# SOLID LIPID NANOPARTICLES

## *Solid Lipid Nanoparticles for the Delivery of Pharmaceutical Actives*

**By:** Andrew Loxley, PhD

### INTRODUCTION

An increasing number of active pharmaceutical ingredients (APIs) under development are poorly water soluble and therefore have poor bioavailability. These are designated Biopharmaceutical Classification System (BCS) class II and class IV APIs.[1-3] Creative formulation efforts are required to produce a finished drug product from these APIs that has acceptable pharmacokinetics. A common formulation approach with such compounds is to focus on creating and stabilizing very small particles of the API in an attempt to increase the surface area available for dissolution in vivo, and hence the rate of dissolution, and consequently plasma or tissue levels of API. Another approach is to create so-called solid solutions of the API.[4]

Biologics (proteins, peptides, oligonucleotides, and SiRNAs) are water soluble but bring their own formulation and delivery challenges. Shelf-life stability and enzymatic degradation are two main areas of concern, and formulation design focuses on stabilizing the API in storage and protecting it from endogenous enzymes until it reaches its therapeutic target. In more advanced formulations, the API is formulated into a delivery vehicle that specifically targets tissue or cells to maximize the therapeutic index.

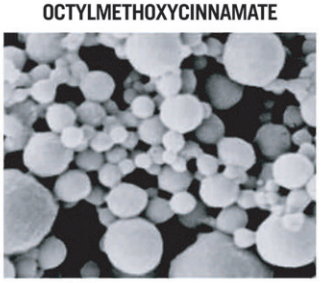
### NANOPARTICLE FORMULATIONS

Many of the aforementioned formulation approaches utilize nanotechnology, that is, the preparation of sub-micron structures containing the API. For BCS class II and IV APIs, the simplest nanoparticle is made of pure API, formed by top-down processes starting with bulk API, such as milling, grinding, homogenization, ultrasonication, and are stabilized in dispersion by the presence of a surfactant.[5] Alternatively, bottom-up "self-assembly" processes can be used, such as anti-solvent precipitation and micellar incorporation by dilution. For example, insoluble APIs may be incorporated into nano-sized vesicles or liposomes, in the form of particles dispersed in the aqueous core of the vesicles, or as molecularly dissolved material in the lipid bilayer.[6] Biodegradable polymers have also been used to form API-loaded nanoparticles or block copolymer micelles or polymersomes, usually by emulsification/solvent-evaporation techniques.[7,8] Biocompatible and biodegradable inorganic nanoparticles can be loaded with API via a microemulsion technique.[9] Biologics and other water-soluble drugs have been incorporated into the aqueous core of liposomes, into the aqueous domains of biodegradable polymer nanoparticles prepared by water-in-oil-in-water emulsion/solvent evaporation, and charge-neutralization nano-complexes made by interaction with oppositely charged polyelectrolyes, or by attachment to gold nanoparticles.[10-13]

Issues with shelf-life stability of the finished product or the need for organic solvents in processing for many of these approaches render them less than ideal.

**FIGURE 1**

SEM OF SLNs CONTAINING OCTYLMETHOXYCINNAMATE

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## SOLID LIPID NANOPARTICLES – MATERIALS & SYNTHESIS

Many biocompatible/biodegradable lipids are solid at room temperature, can be obtained in high purity, are generally recognized as safe (GRAS), and are inexpensive. Some common solid lipids used to make solid lipid nanoparticles (SLNs) include triglycerides (eg, Compritol 888 ATO and Dynasan 112), carnauba wax, beeswax, cetyl alcohol, emulsifying wax, cholesterol, and cholesterol butyrate.

Nano- and microparticles made of these lipids and suspended in water offer an option for formulating both BCS class II and IV APIs as well as biologics that may overcome the issues of shelf-life stability and the cost and toxicity associated with the use of organic solvents. In effect, the concepts of nanoparticles and solid solutions are being combined.

Nanoparticles of these lipids may be made using a templated synthesis from a microemulsion of the molten lipid in aqueous surfactants, by precipitation of the wax from a solution in a non-ionic surfactant on addition of water, or by emulsifying the molten lipid into a hot aqueous surfactant solution with high-shear mixing to obtain the desired submicron particle size.[14-16]

## API ENCAPSULATION IN SLNs

Small molecules can be entrapped within the lipid matrix of the nanoparticles by dissolving or dispersing the material in the molten lipid prior to particle formation. Souto's PhD thesis on the delivery of APIs using SLNs lists more than 100 APIs that have been encapsulated in SLNs.[17]

An SEM of the particles of a typical SLN dispersion (in this case of particles containing the sunscreen octylmethoxycinnamate) is shown in Figure 1. Particles of this type are made at commercial scale for formulation into

FIGURE 2

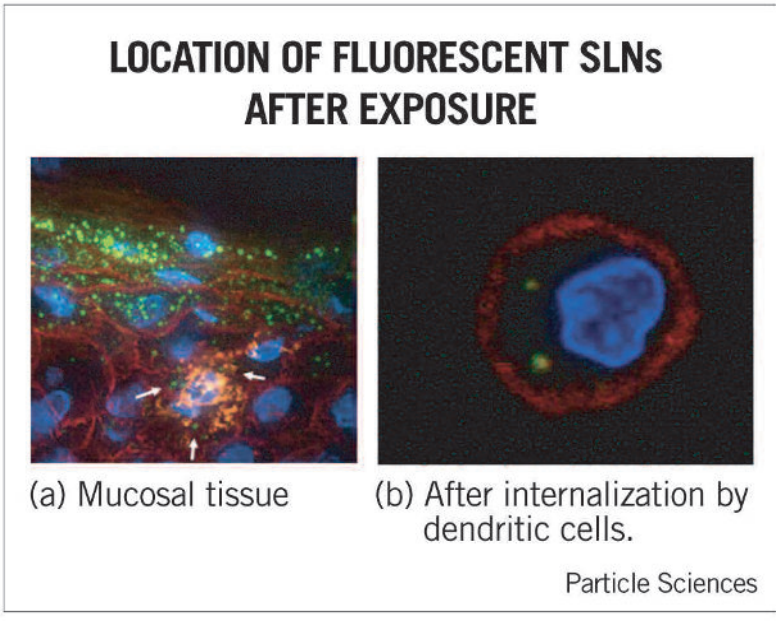


FIGURE 3

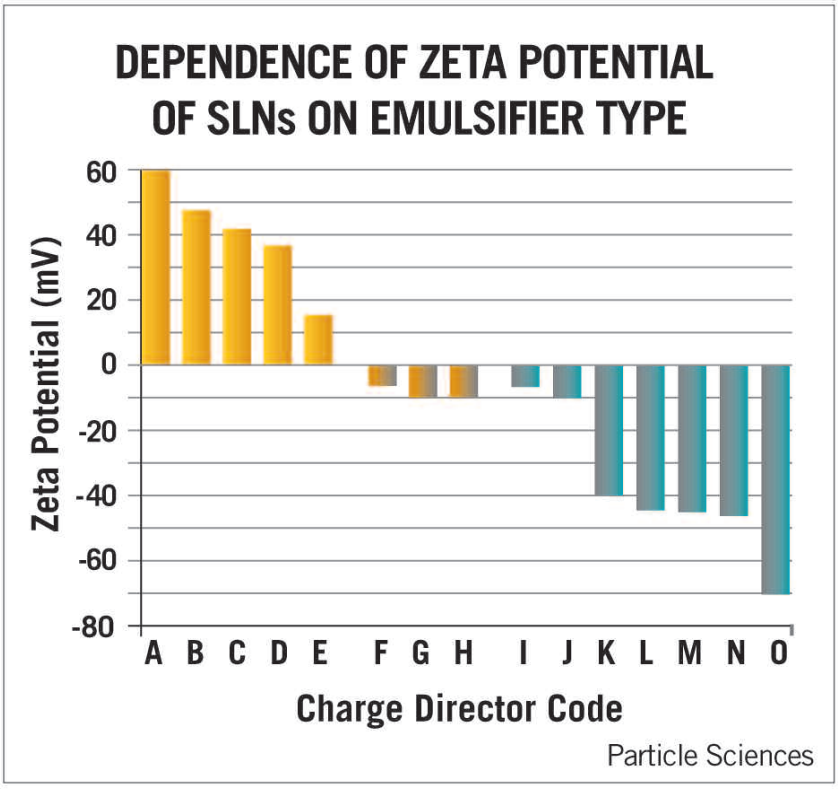

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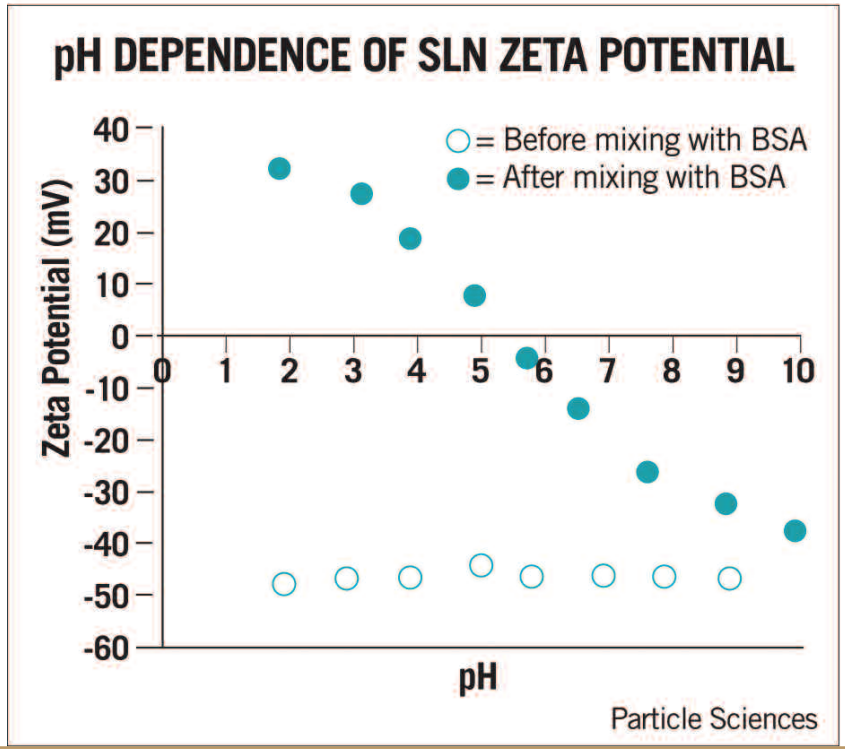
FIGURE 4




topical products to provide UV-protection.

In some cases, the API is not compatible with the lipid and is expelled from the nanoparticle, usually during cooling and solidification. This can lead to undesirable macroscopic crystals of API in the final formulation or phase separation of the particles to structures as complex as "nano-spoons."[18] By mixing liquid lipids with the solid lipid prior to particle formulation, lipid crystallization is hindered or prevented, and a more amorphous nanoparticle internal structure is achieved. In this way, APIs are less likely to be expelled from SLNs during the cooling step of their preparation, and stable SLNs may be formulated with a wider range of APIs.[19]

As one example created at Particle Sciences, fluorescent SLNs have been prepared by adding a fluorescent dye to the molten lipid prior to particle preparation. Green fluorescent SLNs were prepared with pyrromethene 567A dye, and red fluorescent SLNs with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) by a modified preparation technique to accommodate the low solubility of DiI in the molten lipid. Fluorescent SLNs are useful to follow the fate of particles applied mucosally in vivo and determining efficiency of uptake by antigen presenting cells in vitro in the development of a novel HIV vaccine.[20] Tissue samples taken from penile epithelial explants after application of fluorescent SLNs show particles penetrated well into the tissue (Figure 2A), and dendritic cells are shown to internalize green fluorescent SLNs following incubation in vitro (Figure 2B).

The green fluorescent particles were also used in a proof-of-concept study for the development of an inhalable API-loaded SLN dispersion. A fluorescent dye-loaded SLN dispersion was aerosolized using an OTC nebulizer, and the aerosol plume from the mouthpiece was illuminated by UV light. The green fluorescent glow of the plume showed that the SLNs were indeed in the aerosol droplets, and analysis of the condensed aerosol showed that the particle size distribution of the SLNs in the original dispersion was maintained in the droplets. The droplet size of the aerosol was also found to be ideal for delivery to the deep lung (around 5 microns). This work could lead to improved pulmonary delivery of water-insoluble APIs for acute treatment in hospitals where doses may need to be high.

## SURFACE ENTRAPMENT OF APIs WITH SLNs

Instead of incorporating the drug into the particle, an additional way to exploit SLNs is to attach the API to the surface of the particle. The surface properties of SLNs can be varied widely and tailored to the final application. For example, the choice of emulsifier (cationic, non-ionic, anionic, and polymeric) has a strong influence on the surface electrical charge on the nanoparticles, measured by the zeta potential of the particles, as shown in Figure 3.

For SLNs that contain long-chain fatty acids or use them as the emulsifier, the carboxyl groups present at the particle surface can be used to covalently attach proteins and amine-terminated peptides using standard coupling chemistries (such as carbodiimide coupling).

Biologics are generally charged in aqueous solution, and as such are attracted electrostatically to surfaces of opposite charge, and may become strongly attached there as a result. We have found that electrically charged SLNs (cationic or anionic) strongly and irreversibly bind proteins with attachment efficiencies of around 90% (around 650 micrograms protein per mg of SLN solids). Evidence that the protein is attached at the particle surface is provided by the observation that after mixing the SLN and protein and allowing enough time for the protein to adsorb at the particle surface, the pH-dependence of the SLN's zeta-potential goes from that of the naked SLN to that of the pure protein.

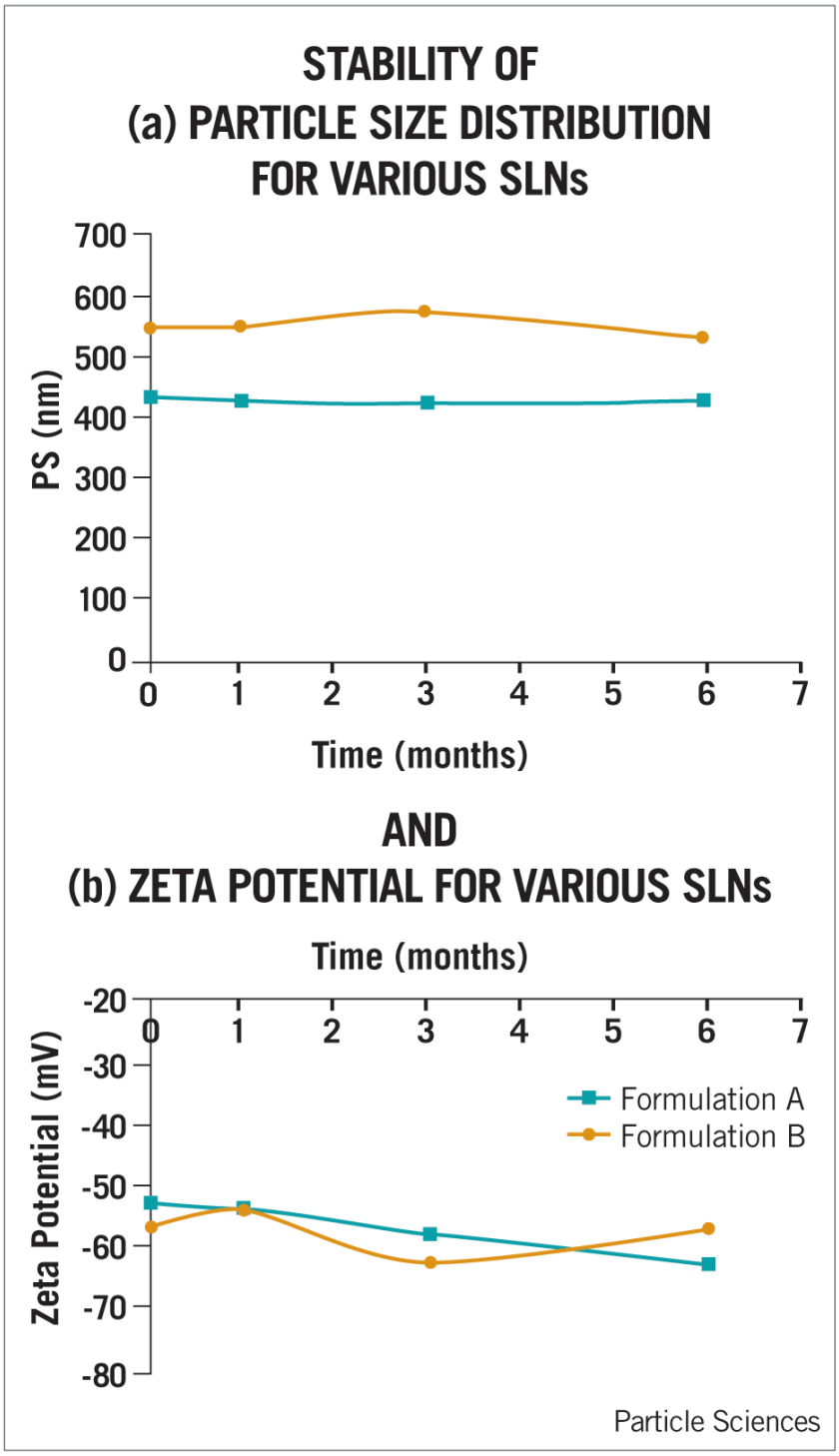
Essentially the SLNs surface properties become dominated by the protein attached there (Figure 4).

Based on the encouraging tissue and cellular uptake results and the ability to efficiently and simply attach proteins to the SLN surface, nanoparticles made of carnauba wax and formulated to carry gp140 (a model HIV antigen) were applied to the vaginal mucosa of mice to evaluate this route of administration as a novel approach to vaccination against HIV. As controls in this experiment, mice were also vaccinated by subcutaneous injection of the SLN-gp140 formulation as well as a formulation using alum, the only particles used in generally approved particle-containing vaccines in the US. The systemic challenge results with the SLNs were equivalent to the alum control (data not shown), indicating that these particular SLNs are potentially promising adjuvants for systemic vaccination.

## STERILIZATION

For parenteral administration, SLN dispersions must be sterile. The mean particle diameter of SLNs is often more than 200 nm, so sterile filtration is not possible in these cases. Autoclaving the finished dispersion is not practical as the lipids melt at temperatures used to terminally heat-sterilize pharmaceutical products, and the molten lipid droplets coalesce as there is no applied shear to prevent this. Options are therefore limited to aseptic manufacturing processes following sterilization of the starting materials (gamma or e-beam irradiation of the final dispersion) or exposure to ethylene oxide gas (EO). Bacterial endotoxins in raw materials need to be monitored, especially when raw materials are of natural origin. It may be possible to lyophilize the SLN dispersion, and this lyophile can be irradiated or exposed to EO. We have demonstrated that lyophilized SLNs made of carnauba wax are readily redispersed, and the original particle size distribution is

FIGURE 5



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recovered. Of course, SLN with appropriately small particle size can be sterilized using filtration.

## STABILITY

The shelf-life stability of SLNs can be very good. Lipids can be chosen that do not hydrolyze in aqueous suspension (another advantage over nanoparticles made from polymers, such as PLGA, which hydrolyzes with a rate that is dependent on polymer structure, and therefore must be lyophilized for practical use). The very small particle size and density close to unity of SLNs means gravity has little effect on the particles in dispersion, and Brownian motion is sufficient to maintain colloidal dispersion without creaming or sedimentation. Any such separation can usually be completely reversed by gentle agitation, even if it is observed. The particle size distribution and zeta potential remains stable over time (Figure 5) as neither Ostwald ripening nor particle dissolution occur in these systems, and the surface charge determining moieties are immobile. For SLNs made with natural lipids, and not made by an aseptic process, they can be prepared with long-term stability against biological growth using standard preservatives when tolerable.

## SUMMARY

SLNs are easily prepared nanoparticles made from inexpensive, safe, stable, and biodegradable materials and can be loaded internally or externally with APIs for controlled delivery. As such, they offer a highly versatile platform and one that should be considered when working with APIs that present solubility and/or bioavailability challenges.

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## BIOGRAPHY

**Dr. Andrew Loxley** is Director of New Technologies at Particles Sciences Inc., a contract research organization in Bethlehem, PA, specializing in pharmaceutical formulation development. He leads a variety of projects, many based on novel and proprietary nanotechnologies, in fields from HIV vaccine and microbicide development to gene-silencing SiRNA delivery. Prior to joining Particles Sciences, he led the development efforts in next-generation lithium ion batteries at A123 Systems Inc, electrophoretic displays at EINK Corp., and latex-based adhesives at Synthomer Ltd. He earned his BSc in Chemistry from the Univeristy of Sussex and his PhD in Physical Chemistry focusing on microencapsulation from the University of Bristol.