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Review Article

Nanoparticle Formulation for Hydrophilic & Hydrophobic Drugs

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ABSTRACT

Nanoparticle formulations have many advantages over traditional dosage forms, such as enhanced dissolution properties and the potential for intracellular drug delivery. Specifically, pure drug nanoparticles, polymeric nanoparticles and polyelectrolyte complexes offer some encouraging results for delivering drugs to various organs and through various routes. Traditional techniques such as spray drying and grinding, and more recent advances in supercritical fluid extraction, precipitation, and double solvent evaporation have been employed to produce nanoparticle formulations for delivery of hydrophilic & hydrophobic drugs here, the benefits of nanoparticle formulations and current progress are compared in light of the practical encumbrances of producing formulations, and possible toxicological effects of these materials.

Keywords: nanotechnology; hydrophilic and hydrophobic drugs; nanoparticle formulations; nanoparticle toxicity.

INTRODUCTION

The colloidal carriers based on biodegradable and biocompatible polymeric systems have largely influenced the controlled and targeted drug delivery concept (Vyas and Khar *et al, 2002*). Nanoparticles are sub-nanosized colloidal structures composed of synthetic or semi-synthetic polymers (Vyas and Khar *et al, 2002*). The poor efficiency of conventional anticancer drugs can be explained by the special solid tumors. First, the stromal component may represent as much as 90 % of the tumor mass, depending on the tumor type. Of course, this interstitial space may play the role of the reservoir for some drugs, but it also increases the distances between most of the tumor cells and the blood vessels, increasing the distances the drugs have to cross. Secondly, vasculature within the tumor is not homogeneous and in some area, tumor cells are not available to blood supply. Thirdly, the absence of well-defined lymphatic network is responsible for high pressure gradients in the interstitial, which hinders the convective flow. Diffusion remains the only transport available for the drug which considerably limits the interest of regions either well or poorly accessible to therapy. Therefore, there is a need for a new therapy that would be able to concentrate the drugs closely to the tumor site, and avoid their too large distribution (Vyas and Khar et al*, 2002*; Donald L. Wise et al*,* 2004).

The first and main interest of nanoparticulate carriers for chemotherapeutic agents was to avoid drug distribution in organs such as the heart and the kidneys where a toxic effect could be observed (C.Verdun et al*,* 1996). Indeed after 4th administration, colloidal particles concentrate rapidly in the liver due to the opsonization process, increasing the liver concentration of the drug itself, where as traces can be found in the kidney and heart. The use of large particles (generally larger than 5 µm) leads to accumulation in the lung, due to a capillary filtration effect (H.sato et al, 1996).

Because bone marrow presents a sinusoidal endothelium, nanoparticles can also accumulate in this organ. Except in the case where the particle surface has been modified to target the bone marrow (L.Illum et al, 1984). It is apparent that the polymers, the building blocks of nanoparticulate composition, belong to the natural and synthetic origins. Some of them have already been exploited for their biomedical applications. Obviously, the literature is abounding with concern their safety, toxicology and biodegradable consideration (Vyas and Khar et al*, 2002*).

Many attempts to improve the gastrointestinal uptake of poorly absorbable drug like insulin have been carried out (F. Cui et al, 2006; Z. Ma et al, 2005). The advantages of nano-encapsulation include the enhanced stability of labile drugs, controlled drug release and an enhanced drug bioavailability owing to the fact that particles in the nano-size range are efficient in crossing permeability barriers (Sharma, A et al., 2004).

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ATTRIBUTES OF NANOPARTICULATE SYSTEMS

Nanoparticulate delivery systems provide a better penetration of the particles inside the body. Their size allows delivery via intravenous injection and therefore they can be used for intramuscular or subcutaneous applications. The nanoscale of these particulate systems also minimizes the irritant reactions at the injection site. Additionally, they exhibit greater stability, in both longer shelf storage lives and uptake times. These synthetic systems are also extremely versatile, and can be designed to elicit the desired kinetics, uptake, and response from the body. The therapeutic agent is often complexed to the carrier to protect both the drug and the non-targeted areas of the body. The biocompatibility of these synthetic systems is of extreme importance. It must be ensured that these nanoparticles are not taken up by undesired systems to exhibit toxic side effects. They must only pass through the lipid membranes of cells or the blood-brain barrier when desired. There are two main synthetic classifications of nanoparticles that will be further discussed (Grenha A et al, 2005; H.S. Kas et al, 1997).

ADVANTAGES 0F NANOPARTICLE FORMULATIONS IN HYDROPHILIC AND HYDROPHOBIC DRUGS - ANTI-CANCER DRUG DELIVERY

Nanoparticle pharmaceuticals offer several advantages over formulations containing larger particles. For example, as the size of a particle decreases, a greater number of its molecules will be found at its surface rather than inside the particles, giving nanoparticles a large surface area to the volume ratio (Pison U et al, 2006), this increase of total surface area leads to an increase dissolution velocity, as described by Noyes-Whitney equation (Sung JC et al, 2007). Additionally, the saturation solubility of a particle increases as the particle size decreases, which is described by the Kelvin and Ostwald-Freundlich equation (Sung JC et al, 2007). Interestingly, this size dependence only becomes apparent after the particle size falls below approximately 1 µm (Sung JC et al, 2007), making it entirely unique to nanoparticles. These phenomena make the nanoparticle formulation a high effective means to enhance mass transfer from the particle to the surrounding medium (Sung JC et al, 2007). For this reason nanoparticle formulation have been used to enhance the bioavailability of insoluble hydrophobic drugs. By suspending the drugs as nanoparticles, one can achieve a dose that is higher than that of a solution, which is thermodynamically limited by the aqueous solubility of drug (Pison U et al, 2006).

These properties of nanoparticles can be exploited in many ways to enhance a drug formulation. For example, it is some time desirable to increase the octanol-water partition coefficient (Log P. where P = (Coctanol/Cwater) of drug by modifying their structures with the addition of on aliphatic tail group. The log P value describes the ratio at which a drug will dissolve in each phase of an octanol-water system, and hence is a measure of the hydrophobicity of drug. Of course, hydrophobicity decreases solubility and facilitates the formation of drug nanoparticle as suspension (Muller RH et al, 2001).

NANOPARTICLE PROCESSING METHODS FOR HYDROPHILIC & HYDROPHOBIC DRUGS

Drug nanoparticle formulations are usually created in one of two ways. Particles may be precipitated out of solution (bottom-up), or they are milled from larger particles (top-down) (Rabinow BE et al, 2004). In both mechanisms, the total surface area increases which increases the free energy of the particles. The system compensates for this increase in free energy by dissolving crystalline nuclei and precipitating onto other particles in a process known as Ostwald Ripening (Lindfors L et al, 2006) or by agglomerating smaller particles. Many chemical processing technologies have been used to produce nanostructured materials suitable for pulmonary delivery. Some processes that are currently under investigation involve wet milling (Rabinow BE et al, 2004; Merisko-Liversidge E et al, 2004), supercritical fluid extraction (Chattopadhyay P et al, 2007), spray drying (Hu J, Johnston KP et al, 2004; V et al, 2006) electro-spray (YeoLY et al, 2005; Ijsebaert Jc et al, 2001) high-pressure homogenization (Verhoff FH et al, 2003) and recrystallization via solvent displacement (Kleemann E et al, 2005). Wet milling is a process that utilizes either ceramic or metallic milling media to grind a suspension of insoluble drug and surfactant (Merisko-Liversidge et al, 2004) have shown that this technique can be used to produce Zn insulin nanoparticles using a roller mill that contains ceramic milling media and surfactants. The authors reported producing insulin nanoparticles less than 200 nm size (Merisko-Liversidge E et al, 2004). Use of this technique for other poorly water soluble drugs has been vindicated by SkyePharma, as evidenced by a recent patent (Verhoff FH et al, 2003).

Spray drying is a process that forces fluid through a nozzle, producing a mist that is dried to produce a fine powder. The technique employs a variety of different types of nozzles, some of which use ultrasound or air-jet shear to nebulize drug suspensions. Supercritical fluid extraction is a technique that is currently being developed for use in nanoparticle drug formulations. It uses supercritical fluid to extract a solvent from a drug emulsion or solution, leaving behind a suspension of drug particles (Chattopadhyay P et al, 2007). These processes are advantageous because they generally offer better scalability, and are therefore industrially relevant.

In addition to chemical processing technologies, multiple recent studies have examined different polymeric nanoparticle fabrication methods (Dailey LA et al, 2006; Pandey R et al, 2003). These techniques generally involve polyelectrolyte complex formation, double

emulsion/solvent evaporation techniques, or emulsion polymerization techniques. Polyelectrolyte complexes use oppositely charged polymers to entrap drugs into a polymeric matrix nanoparticle, which then releases the drug either through polymer degradation or drug diffusion. Double emulsion/solvent evaporation techniques involve dissolving the drug and polymer in an organic solvent, which is then emulsified in an aqueous solution. The organic solvent diffuses out of the polymer phase and into the aqueous phase, and is then evaporated, leaving behind drug-loaded polymeric nanoparticles. Emulsion polymerization is similar to emulsion/solvent evaporation except that monomer is emulsified into droplets and then polymerization is initiated.

A. Spray Drying to Produce Pure Drug Nanoparticles

Spray drying is a process that uses jets of dissolved or suspended drug in an aqueous or other fluid phase that is forced through high pressure nozzles to produce a fine mist. Often, a bulking agent such as lactose will be added to the fluid as well (L. Brannon Peppas et al, 1995). The aqueous or other liquid contents of the mist evaporate, leaving behind a fine powder. A new modification of spray drying, called air nebulization spray drying, uses two wedge-shaped nozzles through which compressed air passes and liquid solutions pass at high velocity. The wedge-shaped nozzle acts as a fluid acceleration zone where the four streams collide at high velocity, producing a shock wave that generates fine droplets. The droplets then descend into a column while being dried into a solid powder by heated air before being collected.

Nanoparticles of water-insoluble model drug were fabricated using chitosan polymer crosslinked with different crosslinking agents like sodium tripolyphosphate and sodium hexametaphosphate by spray drying method. Polyethylene glycol 6000 was also included in the formulation. Different formulations of nanoparticles were prepared using different concentrations of crosslinking agents and polyethylene glycol in the nanoparticles. The average particle size ranged between 249 nm to 951 nm. Zeta potential of nanoparticles ranged between 26.0 mV up to 60.8 mV. Encapsulation efficiency ranged between 58%-96%. The nanoparticles were spherical with solid dense structure. *In vitro* drug release study was carried out in phosphate buffered saline solution pH 7.4 for 10hrs. The analysis of regression values of Higuchi plot suggested diffusional mechanism follows Fick's law of diffusion. Drug polymer interaction was absent as evidenced by FT-IR spectra and DSC thermograms. With polyethylene glycol inclusion shows interaction between lomustine and PEG. Cell viability assay (MTT Assay) have shown that the DNPs were able to reduce the tumour cell proliferation and increased cell viability significantly ($p < 0.05$) as compared to pure drug in L 132 human lung cancer cell line (Berscht PC et al,1994).

B. METHODS TO PRODUCE POLYMERIC NANOPARTICLE FORMULATIONS

Several studies have examined different polymeric nanoparticle formulation techniques. These techniques generally involve polyelectrolyte complex formation, double emulsion/solvent evaporation techniques (Shi L, Plumley CJ et al, 2007; Pandey R et al, 2003; Bivas-Benita M et al, 2004)) or emulsion polymerization methods (Zhang Q et al, 2001). In polyelectrolyte complex formation, a polycation and a polyanion are dissolved (usually in water), then mixed under moderate shear to generate nanoparticles. Alternatively, a single polyionic material can be complexed with an oppositely charged drug and then gelled with an oppositely charged, multivalent salt. Emulsion polymerization methods involve adding monomer to an aqueous surfactant-containing solution and applying shear, which is followed by polymerization of the dispersed phase. For example, this technique sometimes employs (poly) cyanoacrylates, which polymerize in water, thus eliminating the need for an initiator (Zhang Q et al, 2001).

Ionotropic gelation method is used to produce nanoparticles (Grenha A et al, 2005). Chitosan and tripolyphosphate (TPP) were dissolved in aqueous solutions and mixed under mild stirring to spontaneously precipitate nanoparticles. Insulin was dissolved in 0.01M NaOH solution and added to the TPP solution before being added to the chitosan solution. Particles with sizes ranging from 300 to 500 nm and with zeta potentials between +32 and +45 mV were produced in this manner. The authors found that increasing the chitosan to TPP mass ratio decreased the process yield (mass of particles produced over total mass), but increased the size and zeta potential of the particles (Grenha A et al, 2005). The particles decreased in size when incubated in a lysozyme solution (due to hydrolysis of the β-(1–4) glycosidic linkages in chitosan), suggesting that they will degrade in the pulmonary epithelium and release their drug contents. These particles were then incorporated into microparticles by incubating them in lactose and mannitol excipient solutions and spray drying. This process produced microparticles with aerodynamic diameters between 2 and 3 μm, which are suitable for pulmonary delivery 55. The microparticles rapidly dissolved in aqueous solution, leaving behind a suspension of nanoparticles. The authors conclude that these particles could potentially be used to effectively deliver therapeutic macromolecules to the lungs and promote pulmonary absorption (Grenha A et al, 2005).

A new nanoparticulate delivery system for amphotericin B (AmB) has been developed by means of the polyelectrolyte complexation technique. Two opposite charged polymers were used to form nanoparticles through electrostatic interaction, chitosan (CH) as a positively charged polymer and dextran sulfate (DS) as a polymer with a negative charge, together with zinc sulfate as a crosslinking and hardening agent. The AmB

nanoparticles obtained possessed a mean particle size of 600–800 nm with a polydispersity index of 0.2, indicating a narrow size distribution. The measured zeta potential of the nanoparticle surface was approximately −32mV indicating a strong negative charge at the particle's surface. Scanning electron microscopy revealed spherical particles with a smooth surface. Drug association efficacy of up to 65% was achieved. Dissolution studies demonstrated a fast release behavior suggesting that AmB exhibits only moderate interaction with the weakly crosslinked polymers of the nanoparticles. Although, electronic absorbance spectra showed that the aggregation state of AmB was modified within the nanoparticles, a reduction of nephrotoxicity was observed in an *in vivo* renal toxicity study (Waree Tiyaboonchai et al, 2007).

The aim of the present study was to probe the structural integrity of insulin after being entrapped into chitosan/alginate nanoparticles produced by ionotropic polyelectrolyte pre-gelation. By manipulating the alginate:chitosan mass ratio and the pH during nanoparticle production, desired nanoparticles with a mean size of 850 (±88) nm and insulin association efficiency of 81 (±2)% were obtained. Insulin secondary structure was assessed by Fourier transform infrared (FTIR) and circular dichroism (CD) after entrapment into nanoparticles and after release from the particles under gastrointestinal simulated conditions. FTIR second-derivative spectra and area-overlap compared to an insulin standard confirmed that no significant conformational changes of insulin occurred in terms of a-helix and b-sheet content. Far-UV-CD spectra corroborated the preservation of insulin structure during the nanoparticle production procedure. The presented nanoparticulate system is a promising carrier for insulin oral delivery since it preserves insulin structure and therefore also, potentially, its bioactivity (B. Sarmento et al, 2004).

C. SUPERCRITICAL FLUID EXTRACTION TO PRODUCE PURE DRUG NANOPARTICLES

Supercritical fluid extraction is a chemical process that uses a supercritical fluid (often scCO2) to extract solvent or other impurities from a suspension. Supercritical carbon dioxide usage has gained popularity recently because it is an environmentally benign solvent that can be harmlessly vented into the atmosphere (Snavely WK et al, 2002). Additionally, because carbon dioxide is a gas at ambient temperature and pressure, it can simply be flashed vaporize at atmospheric pressure, leaving behind any extracted solvent and impurities. This eliminates the extraction medium stripping step from the process, making it more economical and less energy intensive than traditional extraction and stripping processes. Recently, continuous supercritical carbon dioxide extraction process used to produce solid lipid nanoparticle suspensions for pulmonary delivery (Chattopadhyay P et al, 2007). In this process, supercritical carbon dioxide was used to extract organic solvent from an oil in water emulsion containing one of three lipids (tripalmitin, tristearin, or gelucire 50/13), and one of two model drugs (indomethacin or ketoprofen) (Chattopadhyay P et al, 2007). One of the aforementioned lipids and a selected drug was dissolved in chloroform with a soy lecithin surfactant, then dispersed into an aqueous solution containing sodium glycocholate and homogenized under high pressure to produce the emulsion (Chattopadhyay P et al, 2007). This reportedly created an emulsion with a mean droplet size ranging between 30 and 100 nm, which were introduced into an extraction column counter currently to a stream of supercritical carbon dioxide. The scCO2 extracted the organic solvent from the dispersed droplets, leaving behind solid lipid, drug-containing particles in an aqueous suspension (Chattopadhyay P et al, 2007). This processing method produced nanoparticles with a volume mean diameter between 10 and 30 nm, and a drug loading efficiency between 80% and 90% for the gelucire particles and 10% for the tripalmitin particles. Nebulized droplets were produced from the suspensions within an aerodynamic diameter range between 2 and 4 mm, which is within the respirable range (Chattopadhyay P et al, 2007). Thus, supercritical fluid extraction might be an effective means to produce drug-loaded nanoparticles within a suitable size range for pulmonary delivery as a nebulized aerosol.

D. POLYMERIC DOUBLE EMULSION SOLVENT EVAPORATION METHOD

The present study was designed to improve the oral bioavailability of two clinically important antifungal drugs—clotrimazole and econazole. Each drug was encapsulated in nanoparticles of a synthetic polymer (polylactide-co-glycolide, PLG) or a natural polymer (alginate stabilized with chitosan). The nanoparticles were prepared by the emulsion–solvent-evaporation technique in case of PLG and by the cation-induced controlled gelification in case of alginate. Drug encapsulation efficiency was better (>90%) for the alginate formulation compared with the PLG formulation (nearly 50%). The formulations were orally administered to mice and the drugs were analyzed in plasma by a validated HPLC technique. The biodistribution/ pharmacokinetic data suggested that there was a controlled drug release for 5–6 days with each of the formulations, compared with unencapsulated drugs, which were cleared within 3–4 h of oral/intravenous administration. There was a striking improvement in the relative and absolute bioavailability of each drug. Further, the drugs were detected in the tissues (lungs, liver and spleen) till 6–8 days in case of nanoparticles whereas free drugs were cleared by 12 h. Overall, the alginate formulation appeared to be better than the PLG formulation. The results emphasize the power of nanotechnology to make the concept of enhancement in oral bioavailability of azole antifungal drugs come to reality (Rajesh Pandey et al, 2005).

TOXICITY CONSIDERATION OF NANOPARTICLE FORMULATIONS

Nanotechnology is considered to be one of the world's most promising new technologies, able to impact all phases of life, just as the industrial revolution did in the past two centuries. Utilizing the quantum properties of atoms and molecules, nanotechnology proposes the construction of novel molecular devices possessing extraordinary properties. However, both epidemiological and toxicological studies have contributed to a body of evidence suggesting that nano or ultrafine particles may induce or exaggerate a number of adverse biological effects. It has been suggested that nanoparticles may interfere with a number of molecular processes that should be considered before such particles are brought into wide commercial use (Borm P et al, 2002).

In addition to the possible inherent toxic effects of nanoparticles, some materials used to formulate nanoparticles may have toxic effects and therefore may not be viable for developing therapeutic products. For example, the toxicity of polycyanoacrylates has been demonstrated by Brzoska et al. The authors prepared poly(butyl) cyanoacrylate and poly(hexyl) cyanoacrylate nanoparticles in a method similar to that described by (Zhang et al, 2001) using either Dextran 70 stabilizer or poloxamer-188 (Snavely WK et al, 2002). They determined that both types of nanoparticles caused an increase in lactate dehydrogenase (LDH) activity in human pulmonary epithelial cells. The degree of toxicity was greater for the poly(butyl) cyanoacrylate and independent of the stabilizer used, which stands to reason because shorter chain polycyanoacrylates have been associated with higher cytotoxicity (Brzoska M et al, 2004). The degree of toxicity also increased with increasing nanoparticle concentration (Brzoska M et al, 2004), most likely due to the subsequent increase in polycyanoacrylate concentration. Polyethyleneimine (PEI) has also demonstrated cytotoxicity in lung cells (Kleemann E et al, 2005). When PEI-DNA complexes were used to deliver DNA to lung epithelial cells, cell viability decreased as the PEI-DNA ratio increased (Shi L et al, 2007). Despite this, Dailey et al (Verhoff FH et al, 2003) have shown that PLGA nanoparticles induce less inflammation than polystyrene particles of similar size when delivered to the lungs. Based on this observation, nanoparticle toxicity in the lungs may be more dependent on material choice than particle size. Therefore, there may be alternative polymers that can be investigated for use in pulmonary nanoparticle drug formulations that could mitigate toxicity.

CONCLUSION AND FUTURE ASPECT

Nanoparticle drug formulations offer many advantages over traditional formulation for various hydrophilic and hydrophobic drugs. For example, the bioavailability of poorly water-soluble drugs can be greatly enhanced by the large surface area of drug nanoparticle formulations. Additionally, nanoparticles can be formulated in such a way to offer enhanced control over the morphology of dry powder drug formulations and the ability to produce structures with both a low-density microstructure for delivery of hydrophilic and hydrophobic drugs and nanostructure for enhanced dissolution and bioavailability.

The literature suggests many different formulation approaches for drugs that use a variety of excipients to fabricate nanoparticles or nanoparticle complexes suitable for hydrophilic and hydrophobic drugs. Many chemical processing techniques such as supercritical fluid extraction and spray drying have been successfully used for therapeutic nanoparticle processing. Additionally, polyelectrolyte complexation, double emulsion/solvent evaporation, and emulsion polymerization offer a range of formulation options. This diverse array of techniques has demonstrated the ability to effectively produce nanoparticles with a high degree of control over particle properties; however residual solvents, cytotoxic excipients, low drug loading efficiencies and scale-up issues might limit their commercial applications. With the perfection of nanoparticle formulations for the delivery of hydrophilic and hydrophobic drugs, the various route that may become a preferred for the delivery of many more local and systemic therapeutic interventions.

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