solution fails to abate a pain response (tail twitch) after 4 h (Pace et al., 1999).

The long-term stabilization of nanosuspensions is an uphill battle against the thermodynamics of a metastable, dispersed system. All suspensions will eventually coalesce. Stability therefore rests on the ability to kinetically impede this process. Instability can result from a shift in size distribution to larger particles (Ostwald ripening), irreversible agglomeration, secondary and polymorphic nucleation. The first phenomenon is a consequence of Eq. (1), in which smaller particles must have higher saturation solubility than large particles. This concentration gradient causes growth of large particles at the expense of dissolving smaller ones. In order to obtain a stable suspension, the considerable potential energy created by the large interface between the solid and the surrounding medium must be reduced by adding surface-active agents. Surface stabilization may be achieved by using charged

[12] MARCAINE is a registered trademark of Hospira, Inc.

amphiphiles that migrate to the solid–liquid interface and provide an electrostatic barrier to particle agglomeration. Non-ionic polymers may also aid in surface stabilization. Polymeric surfactants such as poloxamer 188, a triblock co-polymer of ethylene glycol and propylene glycol, are very effective non-ionic stabilizers because of multiple attachment of hydrophobic domains at the particle surface. Entropically, the probability of detachment of all of these hydrophobic moieties is very low at room temperature, thus providing a strong surface affinity (Alexandridis and Hatton, 1995). Non-ionic surfactants may also create a hydration zone, a layer of tightly bound water molecules around each particle. When two particles meet, work is required to dislodge this water layer because of osmotic forces. Other entropic factors are also involved. The hydrophobic domains of the surfactant associate with the particle surface, with pendant hydrophilic domains extending into the aqueous medium. Attraction between particles necessitates the intertwining of these pendant chains leading to a restriction in chain mobility, and hence an unfavorable lowering of entropy (Lee et al., 2000b). This type of stabilization (steric), may provide an effective barrier to aggregation. A combination of non-ionic and electrostatic stabilization is often required to achieve desired shelf life. Glycol copolymers suffer however, from reduced solubility in water at high temperatures, which leads to particle aggregation. This results from thermally induced cleavage of hydrogen bonds between the hydrated polymer and water, leading to formation of visible polymer aggregates ("cloud point"). The ability to autoclave such formulations is limited if the cloud point lies below the sterilization temperature (121 °C). Addition of cloud point modifiers, usually anionic surfactants such as sodium dodecyl sulfate, may raise the cloud point and enhance stability at high temperature (Lee et al., 2000a). Polysorbates (Tweens), poloxamines and poloxamers have been used as non-ionic surfactants. Bile salts (e.g., sodium cholate) and alkyl sulfonates (sodium dodecyl sulfate, sodium dioctylsulfosuccinate, and sodium lauryl sulfate, for example) have been effectively used as ionic surfactants.

Aggregation arises from the natural tendency of the dispersed system to reduce excess surface free energy. In stable dispersions, particle collisions do not cause aggregation because of repulsion forces. From the aspect of buoyancy, stabilized particles smaller than approximately 100 nm will remain dispersed indefinitely, and larger particles will sediment because buoyant forces on these particles cannot overcome gravity. Sedimentation may not be deleterious, as simple shaking may again create a homogenous mixture. In a poorly stabilized dispersion, however, interparticle repulsive forces due to osmotic, entropic and electrostatic barriers, cannot overcome the inertia of particle collision. Surfactant rearrangement around the particles creates an aggregate with lower surface energy. Eventually, formation of heavy aggregates leads to rapid sedimentation. Points of surface contact between particles have a high *negative* (concave) radius of curvature, see Eq. (1) and thus a lower solubility than convex surfaces. This leads to solute buildup at these junctions and the "knitting" together of particles (caking).

Secondary nucleation occurs in a supersaturated solution from crystals of the solute already present in the crystallization medium. Temperature fluctuations may cause some of the drug to dissolve at peak temperatures and recrystallize from a seeded supersaturated medium upon cooling. Nucleation of a more stable polymorph may also occur, and can be seeded from metastable phases. This transformation may occur rapidly or slowly, and may be reversible (enantiotropic) or irreversible (monotropic). Polymorphism may not only alter physical characteristics of a drug, but its pharmacological parameters as well. A classic example is chloramphenicol palmitate, which is inactive in its most stable form (Form A). Monotropic conversion of the desired metastable form (Form B) to Form A can be induced by temperature or by seeding (Byrn et al., 1999b). Ball milling has also been found to induce this polymorphic conversion (Otsuka and Kaneniwa, 1986).

4. Methods for preparing solid drug nanoparticles

4.1. Homogenization

Effective size reduction generally requires high-energy input, resulting in enormous impact forces. When a suspension is homogenized, fluid shear, particle collision and cavitation are critical high-energy parameters. Microfluidization and piston-gap homogenization have been used with success. However, piston-

gap homogenization generally imparts greater turbulent energy by cavitation. In a piston-gap homogenizer, the starting suspension or slurry is pumped through a narrow gap at high pressure (15,000–30,000 psi). The ram pressure created behind the piston is converted to kinetic energy (high flow) as the homogenizate passes through the gap. To maintain constant energy, this high flow is compensated by a dramatic decrease in static pressure. The static pressure drop is great enough to fall below the vapor pressure of water, causing boiling to occur. As the suspension exits the gap, the pressure suddenly rises to ambient pressure and the vapor bubbles implode (cavitation). This implosion generates a considerable local energy as turbulent flow and heat. In fact, the energy density due to cavitational bubble collapse can be enormous, with "hot spots" reaching roughly 5000 °C, and pressures of 500 atm (Suslick, 1990). Acoustic shock waves from cavitation cause particles to collide with one another with enough energy to cause significant size reduction. As the particle size is reduced, progressively larger amounts of energy must be delivered to sustain further size reduction. A critical size limit is reached at a fixed rate of energy input (power). As particles are reduced in size, particle hardness must be overcome by application of inertial forces distributed over an increasing particle population. Cleavage may be favored along crystal dislocations, and theoretically the number of crystalline defects per particle may be reduced as the particle size is decreased. Furthermore, total surface area at the solid–liquid interface increases inversely with the square of the particle diameter at fixed drug mass. Excess surface free energy is proportional to surface area and hence represents additional work that must be applied in the comminution process. Often, the number of homogenization cycles can be reduced by size reduction of the feed material using grinding or milling. Pre-wetting of the slurry by blending of a mixture of drug powder with aqueous surfactant prior to homogenization may also reduce particle aggregation during processing, and maintain free flow of suspension through the homogenizer, eliminating clogging of piston and check valves.

4.2. Precipitation

Precipitation has been applied for many years in the preparation of small particles, particularly in the development of photographic films (Illingsworth, 1972; Musliner, 1974), and within the last decade in the preparation of submicron particles for drug delivery (Sjostrom et al., 1993a,b; Gassmann et al., 1994). Typically, the drug is first dissolved in a solvent, and this solution is mixed with a miscible antisolvent. Mixing processes vary considerably. For example, Violante and Fischer (1989) at the University of Rochester relied on infusion of the antisolvent into the solvent, thus ensuring low supersaturation during mixing. The authors emphasized that through careful control of this addition process it was possible to obtain a narrow particle size distribution. On the other hand, many of these "hydrosols" may also be prepared by rapid addition of solvent to antisolvent and mixing. This ensures sudden high supersaturation, resulting in rapid nucleation and the formation of many small nuclei (Mahajan and Kirwan, 1993). Upon solvent removal, the suspension may be sterile filtered and lyophilized. Simple precipitation methods, however, have numerous drawbacks. It is very difficult to control nucleation and crystal growth to obtain a narrow size distribution. Often a metastable solid, usually amorphous, is formed which is converted to more stable crystalline forms (Violante and Fischer, 1989; Mullin, 2001a). Once nucleation occurs, crystal growth is spontaneous and difficult to control. Furthermore, non-aqueous solvents utilized in the precipitation process must be reduced to toxicologically acceptable levels in the end product.

Precipitation has also been coupled with high-shear processing. The NANOEDGE[13] process (Baxter Healthcare Corporation) relies on the precipitation of friable materials for subsequent fragmentation under conditions of high shear and/or thermal energy (Kipp et al., 2003; Chaubal et al., 2003). This is accomplished by a combination of rapid precipitation and high-pressure homogenization. Rapid addition of a drug solution to an antisolvent leads to sudden supersaturation of the mixed solution, and generation of fine crystalline or amorphous solids. Precipitation of amorphous material may be favored at high supersaturation when the solubility of the amorphous state is exceeded. Furthermore, high supersaturation often favors formation of slender, needle-like crystals. This is in accord with the general Ostwald–Mier theory wherein supersaturation favors nucleation rather than crystal growth (Mahajan and

[13] NANOEDGE is a registered trademark of Baxter International Inc. and its subsidiaries.

Kirwan, 1993). From Eq. (1), if the density of the particle (ρ) and the solid–liquid interfacial tension (γ) are constant, a high supersaturation (left-hand side of Eq. (1)) may be compensated by a lower critical nucleation radius, r. Thus homogeneous nucleation in the highly supersaturated environment produced from rapid mixing will favor the generation many small nuclei that quickly grow and deplete the drug in the supernatant phase. This should result in formation of many small crystals (Estrin, 1993). The precipitated nanosuspension is stabilized during processing by surfactants that are added to the drug solution, antisolvent, or both. These additives may also inhibit crystal growth. It is also known in crystallization studies that as supersaturation is progressively increased, the interface morphology eventually leads to needle-like, or dendritic growth (Mullin, 2001b). Because of their small size and shape, particles produced by rapid precipitation are often more friable than the starting material and hence more susceptible to fragmentation (Kipp et al., 2003). Modeled on a cantilever analogy (Ashby and Jones, 1996), the yield force required to break individual crystals must vary inversely with the second to third power of the crystal thickness. If lattice defects, inclusions, or amorphous domains are also introduced, than the yield force should further decrease. One distinct disadvantage to this process is the need to remove solvent after homogenization. This may be accomplished by various means including ultrafiltration and centrifugation.

5. Potential clinical advantages of parenteral nanosuspensions

As with liposomes, nanosuspensions of solid drug may facilitate the delivery of larger quantities of drug at lower toxicity than would otherwise be possible by micellar dispersions or solutions. In a study by Peters et al. (2000), the efficacy of an intravenous liposomal preparation against *Mycobacterium avium* was compared to that of a clofazimine nanosuspension that was prepared by high-pressure, piston-gap homogenization. The drug was insoluble in aqueous formulations and was found to be toxic at therapeutically significant doses. The liposomal formulation had been found to be highly effective in artificially induced *M. avium* infections. However, the degree of drug loading per volume was appreciably lower in the liposomal preparation than in the nanosuspension. The clofazimine nanosuspension (20 mg/kg), delivered intravenously to mice, was as effective as the liposomal formulation in targeting the RES, and concentrations of clofazimine in the lung, liver and spleen were comparable. Moreover, these concentrations far exceeded the MIC for most strains of *M. avium*.

For highly insoluble drugs, release from intravenously delivered nanoparticles may be greatly extended. In another study, antifungal activity was determined for a 1% itraconazole nanosuspension in immunocompromised rats (Wong et al., 2002; White et al., 2003). A marketed itraconazole solution, SPORANOX IV (manufactured by Janssen Pharmaceutica Products, L.P.), was selected as a control. SPORANOX IV is a concentrate that contains an inclusion complex between itraconazole and 2-hydroxypropyl-β-cyclodextrin. The concentrate must be diluted immediately prior to injection. The control formulation exhibited significant acute toxicity above 10 mg/kg, and a formulation LD_{50} under 40 mg/kg when administered by bolus injection into the caudal vein. On the other hand, a 1% itraconazole nanosuspension could be administered in amounts up to 320 mg/kg without any animal mortality. After injection, the 20 mg/kg solution was rapidly cleared from blood plasma ($t_{1/2}$ less than 10 h for elimination of itraconazole and its first metabolite, hydroxyitraconazole; see Fig. 2) Following injection of the 80 mg/kg nanosuspension, drug concentration in plasma exhibited a precipitous drop and subsequent resurgence to a C_{max} of approximately 2.8 μg/mL, corresponding to approximately 24 h after injection. Within this interval (0.5–140 h), the $t_{1/2}$ was approximately 60 h, and drug was not entirely cleared from the plasma until at least 140 h. These data are consistent with RES uptake of nanoparticles, followed by extended release. The exact mechanism for this rebound effect is as yet unknown. The ability to administer more drug with reduced toxicity enabled dosing of the nanoparticulate at 80 mg/kg per day, given every other day, to immunocompromised rats that had been infected with *Candida* innocula. Due to its toxicity however, the solution could only be administered at a concentration of 10 mg/kg, and because of rapid clearance had to be given daily. Body weight was monitored daily, the animals were sacrificed after completion of

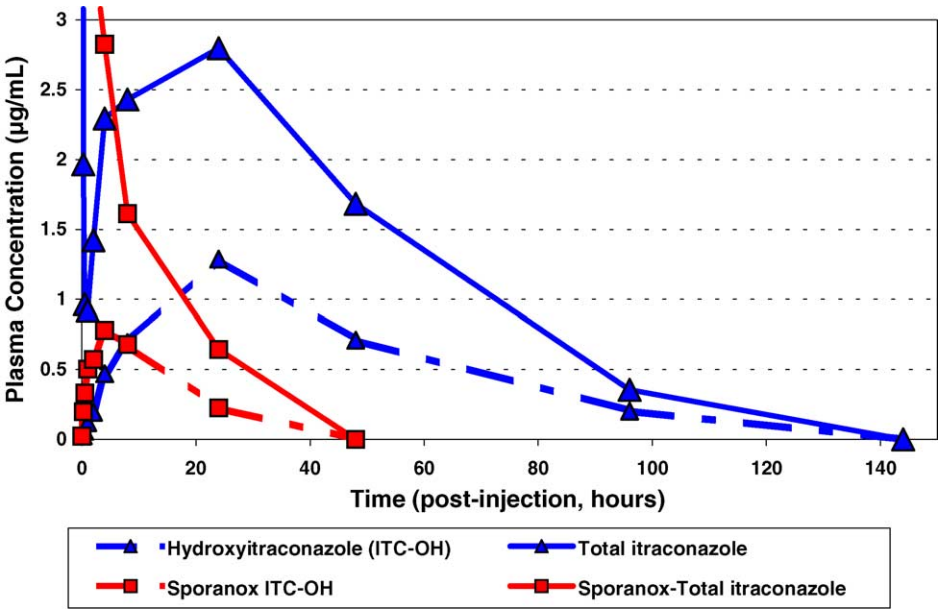

Fig. 2. Plasma concentration of itraconazole in rats versus time after injection into caudal vein: (a) solid line, solid squares: total itraconazole (itraconazole + hydroxyitraconazole), commercial solution of itraconazole:2-hydroxypropyl-β-intraconazole complex (SPORANOX IV); (b) dashed line, solid squares: hydroxyitraconazole, SPORANOX IV; (c) solid line, solid triangles: total itraconazole (1% itraconazole nanosuspension); (d) dashed line, solid triangles: hydroxyitraconazole, 1% itraconazole nanosuspension.

the regimen (11 days), and kidney tissue was analyzed for drug concentration and fungal spore count (cfu/g tissue). Compared to the solution, the ability to safely administer more itraconazole as the nanosuspension permitted delivery of more drug to the kidneys (see Fig. 3). At doses of 40 and 80 mg/kg, fungal spores could not be detected in kidney tissue at the completion of the 11-day regimen. However, in the control group, significant fungal counts remained at the end of the study (see Fig. 3). It should be emphasized that this study only focused on uptake by the kidney, and differential uptake by various tissues may affect overall in vivo efficacy.

For the itraconazole nanosuspension, toxicity reduction and efficacy enhancement were consistent with prolonged release (significantly higher AUC and $t_{1/2}$ than that of the itraconazole–cyclodextrin solution), combined with a greatly reduced C_{max}, resulting in a sustained level well above the minimal inhibitory concentration (MIC_{90}) for *C. albicans* (0.3 ppm) (Carrillo-Munoz et al., 1999). The pharmacokinetic profile may be rationalized as occurring by splenic and hepatic sequestration. It is interesting to note that the MFC (minimal fungicidal concentration) of itraconazole is greater than 10 ppm for *C. albicans* (Carrillo-Munoz et al., 1999), a value which was exceeded in kidney tissue upon administration of the itraconazole nanosuspension (40 and 80 mg/kg doses), but which was clearly not attained with the solution product (see Fig. 3). Furthermore, injection of solution resulted in a brief exposure to high drug levels in plasma, with rapid clearance (see Fig. 2). Frequently drug toxicity is associated with a high peak serum level (e.g., C_{max} of amphotericin B), while efficacy results from prolonged drug bioavailability (Andes, 2003). The observed toxicity of the commercial formulation may occur within this initial interval during which plasma levels are high.

The chronic effects of uptake and storage of solid drug particles in macrophages are not well known, and obviously drug-dependent. Therefore this mechanism of depot release may preclude the use of some drugs in which a combination of organ toxicity and exposure during particle sequestration presents untoward toxicity.

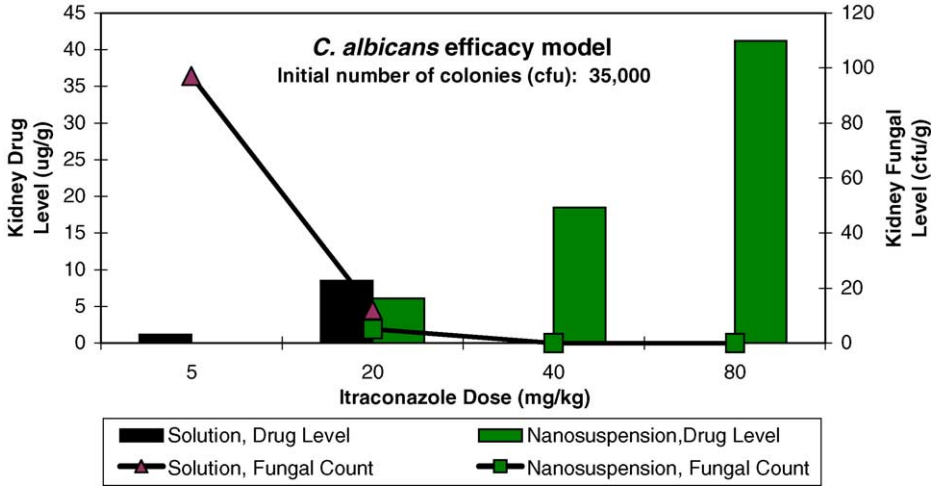

Fig. 3. Results of kidney necropsy at the end of 11-day treatment of rats with intraconazole:2-hydroxypropyl-β-cyclodextrin (SPORANOX IV) solution vs. 1% itraconazole nanosuspension. Graph of drug concentration in kidney tissue (bar height) vs. itraconazole dose administered by bolus injection into the caudal vein of the rat (*x*-axis), and fungal spore counts in excised kidney tissue after treatment with SPORANOX IV (solid triangles in line graph) and fungal spore counts in excised kidney tissue after treatment with a 1% (w/v) itraconazole nanosuspension (solid rectangles in line graph).

6. Conclusion

Aqueous insolubility is clearly recognized throughout the pharmaceutical industry as a major problem. Approaches to address this deficiency have been developed, but these are limited by toxicity of the vehicle, drug toxicity, or the dosage limitation that is inherent in each technology. Carrier-free, solid drug nanoparticulate may offer an alternative that advantageously leverages insolubility and enables high drug loading and delivery, potentially via numerous administration routes, including oral and parenteral. It may have advantages over association colloids (micellar solutions) because the level of surfactant per amount of drug can be greatly minimized, using only that necessary to stabilize the solid–fluid interface. If drug release is gradual so that build up of circulating drug does not exceed its toxicity threshold, larger quantities may be delivered in a single dose than otherwise would have been possible by alternative methods. Moreover, there may be promising applications for intravenous administration of pure, submicron drug particles that can be accumulated in phagocytic cells. Such solid nanosuspensions that slowly dissolve may enable passive targeting of the lung, liver and spleen via the RES, followed by sustained release. For acute treatment of infectious diseases, prolonged release of large quantities of drug above the MIC, yet below the toxicity limit, may offer an important tool in effective eradication of the infectious organism.

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