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(54) Title: SOLVENT AND WATER-FREE LIPID-BASED NANOPARTICLES AND THEIR METHODS OF MANUFACTURE

(57) Abstract: A water and solvent free Continuous Manufacturing platform for Nanoparticle Drug delivery systems (COMMAND), such as, self-dispersing solid lipid nanoparticles and proliposomal nanoparticles, and the nanoparticles made by such platform. Byremoving water and solvent from conventional processes, the formation of dry, solvent free self-dispersing solid lipid nanoparticles and dry, solvent free solid proliposomal nanoparticles can be performed using the COMMAND platform.



WO 2020/144377 A1

SOLVENT AND WATER-FREE LIPID-BASED NANOPARTICLES AND THEIR METHODS OF MANUFACTURE

BACKGROUND OF THE INVENTION

Nanotechnology in medicine

Poor water solubility and poor membrane permeability of New Chemical Entities (NCEs) under development is a common issue facing the whole pharmaceutical industry. It also results in a significant drug delivery problem to the early-stage medicine research and development. It has been reported that over 90% of new chemical entities are classified as poorly water soluble.

Nanomedicines present a solution to this issue. This is a high risk, high return market that has enjoyed unprecedented growth over the last five years. Nanomedicines can be used to solve fundamental issues related to the poorly water-soluble and poorly permeability of new chemical entities. The global nanomedicine market was valued at \$135 billion in 2015 with a predicted annual growth rate of over 10%, leading to a projected value of \$220 billion by 2020. Nanomedicine therapeutics are starting to gain significant attention among multinational pharmaceutical companies, of which, in 2015 alone, there were over 650 compounds in development with 60% of the nanoparticle delivery vehicles utilizing liposomal systems (~ \$110 billion industry).

There are several examples of Food and Drug Administration-approved nanomedicines manufactured using various processes via top-down and bottom-up approaches: large drug particles being processed to nanoparticle sizes; chemical/physical synthesis of nanoparticles via molecular assembling; using advanced techniques such as high-pressure homogenization/nanomilling, microfluidic devices; and traditional thin-film hydration/nanoemulsion methods. However, the production efficiency and commercialization success rate for current nanomedicines based on liposome and nanoemulsion is extremely low, e.g. only ~20 products are currently available for clinical use in a \$100 billion industry (2018).

Furthermore, despite widespread research, it is well recognised that the chemical and physical parameters of nanoparticle drug delivery systems will significantly affect the clinical target performance, the achievement of large-scale and reliable production, under clinical manufacturing conditions at low cost, is often not addressed. The current processes used for manufacturing nanomedicines suffer from many significant manufacturing issues, including: i) multi-step water/solvent-based batch processes; ii) the need for particle size reduction often involving specialised tools and equipment (such as liposome extrusion, ultrasonication and high-pressure homogenization); iii) limited batch sizes, iv) difficulty to scale-up and v) significant capital investment. These problems drive up the cost, limit production and hinder development of new medicines. As previously reported, a typical capital investment for setting up a large scale production facility for these liposomal nanomedicine would require approximate \$300 million or more (ref)

These manufacturing issues have been exemplified by the global shortage of Doxil® (an anti-cancer therapy that is over 20 years old); which resulted from the closure of a sterile injectables production site (Boehringer Ingelheim) quoting manufacturing challenges as the principle reason. Additional

investment of \$400 million with annual maintenance of \$100 million was the estimated cost. Global shortages of this anti-cancer treatment lasted for more than two years. Subsequently, FDA approved the abbreviated drug product applications from both Sun pharmaceuticals and Dr Reddy's resulting in the taken over of generic doxorubicin HCL liposomal formulations (~ \$ 1 Billion market).

Another example was the gold-standard liposomal amphotericin B nanomedicine AmBisome® (over 20 years old). The current global access to this product depends heavily upon the single access policy from Gilead Science. AmBisome was originally developed for anti-fungal therapy and has now expanded its application to the treatment of Visceral Leishmaniasis (VL) and HIV-VL co-infected patients. Current estimates suggest that globally almost 300,000 new cases of VL are diagnosed per year. However, only 50,000 cases were treated between 2011-2016 and about diagnosed 1.45 million patients were unable to access this optimum therapy. Despite significant efforts made by health organisations, charities and the manufacturer, a massive cost reduction for AmBisome® at large quantities is still unrealistic due to the difficulties and complexities associated with large-scale manufacturing. In fact, *"the control of Leishmaniasis in East Africa and South East Asia has become a formidable request for World Health Organisation"* (WHO,

http://www.who.int/neglected_diseases/news/Leishmaniasis-timeline-of-facts-and-the-quest-disease-control/en/). Global health report on Syria Leishmaniasis during the war.

It is clear that the massive gap between supply and demand in nanomedicine has created a difficult situation for both patient and health organisations, particularly for resource constrained countries where accessing to cheap medications is already difficult. Innovations and subsequently revolutions for a scalable manufacturing platform that is capable of consistently producing high quality nanomedicine at low cost.

Nanotechnology in cosmetics, skincare and agriculture

The applications of lipid-based nanoparticle systems can also be found in the areas of cosmetics, skincare and agriculture. Liposomes and solid lipid nanoparticles are widely researched in the cosmetic and skincare industry as delivery vehicles and have been found to be better than using the active cosmetic ingredients alone. The research & development landscape is very promising and the possibilities offered by lipid-based nanoparticle technology in several applications are being actively explored. For example, L'Oreal holds a very high number of patent associated with nanotechnology for skincare. These lipid-based nanoparticles can enhance the skin hydration, bioavailability and stability of the agents through the encapsulation. Examples of marketed products such as Revitalif by L'Oreal and Skin Caviar by La Prairie etc. utilising lipid-based nanoparticles for anti-aging purposes are normally fetching a very high price (>\$500 per 50 mL).

For agriculture sector, the current efforts in nanotechnology applications have been ongoing for largely a decade, searching for solutions in sustainability, food security and climate changes. Nanotechnologies aim in reducing the amount of sprayed chemical product by smart delivery, enhancing nutrient absorption and bioavailability of pesticides in crops, improving the animal production/reproduction and veterinary disease control are the main beneficial areas. For example, the global animal feed additives market has reached \$16 Billion with a CAGR of 4.5% (2014) and was projected at \$22 Billion by 2022. Feed additives are used in animal nutrition for purposes of improving

the quality of feed, the animals' performance and health. The use of nanoparticle or nanoencapsulation technology can significantly enhance the absorption of feed additives such as vitamins, amino acids and fatty acids and sustainability of animal farming.

However, the lipid-based nanoparticle applications in cosmetics, skincare and agriculture so far do not demonstrate a sufficiently high economic interest due to the nature of the complexity and high costs of large-scale manufacturing.

SUMMARY OF THE INVENTION

Disclosed herein is a water and solvent free Continuous Manufacturing platform for Nanoparticle Drug delivery systems (COMMAND) which directly addresses the research and manufacturing problems associated with lipid-based nanoparticles (proliposomal nanoparticle and self-dispersing solid lipid nanoparticle) disclosed above.

In one embodiment the present invention is a solid proliposomal nanoparticle comprising less than about 5% w/w water and/or less than about 1% w/w organic solvent

In another embodiment the present invention is a solid proliposomal nanoparticle comprising at least one phospholipid bilayer and a hydrophilic matrixexcipient, wherein the hydrophilic matrixexcipient is present inside the core of the phospholipid bilayer.

In one embodiment the present invention is a solvent and water free, continuous process to make self-dispersing solid lipid nanoparticles.

In one embodiment the present invention is a solvent and water free, continuous process to make solid proliposomal nanoparticles.

In another embodiment, the present invention is a water and solvent free process for making a self-dispersing solid lipid nanoparticle, the process comprising: a) combining: i) solid lipid; ii) surfactant; iii) solid hydrophilic matrix; and iv) optionally an active pharmaceutical ingredient; b) heating the combination of a) to between about 40 C and about 200 C to form a continuous liquid phase comprising the self-dispersing lipid particle and hydrophilic matrix; c) reduce the particle size of self-dispersing lipid particle to nanosize by stirring, mixing and/or homogenizing; and d) cooling the liquid phase of c) to form a solid comprising the self-dispersing solid lipid nanoparticle suspended within the hydrophilic matrix.

In another embodiment, the present invention is a water and solvent free process for making a solid proliposomal nanoparticle, the process comprising: a) combining: i) solid phospholipid; ii) sterol; iii) solid hydrophilic matrix; and iv) optionally an active pharmaceutical ingredient; b) heating the combination of a) to between about 40 C and about 200 C to form a continuous liquid phase comprising the solid proliposomal particle and hydrophilic matrix; c) reduce the particle size of solid proliposomal particle to nanosize by stirring, mixing and/or homogenizing; and d) cooling the liquid phase of c) to form a solid comprising the solid proliposomal nanoparticle suspended within the hydrophilic matrix.

The processes of the invention are illustrated in Figure 1 which shows a comparison with conventional processes. By removing the water and solvent out of the entire processes, the formation of dry self-

dispersing solid lipid nanoparticles and solid proliposomal nanoparticles can be transformed using the present invention.

In one embodiment, the processes of the present invention can be used to replace the traditional water/solvent based processes and offer significant advancements on flexibility and cost-saving production for scalable nanomedicine manufacturing.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1, schematic diagram of conventional solvent/water-based nanoparticle manufacturing platform comparing to the COMMAND platform of the invention.

Figure 2, illustration of self-dispersing solid lipid nanoparticles (a) and proliposomes (b) manufactured via continuous flow water/solvent-free platform of the invention.

Figure 3, illustration of the twin-screw extrusion platform for the generation of drug encapsulated liposome nanoparticles; a molten emulsion of lipids (blue) is created in the pool of hydrophilic matrix (yellow) and crystalline drugs (red) are dissolved and encapsulated within the molten lipids.

Figure 4, environmental scanning electron microscopy images (FEI Quanta FEG, Oxford Ex-ACT) of reconstituted liposomal nanoparticles from proliposome solids manufactured by COMMAND platform containing drug naproxen (a) and amphotericin B (b).

Figure 5, transmission electron (JOEL JEM 1400 Plus, JEOL USA) microscopy images of liposomal nanoparticles containing drugs naproxen (a, scale bar=50nm), ketoconazole (b, scale bar= 200nm) and amphotericin B (c,d); the liposomal nanoparticles were reconstituted from proliposomes manufactured by COMMAND platform. Examples of single/multi- bilayer(s) of phospholipids can be obtained within the proliposome products manufactured by COMMAND platform (e, f).

Figure 6, UV absorbance from *Candida Albican* media as a function of amphotericin B concentration.

Figure 7, direct comparison of in vitro minimum fungicidal concentrations (MFCs) between marketed AmBisome formulation and liposomal amphotericin B nanoparticles manufactured by COMMAND technology.

Figure 8, stability characterizations of (a) particle size (b) PDI and (c) zeta potential for manufactured nanoliposomal drug delivery system suspended in buffer solution for 28 days at temperature 4°C, 20°C and 37°C.

Figure 9, HCT116 cell toxicity assay on reconstituted liposomes from COMMAND platform at various concentrations for 48 hours; the cell viability is assessed by CellTiter-Glo® luminescent cell viability assay.

Figure 10, cell uptake comparison between reconstituted liposomal nanoparticles (via COMMAND, a) and mPEG-PLGA nanoparticles (b) using confocal laser microscopy (Nikon eclipse TE2000-U); the cell nuclei was dyed by DAPI blue and liposomal and mPEG-PLGA nanoparticles were loaded with Nile red; the average size of these particles are controlled at 110 nm with similar concentrations.

Figure 11, quantitative comparison of uptake of lipid formulation vs mPEG-PLGA nanoparticles: HCT116 cells were seeded and Nile red loaded treatments added at various time-points before three-fold washing with ice cold PBS. Cells were then acid stripped, washed and lysed.

Figure 12, 3-D plot describing the effects of lipid type (OA and CT), the of blank self-dispersing solid lipid nanoparticles (A), felodipine-loaded self-dispersing solid lipid nanoparticle (A) and hot-melt extruder screw configurations (B) on the particle sizes of reconstituted solid lipid nanoparticles; F1 and F2 are the two type of screw configurations (SC1 and SC2) for COMMAND platform.

Figure 13, 3-D plot depicting the effects of screw configuration and drug loading on the particle size (A) and PDI (B) of self-dispersing solid lipid nanoparticle formulations, F1 and F2 are two types of screw configurations used in COMMAND.

Figure 14, cumulative % drug release profiles (in vitro) of ketoconazole-loaded (a) and naproxen-loaded self-dispersing solid lipid nanoparticle formulations in PBS pH 6.8 at 37 °C. F1 and F2 represent the self-dispersing solid lipid nanoparticles prepared with different screw configurations of SC1 and SC2 at 15% w/w ratio for CT/lipids.

DETAILED DESCRIPTION

DEFINITIONS

As disclosed herein, “solid” means a material which, at room temperature, is characterized by structural rigidity and resistance to changes of shape or volume.

As disclosed herein, a “nanoparticle” is a particle with dimensions between about 1 nanometres (nm) and about 1000 nm in size.

As disclosed herein, a “solid lipid nanoparticle” is a solid nanoparticle comprising a solid lipid core, said core being stabilized by interfacial layers. The interfacial layers are made from materials selected from the group comprising surfactant, phospholipid and/or a mixture of both.

As disclosed herein, a “self-dispersing solid lipid nanoparticle” is a solid nanoparticle comprising a solid lipid nanoparticle and hydrophilic matrix wherein the hydrophilic matrix can rapidly dissolve in an aqueous solution to give a solid lipid nanoparticle in an aqueous suspension. The illustration of self-dispersing solid lipid nanoparticle is presented in Figure 2a. The self-dispersing solid lipid nanoparticles have a particle size ranging from about 1 nm to about 1000 nm, about 1 nm to about 500 nm, about 1 nm to about 200 nm, or about 1 nm to about 100 nm, or about 50 to 100 nm or about 80 to 100 nm.

As disclosed herein, the terms “liposome” and “liposomal particle” can be used interchangeably and mean a particle comprising at least one phospholipid bilayer. The phospholipid bilayer comprises, for example, phospholipid and sterol.

As disclosed herein, the terms “nanoliposome” and “liposomal nanoparticle” can be used interchangeably and mean a nanoparticle comprising at least one phospholipid bilayer at the size ranged disclosed above. The phospholipid bilayer comprises, for example, phospholipid and sterol.

As disclosed herein, the terms “nanoproliposome”, “proliposomal nanoparticle” and “proliposomal nanoparticle” can be used interchangeably and mean a nanoparticle comprising at least one phospholipid bilayer and hydrophilic matrixmatrix.

This definition is very different from prior art (WO2018/089759 A1 or WO2017/120586 A1).

In one embodiment of the invention, in the proliposomes of the invention the hydrophilic matrix can be present both inside and outside of the phospholipid bilayer as illustrated in Figure 2b, therefore

enhanced liposomal stability is expected. The hydrophilic matrix outside of the liposomal bilayer can rapidly dissolve in an aqueous solution to give a liposomal nanoparticle in an aqueous suspension. In one embodiment, a phospholipid and a sterol form at least one layer of the bilayer structure. In another embodiment, a proliposome of the present invention can comprise multiple phospholipid bilayers (as shown in Figure 5 TEM images). The “nanoproliposome” and “proliposomal nanoparticle” and “solid proliposomal nanoparticle” have a particle size ranging from about 1 nm to about 1000 nm, about 1 nm to about 500 nm, about 1 nm to about 200 nm, or about 1 nm to about 100 nm. The deliberate design of our proliposomal nanoparticles will offer much better product quality and controlled nanoliposome particle size distribution upon reconstitution.

As disclosed herein, a “hydrophilic matrix” is a crystalline solid with melting temperature between about 40°C to about 200°C. In one embodiment, the hydrophilic matrix is selected from the group comprising sugar (e.g. fructose, glucose), and sugar derivatives, e.g., sugar alcohol (e.g. sorbitol, xylitol, erythritol) and amino sugar (e.g. glucosamine, meglumine).

As disclosed herein, “COMMAND” is a solvent/water-free Continuous MANufacturing platform for Nanoparticle Drug delivery systems.

PARTICLES

In one embodiment, the present invention is a self-dispersing solid lipid nanoparticle and/or proliposomal nanoparticle produced by the processes and methods of the invention as disclosed herein without any involvement of water or organic solvent. The resulted self-dispersing solid lipid nanoparticle and proliposomal nanoparticle are dry solids.

As a direct comparison to other post-dried proliposomes, for example in US 2004/0175417 A1 for the preparation of amphotericin B proliposomes, the water contents was measured to be about 8-10% w/w.

In one embodiment, the present invention is a self-dispersing solid lipid nanoparticle and/or proliposomal nanoparticle comprising less than about 5% w/w water, less than about 3% w/w water, less than about 0.2% w/w water, less than about 0.1% w/w water, or less than about 0.05% w/w water.

In one embodiment, the present invention is a self-dispersing solid lipid nanoparticle and/or proliposomal nanoparticle comprising less than about 1% w/w organic solvent, less than about 0.5% w/w organic solvent, less than about 0.2% w/w organic solvent, less than about 0.1% w/w organic solvent, or less than about 0.05% w/w organic solvent.

In one embodiment, the present invention is a self-dispersing solid lipid nanoparticle and/or proliposomal comprising less than about 1% w/w organic solvent, less than about 0.2% w/w organic solvent, less than about 0.1% w/w organic solvent, or less than about 0.05% w/w organic solvent.

In one embodiment, the present invention is a self-dispersing solid lipid nanoparticle and/or proliposomal nanoparticle comprising less than about 5% w/w water and less than about 1% w/w organic solvent, less than about 3% w/w water and less than about 1% w/w organic solvent, less than about 0.2% w/w water and less than about 0.2% w/w organic solvent, less than about 0.1% w/w water

and less than about 0.1% w/w organic solvent, or less than about 0.05% w/w water and less than about 0.05% w/w organic solvent.

In one embodiment the self-dispersing solid lipid nanoparticle and/or proliposomal nanoparticle of the present invention have a mean size range from about 1 nm to about 1000 nm, from about 50 nm to about 600 nm, from about 100 nm to about 600 nm, from about 50 nm to about 500 nm, from about 100 nm to about 500 nm, or from about 200 nm to about 600 nm.

In one embodiment the self-dispersing solid lipid nanoparticle and/or proliposomal nanoparticle of the present invention have a polydispersibility index of less than about 0.5, or from about 0.1 to about 0.2, from about 0.1 to about 0.5, about 0.2 to about 0.5, or about 0.3 to about 0.5.

In one embodiment, the self-dispersing solid lipid nanoparticles of the present invention comprise about 1 % w/w to about 49% w/w lipid nanoparticle and about 50 % w/w to about 98% w/w hydrophilic matrix and about 1 to 10% w/w surfactant.

In one embodiment, the amount of self-dispersing solid lipid nanoparticle is from about 1% w/w to about 49% w/w, from about 1% w/w to about 10% w/w, from about 10% w/w to 20% w/w, from 30% w/w to 49% w/w.

In one embodiment, the proliposomal nanoparticle of the present invention comprise about 1 % w/w to 49.9% w/w phospholipid and 50 % w/w to 98% w/w hydrophilic matrix and 0.1 to 5% w/w of cholesterol. In one embodiment, the amount of phospholipid is from 1 % w/w to about 10% w/w, about 10 % w/w to about 20% w/w, about 20 % w/w to about 30% w/w and about 30 % w/w to about 49.9% w/w according to the final formulation properties and particle size.

MIXTURES

The present invention is a mixture comprising a hydrophilic matrix and the self-dispersing solid lipid nanoparticle and/or proliposomal nanoparticle of the present invention as disclosed herein.

In one embodiment the self-dispersing solid lipid nanoparticle and/or proliposomal nanoparticles are suspended in a hydrophilic matrix.

PROCESSES

Solid lipid nanoparticles and liposomes are mainly formed by wet process (e.g. using solvent and/or water). These wet-based techniques typically utilize an organic solvent or aqueous material to dissolve or disperse the lipids forming nanoemulsion and further evaporate it from the nanoemulsion to obtain the dry solid lipid particles or proliposomes. In this principle, there will be residual organic solvent, water and/or potential solvent/water induced impurities within the dried solid lipid particles or proliposomes, e.g. up to 10% w/w volatile components can be discovered in market products. In a factory setting, the disposal and/or recovery of these contaminated volatile components or water during large scale manufacturing may be environmentally unfriendly and costly. For example, in prior art WO2017/120586 A1 or US 2004/0175417 A1, a small amount of solvent was required to form the initial solution for preparation of proliposome or liposomes.

The processes of the invention do not use any organic solvent or any aqueous material representing a completely transformation to the current pharmaceutical research and manufacture. In the processes of the invention the solid materials, such as, solid lipids and solid hydrophilic matrix are used. These solid materials are liquefied under process conditions known in the art and/or defined herein. More than one material can be liquefied at once and the materials can then be mixed to form a liquid mixture or emulsion via, for example, high speed stirring/mixing, hot melt extrusion (HME), ultrasonication, homogenization or a combination thereof, etc. The hydrophilic matrix forms a continuous phase during the process of the invention, while the lipid phase forms a dispersed phase. In one embodiment, of the invention, during the process of the invention, a homogeneous mixture comprising lipid phase dispersed in hydrophilic matrix at nanoscales is formed. The exact particle size distribution for the lipid phase is highly depended on the design of the formulation and process. In one embodiment of the processes of the present invention, an active pharmaceutical ingredient can also be encapsulated within the lipid phase.

In one embodiment the present invention is a water and/or solvent free process for making a self-dispersing solid lipid nanoparticle system of the present invention, the process comprising a) combining: i) solid lipid; ii) surfactant; iii) solid hydrophilic matrix; and iv) optionally an active pharmaceutical ingredient; b) heating the combination of a) to between about 40 C and about 200 C, about 80 C to about 120 C, about 100 C to about 150 C, about 150 C to about 200 C, or about 50 C to about 150 C to form a continuous liquid phase comprising self-dispersing solid lipid particle and hydrophilic matrix; and c) and preferably providing mechanical energy into the systems via high speed stirring, hot melt extrusion or ultrasonication or a combination thereof to form self-dispersing lipid nanoparticles in hydrophilic matrix; d) cooling the liquid phase of c) to form a solid comprising a self-dispersing solid lipid nanoparticle suspended within the solid hydrophilic matrix.

In one embodiment the present invention is a one step, water and/or solvent free process for making a self-dispersing solid lipid nanoparticle of the present invention, the process comprising a) combining: i) solid lipid; ii) surfactant; iii) solid hydrophilic matrix; and iv) optionally an active pharmaceutical ingredient; b) heating the combination of a) to between about 40 C and about 200 C to form a continuous liquid phase comprising self-dispersing solid lipid nanoparticle and hydrophilic matrix; and c) cooling the liquid phase of b) to form a solid comprising the self-dispersing solid lipid nanoparticle suspended within the solid hydrophilic matrix.

In one embodiment the present invention is a one step, continuous, water and/or solvent free process for making a self-dispersing solid lipid nanoparticle of the present invention, the process comprising a) combining: i) solid lipid; ii) surfactant; iii) solid hydrophilic matrix; and iv) optionally an active pharmaceutical ingredient; b) heating the combination of a) to between about 40 C and about 200 C to form a continuous liquid phase comprising self-dispersing solid lipid nanoparticle and hydrophilic matrix; and c) cooling the liquid phase of b) to form a solid comprising the self-dispersing solid lipid nanoparticle suspended within the solid hydrophilic matrix.

In one embodiment the present invention is a one step, continuous, water and solvent free process for making a self-dispersing solid lipid nanoparticle of the present invention, the process comprising a)

combining: i) solid lipid; ii) surfactant; iii) solid hydrophilic matrix; and iv) optionally an active pharmaceutical ingredient; b) heating the combination of a) to between about 40 C and about 200 C to form a continuous liquid phase comprising self-dispersing solid lipid nanoparticle and hydrophilic matrix; and c) cooling the liquid phase of b) to form a solid comprising the self-dispersing solid lipid nanoparticle suspended within the solid hydrophilic matrix.

In one embodiment the present invention is a one step, continuous, water and solvent free process for making a water and solvent free self-dispersing solid lipid nanoparticle of the present invention, the process comprising a) combining: i) solid lipid; ii) surfactant; iii) solid hydrophilic matrix; and iv) optionally an active pharmaceutical ingredient; b) heating the combination of a) to between about 40 C and about 200 C to form a continuous liquid phase comprising self-dispersing solid lipid nanoparticle and hydrophilic matrix; and c) cooling the liquid phase of b) to form a solid comprising the self-dispersing solid lipid nanoparticle suspended within the solid hydrophilic matrix.

In one embodiment of the present invention, the active pharmaceutical ingredient can be any small organic molecules.

In one embodiment of the present invention, the active pharmaceutical ingredient can be biological molecules such as protein, peptide etc.

In one embodiment of the present invention all of the solid materials are fed into a hot melt extruder in a continuous method. In one embodiment the process of the invention is carried out in one step.

In one embodiment of the present invention, the hydrophilic matrix is crystalline solids or at least partially crystalline solids before and after the process of the invention.

In one embodiment of the present invention the solid lipid is an anionic or cationic lipid.

In one embodiment of the present invention the solid lipid is an anionic or cationic phospholipid (e.g., 1,2-distearoyl-sn-glycero-3-phosphocholine, DSPC; 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, DPPC; 1,2-dimyristoyl-sn-glycero-3-phosphocholine, DMPC; dimethyldioctadecylammonium dromide, DDAB; 1,2-dioleoyl-3-trimethylammonium-propane chloride, DOTAP).

In one embodiment of the present invention the solid lipid is a monoglyceride, fatty acid (e.g., long chain fatty acid), mono-, di-, or triglyceride or natural wax. In one embodiment of the present invention the monoglyceride is glyceryl monostearate or glyceryl behenate. In one embodiment of the present invention the long chain fatty acid is stearic acid or behenic acid. In one embodiment of the present invention the triglyceride is glyceryl tristearate or glyceryl tripalmitate. In one embodiment of the present invention the natural wax is beewax or carnauba wax.

In one embodiment of the present invention the surfactant is a phospholipid selected from the group comprising phosphatidylglycerol, poloxamer or tween. In one embodiment of the present invention surfactant is poloxamer 188 or tween 80.

In one embodiment of the present invention a) further comprises a liquid lipid. In one embodiment of the present invention the liquid lipid is a short chain unsaturated or saturated fatty acid. In one embodiment, when liquid lipid is used in the process of the invention any solvent or water is removed from the raw materials before and/or during process of the invention. In one embodiment of the process of the invention, the process temperature will be generally higher than the evaporation temperature of the solvent or water.

In one embodiment of the present invention in step a) about 1 to about 45% w/w, solid lipid is combined with about 50% to about 98.9% w/w hydrophilic matrix. In one embodiment of the present invention in step a) about 0.1 to about 5% w/w surfactant is used.

In one embodiment the present invention is water and/or solvent free process for making a solid proliposomal nanoparticle of the invention, the process comprising a) combining: i) solid phospholipid; ii) sterol; iii) solid hydrophilic matrix; and iv) optionally an active pharmaceutical ingredient; b) heating the combination of a) to between about 40 C and about 200 C, 8 about 80 C to about 120 C, about 100 C to about 150 C, about 150 C to about 200 C, or about 50 C to about 150 C and optionally providing mechanical energy into the systems via high speed stirring, hot melt extrusion or ultrasonication or a combination thereof to form a continuous liquid phase comprising solid proliposomal nanoparticles suspended in hydrophilic matrix; and c) cooling the liquid phase of b) to form a solid comprising the proliposomal nanoparticle suspended within the solid hydrophilic matrix. In one embodiment the present invention is one step water and/or solvent free process for making a solid proliposomal nanoparticle of the invention, the process comprising a) combining: i) solid phospholipid; ii) sterol; iii) solid hydrophilic matrix; and iv) optionally an active pharmaceutical ingredient; b) heating the combination of a) to between about 40 C and about 200 C to form a continuous liquid phase comprising solid proliposomal nanoparticle and hydrophilic matrix; and c) cooling the liquid phase of b) to form a solid comprising the solid proliposomal nanoparticle suspended within the solid hydrophilic matrix.

In one embodiment the present invention is one step, continuous water and/or solvent free process for making a solid proliposomal nanoparticle of the invention, the process comprising a) combining: i) solid phospholipid; ii) sterol; iii) solid hydrophilic matrix; and iv) optionally an active pharmaceutical ingredient; b) heating the combination of a) to between about 40 C and about 200 C to form a continuous liquid phase comprising solid proliposomal nanoparticle and hydrophilic matrix; and c) cooling the liquid phase of b) to form a solid comprising the solid proliposomal nanoparticle suspended within the solid hydrophilic matrix.

In one embodiment the present invention is one step, continuous, water and solvent free process for making a solid proliposomal nanoparticle of the invention, the process comprising a) combining: i) solid phospholipid; ii) sterol; iii) solid hydrophilic matrix; and iv) optionally an active pharmaceutical ingredient; b) heating the combination of a) to between about 40 C and about 200 C to form a continuous liquid phase comprising solid proliposomal nanoparticle and hydrophilic matrix; and c) cooling the liquid phase of b) to form a solid comprising the solid proliposomal nanoparticle suspended within the solid hydrophilic matrix.

In one embodiment the present invention is one step, continuous, water and solvent free process for making a water and solvent free solid proliposomal nanoparticle of the invention, the process comprising a) combining: i) solid phospholipid; ii) sterol; iii) solid hydrophilic matrix; and iv) optionally an active pharmaceutical ingredient; b) heating the combination of a) to between about 40 C and about 200 C to form a continuous liquid phase comprising solid proliposomal nanoparticle and hydrophilic matrix; and c) cooling the liquid phase of b) to form a solid comprising the solid proliposomal nanoparticle suspended within the solid hydrophilic matrix.

In one embodiment of the present invention the solid phospholipid is a saturated or non-saturated phosphatidylcholine. In one embodiment of the present invention the solid phospholipid is a hydrogenated soy phospholipid. In one embodiment of the present invention the solid phospholipid is dipalmitoylphosphatidylcholine. In one embodiment of the present invention the solid phospholipid is a saturated or non-saturated phosphatidic acid, a saturated or non-saturated phosphatidylethanolamine, or saturated or non-saturated phosphatidylserine.

In one embodiment of the present invention the hydrophilic matrix comprises a sugar or sugar alcohol or amino sugar or other sugar derivatives. In one embodiment of the present invention the hydrophilic matrix is fructose or xylitol or meglumine.

In one embodiment of the present invention in step a) about 1 to about 45% w/w solid phospholipid is combined with about 50 to about 98.9% w/w hydrophilic matrix. In one embodiment of the present invention in step a) about 0.1 to about 5% sterol is used.

In one embodiment, the sterol selected from the group comprising cholesterol, ergosterol, stigmasterol, and androsterone.

In one embodiment the processes of the invention as described herein are performed in one-step. In one embodiment the processes of the invention as described herein are performed in a continuous process. In one embodiment of the present invention, the continuous process includes continuously feeding i) solid lipid; ii) surfactant; iii) solid hydrophilic matrix; and iv) optionally an active pharmaceutical ingredient through a feeder to a hot-melt extruder to prepare a self-dispersing solid lipid nanoparticle composition of the present invention. In one embodiment of the present invention, the continuous process includes continuously feeding i) solid phospholipid; ii) sterol; iii) solid hydrophilic matrix; and iv) optionally an inactive pharmaceutical ingredient through a feeder into a hot-melt extruder to prepare a proliposomal nanoparticle composition of the present invention.

In one embodiment the process of the invention is water free, solvent free, one step, continuous manufacturing method to make the self-dispersing solid lipid nanoparticle and proliposomal nanoparticle compositions of the invention.

In one embodiment of the present invention, a) solid lipids (both anionic and cationic solids), such as, glycerol monostearate (for solid-lipid nanoparticles), or b) solid hydrogenated soy phospholipid (for proliposomal formulations) and c) solid sugar/sugar alcohol based hydrophilic matrix at defined lipid to matrix ratio are fed into the barrel of hot-melt extruder via automated feeder. Poorly water-soluble drugs (solid) are pre-mixed with the lipid-matrix mixtures (solid) and subsequently dissolved into the lipids phase during HME process. At the same processing temperature, the hydrophilic matrix is also melted forming a continuous liquid phase. Due to the immiscibility between hydrophobic lipids and drug with the hydrophilic matrix, a homogeneous liquid containing nano-scale phase separated structures may be formed in the presence of twin-screw shearing. Upon exit of extruder die, the liquid mixture will solidify where the self-dispersing solid lipid nanoparticles or proliposomal nanoparticles are suspended within the hydrophilic matrix. Additional surfactants (solid or liquid), cholesterol or phospholipids may be added into the solid mixture for stabilisation purposes of the nanoparticles. The liquid can be solidified during HME process or after to produce a solid product.

Upon the rehydration of the product, the solid hydrophilic matrix will rapid dissolve and the suspended lipid mixtures will form solid lipid nanoparticles or liposomal nanoparticles.

In one embodiment of the present invention, lipid-based nanoparticle (proliposomal nanoparticles or self-dispersing solid-lipid nanoparticle) drug delivery systems will be self-assembled (Figure 2). By using this novel solvent/water-free platform, we can produce solid sugar-based products containing dried self-dispersing solid lipid nanoparticle or proliposomal nanoparticles in single step without the extra drying and/or particle size reduction (Figure 2).

The finished product can be powder or pellets depending on the use of extruder die head. The solid sugar-based products containing dried self-dispersing solid lipid nanoparticles or proliposomal nanoparticles, can be stored in a desiccated environment, e.g. sealed bottle, at room temperature. This will ease transportation, packaging and storage, particularly in countries with hot climate. For parenteral delivery, the self-dispersing solid lipid nanoparticles and proliposomal nanoparticles of the invention can be rapidly dissolved in a saline solution and the self-dispersing solid lipid nanoparticles and proliposomal nanoparticles of the invention can be reconstituted prior to infusion or injection (similar to other marketed lipid-based nanomedicine formulations). For oral drug delivery, the self-dispersing solid lipid nanoparticles and proliposomal nanoparticles of the invention may be subjected to technologies such as capsule filling, tableting or coating to assist the formation of solid dosage forms. If self-dispersing solid lipid nanoparticles of the invention are used, no special coating is required, whilst, if proliposomal nanoparticles of the invention are used, additional steps to prevent the degradation of liposome inside the stomach can be used.

EXTRUSION

In one embodiment, the process of the present invention uses a high speed mixer.

In one embodiment, the process of the present invention uses a hot melt extruder. As used herein, Hot melt extrusion (HME) is the process of applying heat and shearing to melt a material and force it through an orifice in a continuous process. HME is carried out using an extruder. As used herein, extruders consist of up to four distinct parts:

- i) an opening through which material enters a barrel, that may have a feeder that is filled with the material(s) to be extruded, or that may be continuously supplied to in a controlled manner by one or more external feeder(s),
- ii) a conveying section (process section), which comprises the barrel and one or two screw(s) that transport, and where applicable, mix the material,
- iii) integrated or downstream auxiliary equipment for cooling, cutting and/or collecting the finished product and
- iv) an option of orifice (die) for shaping the material as it leaves the extruder.

In one embodiment the extruder used in the process of the invention is a single screw extruder, comprising one rotating screw at length to screw diameter (L/D) ratio of 40:1. In one embodiment, the extruder used in the process of the invention is a twin-screw extruder, comprising two rotating screws rotating at the same direction (co-rotating).

In one embodiment, the extruder used in the process of the invention is a twin-screw extruder, comprising two rotating screws rotating at the opposite direction (counter-rotating).

In one embodiment of the present invention, the material is melted in the hot melt extruder by frictional heating within the barrel. In one embodiment, the barrel is heated with heaters mounted on the barrel. In one embodiment, the barrel is cooled with water.

In one embodiment processes described herein utilize hot melt extrusion to form self-dispersing solid lipid nanoparticles and proliposomal nanoparticles suitable for use in nutraceutical or pharmaceutical applications.

In one embodiment processes described herein utilize hot melt extrusion to form self-dispersing solid lipid nanoparticles and proliposomal nanoparticles suitable for use in nutraceutical or pharmaceutical applications.

In one embodiment, the length to screw diameter (L/D) ratio of the extruder is 20:1. The total configurable screw element is 20 with mixing elements (60° or 90°) and forward convey elements (FC) in the sequential order of: FCx5 - 90°x3 – FCx2 - 60°x2 – FCx3 – 90°x2 – FCx3.

In one embodiment, the length to screw diameter (L/D) ratio of the extruder is 20:1. The total configurable screw elements is 20 with mixing elements and convey elements at defined ratio about 1:1, or about 1:2, or about 2:1. The sequential order of mixing to convey elements are also defined.

In one embodiment, the length to screw diameter (L/D) ratio of the extruder is 40:1. The total configurable screw element are 10 with mixing elements (60°) and forward convey elements in the sequential order of: FCx10 - 60°x4 – FCx10 - 60°x4 – FCx10 - 60°x2.

In one embodiment the processes of the invention are continuous and scalable.

Due to the principle of continuous process, the optimized HME parameters are based on the relative scale of manufacturing, therefore, it is very common that the scale-up of the COMMAND platform is significant easier in industrial settings. A liquid-based flow relaying on the connection of pipes between each step of the batch process may be completely replaced by COMMAND based one-step process which may offer significant advantages in scale-up.

It is clear that, by using design of experiment (DoE) approach, the critical process parameters and formulation combinations can be identified using reduced number of experiment. The design space for selected formulations may be established and desired product qualities can be controlled through the DoE process.

EXAMPLES

Drug solubility in lipids

Drug solubility/miscibility in lipids are critical for the formation of stable proliposomal and SLN systems containing hydrophobic drugs, as the hydrophilic drug needs to be soluble/miscible within the lipids. While drug solubility in lipids are normally assessed via a trial and error approach in conventional manufacturing platforms, the prediction of this in COMMAND platform needs to be conducted in advance due to the continuous nature of the process. Initial assessments are designed using thermodynamic modelling with small scale experimental approaches such as hot-stage light

microscopy and differential scanning calorimetry methods. The predicted drug-lipids loadings are also validated after manufacture.

Example 1, drug solubility

The solubilities of three drugs: felodipine, naproxen and ketoconazole with two solid lipids glycerol monostearate (GMS) and hydrogenated soy PC (HSPC) were estimated using a thermodynamic approach. The drug in lipid solubilities are summarized in Table 1. Similarly, the solubility of drug in phospholipids can also be estimated. Three drugs felodipine, naproxen and ketoconazole with phospholipids HSPC are summarized in Table 1. The estimated values of drug solubility in lipids can provide guidance on the identification of maximum drug loading in the premix. It also provides necessary information on the design of COMMAND process, particularly for the level of drug to lipids ratio at different temperatures.

Table 1 the prediction drug solubility in lipids at temperature 25°C and 110°C for both solid lipid nanoparticles and nanoliposome systems

Drug solubility in lipids at 25°C			
Lipid types	Felodipine w/w%	Naproxen w/w%	Ketoconazole w/w%
GMS	12	8	31
HSPC	5	38	1
Drug solubility in lipids at 110°C			
Lipid types	Felodipine w/w%	Naproxen w/w%	Ketoconazole w/w%
GMS	20	13	55
HSPC	15	42	8

Example 2, nanoliposomal drug delivery systems via COMMAND

Liposomal amphotericin B system

To demonstrate the suitability of COMMAND for the manufacturing of amphotericin B nanoparticle drug delivery system, the market product AmBisome® was used as a comparison. Similar phospholipids combinations consisting of HSPC and DSPG at 15% w/w to hydrophilic matrix xylitol were used. The drug loading of amphotericin B to total lipids ratio is 10% w/w. In this amphotericin B case, the extruder barrel included two 11-mm screws rotating in the same direction (co-rotating twin-screw) operating screw speed at 200 rpm. The premixed solids were fed into the extruder via powder feeder at feed rate of 8 rpm. The temperature of extruder barrel is also set at 110°C across the five zones with screw rotation. With these process conditions, the lipids and drug within the extrudates were quantified using a combination of high performance liquid chromatography (HPLC) and evaporative light scattering detection (ELSD). The encapsulation of amphotericin B within extrudates was calculated to be 97% ± 0.2 and the recovery of both lipid HSPC and DSPG within the extrudates were calculated to be 92% ± 0.2 and 99% ± 1 showing excellent recovery of both drug and lipids after the process. After reconstitution in water, the resulted amphotericin B liposomal nanoparticles were

quantified by dynamic light scattering method (Zetasizer NanoS, Malvern Instrument, UK) to be around 124 nm with PID of 0.232 (Table 2, Figure 5).

The moisture contents in the proliposomal nanoparticle amphotericin B systems manufactured by COMMAND were tested using Karl Fischer Titration (870 KF Tirino plus, Metrohm AG); the marketed AmBisome® product was also tested with the same technique. The moisture contents were measured to be $10 \pm 0.6\%$ w/w for AmBisome® and $1 \pm 0.5\%$ w/w for proliposome amphotericin B nanoparticles(COMMAND) respectively.

Liposomal naproxen system

In another experiment, the phospholipid DPPC (16-carbon) was mixed with crystalline sorbitol (DPPC to sorbitol at 10%:90% w/w) using universal rotary mixer. A model hydrophobic drug naproxen was also added within the mixture at pre-defined ratio (20%: 80%, naproxen:DPPC, w/w). In this naproxen case, the extruder barrel included two 11-mm screws rotating in the same direction (co-rotating twin-screw) operating screw speed at 200 rpm. The screw configuration was organized with convey elements and 90° mixing elements at 2:1 ratio. The mixed solids were fed into the extruder via powder feeder at feed rate of 8 rpm. The temperature of extruder barrel was also set at 110°C across the five zones with screw rotation speed of 100 rpm. Table 2 demonstrates the ability of COMMAND platform in generation nanoliposome drug delivery systems consisting of phospholipids and cholesterol at various combinations.

The moisture contents in the proliposomal nanoparticle naproxen systems (manufactured by COMMAND) were tested using Karl Fischer Titration (870 KF Tirino plus, Metrohm AG). The moisture content was measured to be $1 \pm 0.5\%$ w/w for proliposome naproxen system.

Scanning electron microscope and transmission electron microscope were both used to characterize the liposomal nanoparticle reconstituted from the sugar based extrudates (COMMAND platform). It is clearly demonstrated that, in Figure 4, 5 and 6, the reconstituted liposomal nanoparticle drug delivery systems are spherical particles in the range of approx. 200-110 nm. The results are showing similar particle size when measured by dynamic light scattering method (Table 2). They are also in the similar size to mPEG-PLGA nanoparticles current manufactured by PolySciTech Inc. using conventional methods

(<https://akinainc.com/polysciotech/products/polyvivo/categories.php>)

Table 2 Assessment of loading capacity, entrapment efficiency, particles size and polydispersity index (PDI) of nanoliposome formulations produced by COMMAND one-step process; particle size and distribution were measured via dynamic light scattering (Zetasizer NanoS, Malvern Instrument, UK)

<u>Formulation</u>	<u>Average Size by intensity</u> <u>(nm)</u>	<u>PDI</u>	<u>EE%</u>
LIPOSOME 1 blank	130.4	0.247	-

LIPOSOME 2 loaded naproxen (20% w/w)	149.5	0.291	-80%
LIPOSOME 3 loaded felodipine (10% w/w)	203.2	0.237	-95%
LIPOSOME 3 loaded amphotecerin (16% w/w)	114.8	0.132	-89%
LIPOSOME 5 loaded ketoconazole (10% w/w)	224.8	0.232	-78%
LIPOSOME 6 loaded insulin (12% w/w)	130.5	0.15	-68%

Example 3, Electron microscopy images on reconstituted liposomal amphotericin B. Scanning electron microscopy was used to assess the reconstituted nanoliposomes manufactured by COMMAND platform. Briefly, after reconstituting the proliposomes (COMMAND), the suspensions (~5mg/mL liposome concentration) were added to double sided copper tape, fixed to an aluminium stub and allowed to dry. Liposomes were then sputter coated with gold and imaged using the FEI Quanta FEG - Environmental Scanning Electron Microscope (E-SEM, Figure 4). The reconstituted liposomal nanoparticle structure was characterized using transmission electron microscope (TEM, JOEL JEM 1400 Plus, USA). The reconstituted liposomal nanoparticles were collected and placed on the TEM grid (copper mesh coated with amorphous carbon), washed with water twice and stained with 2% phosphotungstic acid twice (10 μ l, 30 sec). The grids were left to dry under room temperature and vacuum (2mBar). The dried liposomal nanoparticle samples were discharged under UV light prior to the TEM imaging. Figure 5 shows various liposomal nanoparticle from reconstituted proliposome solids manufactured by COMMAND. The bilayer structure of liposome dyed by the phosphotungstic acid can be clearly seen from TEM images. The particle sizes of these reconstituted liposomes are in similar range to the results obtained from Zetasizer.

The formation of novel phospholipid bilayer structure within the hydrophilic matrixs produced via COMMAND platform has also been characterized by transmission electron microscope (TEM, JOEL JEM 1400 Plus, USA). The images are shown in Figure 5 e and f. Bilayer structures of the phospholipids in the presence of xylitol can be observed in the range from 10 to 200nm (dark spherical particles). These phospholipid bilayer structures remain intact upon the reconstitution of proliposome post-manufacture.

Example 4, Direct comparison of liposomal amphotericin B nanoparticles by COMMAND and market AmBisome *in vitro*

The *in vitro* study on produced liposomal amphotericin B nanoparticles was based on the minimum fungicidal concentration and minimal inhibitory concentrations for *Candida Albicans*. Market product AmBisome was used as comparison.

Inoculum Suspension composition: *Candida Albicans* was cultured on Sabouraud's dextrose agar for 48 hours at 37 °C. 1.3×10^5 CFU/ml *Candida Albicans* suspension was prepared in sterile distilled

water for the test. When preparing the solution, the cell density of the suspension was adjusted to 0.15 by measuring the absorbance in a spectrophotometer at a wavelength of 530 nm. Media: Double strength RPMI 2% G medium. Working solution: Two-fold serial dilutions from 64 mg/L to 0.0195 mg/L for extrudate, ambisome and amphotericin B free drug were prepared in the RPMI media.

Minimum Inhibitory Concentrations (MICs) determination:

The two-fold serial dilutions for drugs were prepared in the 96 well plates. The candida albicans suspension was added into each well and mixed with drug dilutions. Each well contains 100 uL drug and 100 uL fungal suspension. The 96 well plates were incubated at 37 °C for 24 hours. Microdilution plate reader was used to measure the absorbance at 530 nm. The MIC is determined as the lowest concentration giving rise to an inhibition of growth of 90% of that of the drug free control.

Minimum fungicidal concentration (MFCs) determination:

The incubated fungal suspensions in each well below the MIC were transferred on to the Sabouraud's dextrose agar for incubation for 24-48 hours. The MFC is determined as the lowest concentration giving rise to 99.9% fungal cell death.

MIC and MFC on AmBisome and liposomal amphotericin B nanoparticles via COMMAND:

From Figure 6, it can be observed that the growth of Candida Albicans was fully inhibited above the concentration of 0.5 mg/L for all the formulations, with no increase in the UV absorbance, which was corresponding to the cell density. A slight difference presents in their exact MIC as MIC for liposomes via COMMAND technology is 0.25 mg/L whilst MIC for AmBisome is 0.5 mg/L. In Figure 7, the corresponding concentrations of the drugs where with no Candida cells grew on the agar stand for the MFC. At lower concentration, although the growth of cells was inhibited at MIC, the Candida cells were still alive. They were only killed when the concentration reached MFC as MFC for liposomes via COMMAND technology is 1 mg/L whilst MFC for AmBisome is 2 mg/L.

From all these tests, the liposomal amphotericin B nanoparticles manufactured by COMMAND technology offer comparable results to the marketed AmBisome product.

Example 5, direct comparison of nanoliposome via COMMAND and mPEG-PLGA nanoparticle via batch process

For any product manufactured via a new process to be successful it must be at least, as useful/effective as the currently available alternatives. In order to give an indication as to how the HME formulation compared to an alternative delivery system PLGA-mPEG nanoparticle with similar particle size were produced via a solvent/water based conventional method and loaded with Nile red. PLGA nanoparticle made by Polysciences is a blank nanoparticle and commercially available, the main reason to compare to PLGA is because this type of blank nanoparticle has been widely used at the similar nanoparticle size range to the nanoliposome blank particles generated via COMMAND. The cell culture studies show the liposome nanoparticle manufactured using COMMAND platform is comparable to the commercially available blank nanoparticle in terms of cell toxicity and uptake. As shown in Figure 9, there was no significant nanoparticle aggregation on reconstituted nanoliposome suspension. Cell viability (toxicity) was assessed via Cell Titre Glo (CTG) assay. HCT116 cells were seeded in 96-well plates at 2500 cells/well and allowed to adhere. Cells were

subsequently treated with nanoliposome formulation and incubated at 37°C for 48hrs, the concentration was ranged between 0.0125 to 0.2 mg/mL as shown in. The cell viability after the treatment of nanoliposomal drug delivery system (liposome2) was not significantly changed. Following assessment of in vitro cytotoxicity the ability of the lipid formulation to enter the cells was assessed. HCT116 cells were incubated with calcein AM cytoplasmic dye and the nanoliposome particles for 2hrs before washing of the cells, fixing and imaging via confocal microscopy. The nanoliposome particles were readily internalised and located within the cytoplasmic region of the cell (Figure 10).

For cell uptake studies, a comparison of uptake of mPEG-PLGA nanoparticles and the HME lipid formulation was conducted by confocal microscopy (Figure 10). HCT116 cells were treated with equivalent amounts of lipid or polymeric nanoparticle formulation for 1, 2 or 3 hours. Cells were then washed, fixed and imaged. At all time points assessed, there was no discernable difference in the amount of internalisation between the two nanoparticle formulations. This indicates that HME is conceivably an alternative means to conventional methods of producing useful nanoparticle systems. To gain a more quantifiable comparison of uptake the fluorescent signal of the two formulations post internalization was measured (Figure 11). Following treatment over varying time periods cells were acid stripped (to remove any material bound to the surface but not internalized) and then lysed. The fluorescent signal was equilibrated between the two formulations to account for differing levels of entrapment. Again, little difference in uptake between the two formulations was noted over the time points assessed highlighting the possibility of using HME for nanomedicine production.

Example 6, self-dispersing solid-lipid nanoparticle systems via COMMAND

In one experiment, the glycerol monostearate (GMS) and solid surfactant poloxamer 188 (GMS to poloxamer 188 at ratio 90%:10% w/w) were mixed with crystalline sorbitol (GMS to sorbitol at ratio 10%:90% w/w) using universal rotary mixer. A model hydrophobic drug felodipine was also added within the mixture at pre-defined ratio (12%: 88% w/w felodipine: GMS). Extruder barrel may include any number of extruder screws in a variety of configurations depending on the requirements of particle size. In this felodipine case, the extruder barrel included two 11-mm screws rotating in the same direction (co-rotating twin-screw) operating screw speed at 100 RPM. The screw configuration consisted of convey and 90° mixing elements at 1:1 ratio. The mixed solids with starting particle size of micro range were fed into the extruder via powder feeder at feeding rate of 8 rpm. The temperature of extruder barrel was set at 110°C across the five zones with screw rotation rate of 100 rpm.

Examples of these process factors are shown below. Table 2 which demonstrate the generation of self-dispersing solid lipid nanoparticle system that can produce solid lipid nanoparticles (SLNs) upon reconstitutions. Tailorable particle size from 131 – 520 nm with PDI from 0.227 (narrow distribution) to 0.527 (acceptable distribution) for these self-dispersing SLN systems. It is also demonstrated via the change of centrifugation process for SLN, the particle sizes are not significantly affected.

SLNs with and without loading active pharmaceutical ingredients were also prepared using COMMAND platform (Table 3). Oleic acid (OA) and capric triacylglycerol (CT) were also used as the liquid lipids to blend with solid lipid glycerol monostearate (GMS) to form the final solid lipid

nanoparticles. In the case of loading pharmaceutical ingredients (nile red and felodipine), the change on particle size of final reconstituted SLNs was not significant. With the addition of liquid lipids with solid lipids, the particle size was reduced SLN1 and SLN2 vs SLN 3 and SLN4.

The moisture content in the self-dispersing solid lipid nanoparticle (manufactured by COMMAND) were tested using Karl Fischer Titration (870 KF Tirino plus, Metrohm AG). The average moisture content was measured to be $1 \pm 0.8\%$ w/w for all self-dispersing solid lipid nanoparticle systems..

Table 3 Assessment of particles size and polydispersity index (PDI) of solid lipid nanoparticle (SLN, GMS) formulations; measured via photo correlation spectroscopy (Zetasizer NanoS, Malvern Instrument, UK)

Formulation	RCF (G)	Time (mins)	Average Size by intensity (nm)	PDI
SLN1	5000	15	519.7	0.327
SLN2 loaded Nile red (1%)	5000	15	279.8	0.524
	10000	15	255.7	0.431
SLN3	5000	15	176.7	0.327
SLN3 loaded Nile red (1%)	5000	15	168.9	0.362
SLN4 loaded Nile red (1%)	5000	15	150.6	0.374
SLN4 loaded felodipine (12%)	5000	15	131.5	0.227

Example 7, effects of screw configuration and formulation on the qualities of reconstituted SLNs

The use of design of experiment (DOE) techniques can accelerate the selection of formulation combinations and process parameters with reduced number of experiments. This experimental design was adopted to study the effects of critical process factors on the characteristics like particle size and span.

A range of SLN formulations containing drug felodipine (FD), ketoconazole (KZ) and naproxen (NPX) processed with different screw configurations were selected as examples to scoping the effects. A total of 24 trials were conducted based on two screw configurations (selected number of experiments are shown in Table 4). The responses are Zeta-average, PDI, entrapment efficiency (EE) and total loading capacity (LC).

The formulation of SLN after reconstitution is highly relevant to the formulation and process in COMMAND platform. Due to the superiority of using liquid lipid with solid lipid to reduce the particle size of final SLNs GMS with and oleic acid (OA) and caprylic/capric triacylglycerol (CT) were used as combinations for the study. A total of 48 experimental trials with RSM method design were conducted (selected examples are shown in Table 5). Experimental factors that were modified included type of lipids, percentages of lipids, screw configuration and drug loading. Average particle size (by intensity)

and PDI were assessed for these variables. Felodipine was used as model drug. For example, two sets of screw design (SC1 and 2) were selected to produce SLNs using COMMAND. For example, in the current case, SC1 was assembled using 2x60° and 7x90° kneading elements whereas SC2 was assembled at screw configuration of 5x60° and 5x90° kneading elements. The rest elements on screws were assembled using forward convey elements (total elements=20).

Liquid lipid type stated a significant effect on Z-average ($P < 0.0331$) of blank and drug-loaded formulations. OA exhibited Z-average with a lower value than that of CT in free and FD-loaded structured lipid matrix formulations (Figure 12 a). Liquid lipid type also revealed a significant effect ($P < 0.0325$) on the Z-average of formulations possessing different screw configurations. In both SC1 and SC2, the use of OA has resulted a significant lower particle size in comparison to the use of CT (Figure 12 b).

Table 4 Examples of SLN formulations manufactured using COMMAND platform. The effects of screw configurations and formulation for manufactured SLN formulations (reconstituted in PBS pH6.8) were presented using particle size and PDI as the responses.

Run	Liquid lipid % w/w	Liquid lipid type	Drug loaded	Screw configuration	Average particle size by intensity (nm)	PDI
1	20	OA	DF	SC1	376.4 ± 1.0	0.362 ± 0.04
2	30	CT	DF	SC1	507.5 ± 10.7	0.396 ± 0.01
3	15	OA	DL	SC1	413.2 ± 3.0	0.425 ± 0.03
4	20	CT	DF	SC1	445.9 ± 2.3	0.387 ± 0.08
5	20	OA	DL	SC2	263.7 ± 17.2	0.336 ± 0.08
6	15	OA	DF	SC2	296.4 ± 4.6	0.315 ± 0.02
7	20	OA	DF	SC2	270.1 ± 3.5	0.351 ± 0.04
8	30	OA	DL	SC2	257.3 ± 8.9	0.376 ± 0.07
9	20	OA	DL	SC1	385.2 ± 14.5	0.423 ± 0.04
10	20	CT	DL	SC2	332.4 ± 16.2	0.357 ± 0.02
11	15	OA	DL	SC2	283.2 ± 4.7	0.358 ± 0.01
12	15	CT	DF	SC1	413.7 ± 6.5	0.395 ± 0.03
13	30	OA	DF	SC2	333.2 ± 1.5	0.352 ± 0.05
14	30	OA	DF	SC1	254.9 ± 7.2	0.343 ± 0.01

Concerning screw configuration, Z-average was observed to be significantly decreased ($P < 0.0090$) in free and FD-loaded F2 formulation when compared to that of the F1 formulation (Figure 12 a).

Loading of felodipine into structured lipid matrix formulations exhibited a significant effect ($P < 0.0414$) on Z-average.

The Z-average value reduced in SC2 and increased in SC1 formulations after loading of FD into the formulations (Figure 13 a). Regarding liquid concentration, the effect on Z-average was insignificant where the P value was more than 0.05. PDI was found to be decreased in blank SC1 and increased significantly in FD-loaded SC2 formulations ($P < 0.0219$) (Figure 15 B).

Table 5 are the summarised results for entrapment efficiency and final drug loading capacity of three drugs within the SLNs manufactured via COMMAND platform. The highest EE% of 86% for KZ with final drug loading of 26% which is high than most of the results reported in literature.

Table 5 EE% and LC% of FD, KZ and NPx-loaded structured lipid matrix formulations.

Screw configurations	Liquid lipid % w/w	Lipid type	Loaded drug	EE%	LC%
SC1	15	CT	FD	77.10 $\pm$ 0.173	9.23 $\pm$ 0.021
SC1	30	CT	FD	74.03 $\pm$ 0.003	8.86 $\pm$ 0.015
SC1	15	OA	FD	75.51 $\pm$ 0.151	9.04 $\pm$ 0.018
SC1	30	OA	FD	72.72 $\pm$ 0.054	8.71 $\pm$ 0.007
SC2	15	CT	FD	80.84 $\pm$ 0.173	9.70 $\pm$ 0.021
SC2	30	CT	FD	74.30 $\pm$ 0.099	8.92 $\pm$ 0.012
SC2	15	OA	FD	77.17 $\pm$ 0.053	9.26 $\pm$ 0.006
SC2	30	OA	FD	72.91 $\pm$ 0.152	8.75 $\pm$ 0.018
SC1	15	CT	KZ	86.69 $\pm$ 0.027	26.87 $\pm$ 0.008
SC2	15	CT	KZ	86.16 $\pm$ 0.026	26.71 $\pm$ 0.008
SC1	15	CT	NPX	81.88 $\pm$ 0.022	6.55 $\pm$ 0.002
SC2	15	CT	NPX	82.76 $\pm$ 0.008	6.62 $\pm$ 0.001

FD, KZ and NPX are denoted as felodipine, ketoconazole and naproxen. Data are indicated as mean $\pm$ standard deviation (n=3).

Example 9, Drug release study for SNs under sink condition

To further investigate that drugs are encapsulated within the solid lipid core rather than surface of the SLNs, in vitro drug release was conducted for selected formulations. In vitro drug release study of SLNs was investigated via traditional bag dialysis method where SLNs are retained in the dialysis bag and drug molecules are allowed to release into the dissolution media. The dialysis tube (molecular weight cut off: 12,000-14,000 Da, VWR, UK) was immersed in the dissolution medium over a period of 12 h prior to use. The dialysis tube was then filled with a one mL aliquot of prepared extrudate dispersion and the ends of the tube were tightly sealed by clamps to retard any possible leakage. The dialysis tubes were then kept in a glass bottle and 50 ml of phosphate buffer (pH adjusted to 6.8) was added as release medium. The bottles were placed into a horizontal rotary shaker incubated at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ and shaking at 40 rpm (Incubator, Gallenkamp, UK). 2 mL aliquot of release medium was taken at pre-determined time points and replaced with an equal volume of fresh buffer to maintain a constant volume. Cary 50 UV- Visible spectrophotometer (Variant Ltd, Oxford, UK) was employed to measure samples tested at 361, 243 and 232 nm for FD, KZ and NPX, respectively. The regression equation obtained from the calibration curve of drug solutions was utilised to determine the content of drug in the tested samples. All experiments were performed in triplicate.

For drug FD and KZ, sustained release profiles were observed whilst for drug NPX, immediate release profile was obtained (Figure 16-18). It was also shown that the use of NLCs can alter the drug release profile of model drugs. Furthermore, the formation of SLNs are also capable of increasing the drug solubility for certain poorly water-soluble drugs (KZ and FD, Figure 17,18).

CLAIMS

1. A solid proliposomal nanoparticle comprising less than about 5% w/w water and less than about 1% w/w organic solvent.
2. The solid proliposomal nanoparticle of Claim 1, comprising less than about 3% w/w water.
3. The solid proliposomal nanoparticle of Claim 1 or 2, comprising less than about 1% w/w water.
4. The solid proliposomal nanoparticle of Claim 1, 2 or 3, comprising less than about 0.5% w/w water.
5. The solid proliposomal nanoparticle of any of the preceding claims, comprising less than about 0.2% w/w organic solvent.
6. The solid proliposomal nanoparticle of any of the preceding claims, comprising less than about 0.1% w/w organic solvent.
7. The solid proliposomal nanoparticle of any of the preceding claims, comprising less than about 0.05% w/w organic solvent.
8. The solid proliposomal nanoparticle of the preceding claims comprising less than about 0.2% w/w water and less than about 0.2% w/w organic solvent.
9. The solid proliposomal nanoparticle of Claim 8, comprising less than about 0.1% w/w water and less than about 0.1% w/w organic solvent.
10. The solid proliposomal nanoparticle of Claim 9, comprising less than about 0.05% w/w water and less than about 0.05% w/w organic solvent.
11. A solid proliposomal nanoparticle comprising at least one phospholipid bilayer and a hydrophilic matrix, wherein the hydrophilic matrix is present both inside and outside of the phospholipid bilayer.
12. The solid proliposomal nanoparticle of Claim 11, comprising less than about 2% w/w water and less than about 0.05% w/w organic solvent.
13. The solid proliposomal nanoparticle of any one of Claims 1-12, wherein the solid proliposomal nanoparticle comprises about 1 to about 49.9% w/w phospholipid and about 50 to about 98% w/w hydrophilic matrix.

14. The solid proliposomal nanoparticle of Claim 13, wherein the solid proliposomal nanoparticle further comprises about 0.1 to about 5% w/w sterol.
15. A mixture comprising a hydrophilic matrix and the solid proliposomal nanoparticle of any one of Claims 1-14.
16. The mixture of Claim 15, wherein the solid proliposomal nanoparticle is suspended in the hydrophilic matrix.
17. The mixture of Claim 16, wherein the solid proliposomal nanoparticle has a mean size range from about 1 nm to about 200 nm.
18. The mixture of Claim 16, wherein the solid proliposomal nanoparticle has a polydispersibility index of less than about 0.5.
19. A mixture comprising a hydrophilic matrix and a self-dispersing solid lipid nanoparticle comprising less than about 2% w/w water and less than about 0.05% w/w organic solvent.
20. The mixture of Claim 19, wherein the self-dispersing solid lipid nanoparticle is suspended in the hydrophilic matrix.
21. The mixture of Claim 19, wherein the self-dispersing solid lipid nanoparticle has a mean size range from about 1 nm to about 100 nm.
22. The mixture of Claim 19, wherein the self-dispersing solid lipid nanoparticle has a polydispersibility index of less than about 0.5.
23. The mixture of Claim 19, wherein the self-dispersing solid lipid nanoparticle comprises about 1 to about 49% w/w lipid nanoparticle and about 50 to about 98% w/w hydrophilic matrix.
24. The mixture of Claim 23, wherein the self-dispersing solid lipid nanoparticle further comprises about 1 to about 10% w/w surfactant.
25. A water and solvent free process for making a self-dispersing solid lipid nanoparticle, the process comprising:
 - a) combining:
 - i) solid lipid;
 - ii) surfactant;
 - iii) solid hydrophilic matrix; and
 - iv) optionally an active pharmaceutical ingredient;

b) heating the combination of a) to between about 40 C and about 200 C to form a continuous liquid phase comprising the self-dispersing solid lipid nanoparticle and hydrophilic matrix; and
c) cooling the liquid phase of b) to form a solid comprising the self-dispersing solid lipid nanoparticle suspended within the hydrophilic matrix.

26. The process of Claim 25, wherein the solid lipid is fatty acid.

27. The process of Claim 25, wherein the solid lipid is mono-, di-, or tri-glyceride.

28. The process of Claim 25, wherein the solid lipid is a natural wax.

29. The process of Claim 25, wherein solid lipid is a long chain fatty acid selected from stearic acid or behenic acid.

30. The process of Claim 25, wherein solid lipid is a monoglyceride selected from glyceryl monostearate or glyceryl behenate.

31. The process of Claim 27, wherein the triglyceride is glyceryl tristearate or glyceryl tripalmitate.

32. The process of Claim 28, wherein the natural wax is beeswax or carnauba wax.

33. The process of Claim 25, wherein a) further comprises a liquid lipid.

34. The process of Claim 33, wherein the liquid lipid is a short chain unsaturated or saturated fatty acid.

35. The process of Claim 25, wherein the surfactant is a phospholipid.

36. The process of Claim 35, wherein the phospholipid is phosphatidylglycerol, poloxamer or tween.

37. The process of Claim 35 or 36, wherein the phospholipid is poloxamer 188 or tween 80.

38. The process of Claim 25, wherein in step a) about 1% w/w to about 49% w/w solid lipid is combined with about 50% w/w to about 98% w/w hydrophilic matrix.

39. The process of Claim 38, wherein in step a) about 1% w/w to about 10% w/w surfactant is used.

40. A water and solvent free process for making a solid proliposomal nanoparticle, the process comprising:

a) combining:

- i) solid phospholipid;
 - ii) sterol;
 - iii) solid hydrophilic matrix; and
 - iv) optionally an active pharmaceutical ingredient;
- b) heating the combination of a) to between about 40 C and about 200 C to form a continuous liquid phase comprising the solid proliposomal nanoparticle and hydrophilic matrix; and
- c) cooling the liquid phase of b) to form a solid comprising the solid proliposomal nanoparticle suspended within the hydrophilic matrix.

41. The process of Claim 40, wherein the solid phospholipid is a saturated or non-saturated phosphatidylcholine.

42. The process of Claim 40, wherein the solid phospholipid is a saturated or non-saturated phosphatidic acid.

43. The process of Claim 40, wherein the solid phospholipid is saturated or non-saturated phosphatidylethanolamine.

44. The process of Claim 40, wherein the solid phospholipid is saturated or non-saturated phosphatidylserine

45. The process of Claim 25 or 40, wherein the hydrophilic matrix comprises a sugar or sugar alcohol or amino sugar or other sugar derivatives.

46. The process of Claim 40, wherein the hydrophilic matrix is fructose or xylitol or meglumine.

47. The process of Claim 40, wherein in step a) about 1 % w/w to about 49.9% w/w solid phospholipid is combined with about 50 % w/w to about 98% w/w hydrophilic matrix.

48. The process of Claim 47, wherein in step a) about about 0.1 % w/w to about 5% w/w sterol

49. The process of Claim 49, wherein the sterol is selected from the group comprising cholesterol, ergosterol, stigmasterol, and androsterone.

50. The process of Claim 25 or 40, wherein the combination in step a) is passed through a high speed mixer.

51. The process of Claim 25 or 40, wherein the combination in step a) is passed through a hot melt extruder.

52. The process of Claim 51, wherein the hot melt extruder comprises two rotating screws.
53. The process of Claim 25 or 40, wherein the process produces a self-dispersing solid lipid nanoparticle or a solid proliposomal nanoparticle having a mean particle size of from about 1 nm to about 200 nm.
54. The process of Claim 25 or 40, wherein the process produces a self-dispersing solid lipid nanoparticle or a solid proliposomal nanoparticle having a polydispersibility index of less than about 0.5.
55. The process of Claim 25 or 40, wherein the process is a continuous process.
56. A self-dispersing solid lipid nanoparticle or solid proliposomal nanoparticle made by the process of any one of claims 25-55.



Figure 1 The schematic diagram of conventional solvent/water-based nanoparticle manufacturing platform comparing to the COMMAND platform described in this invention

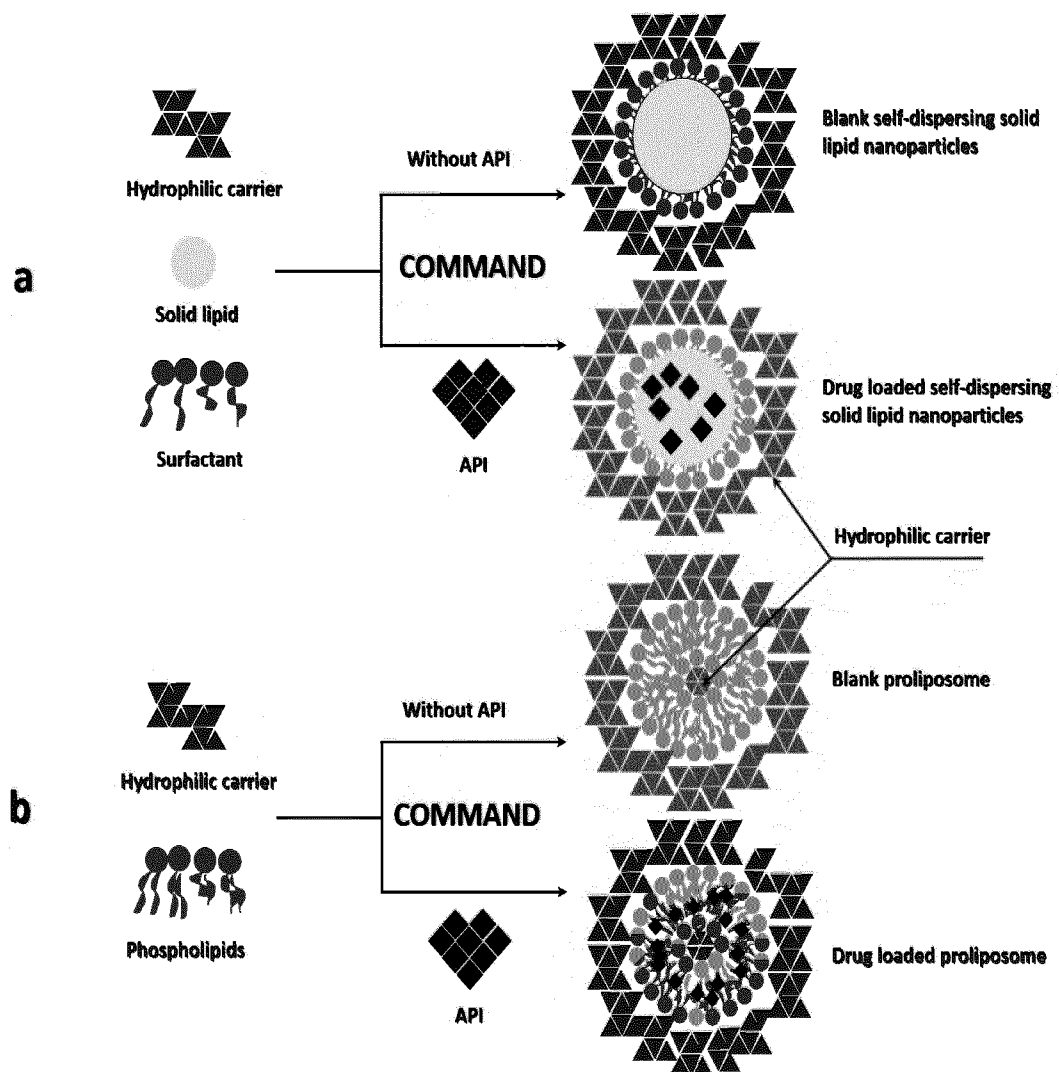


Figure 2

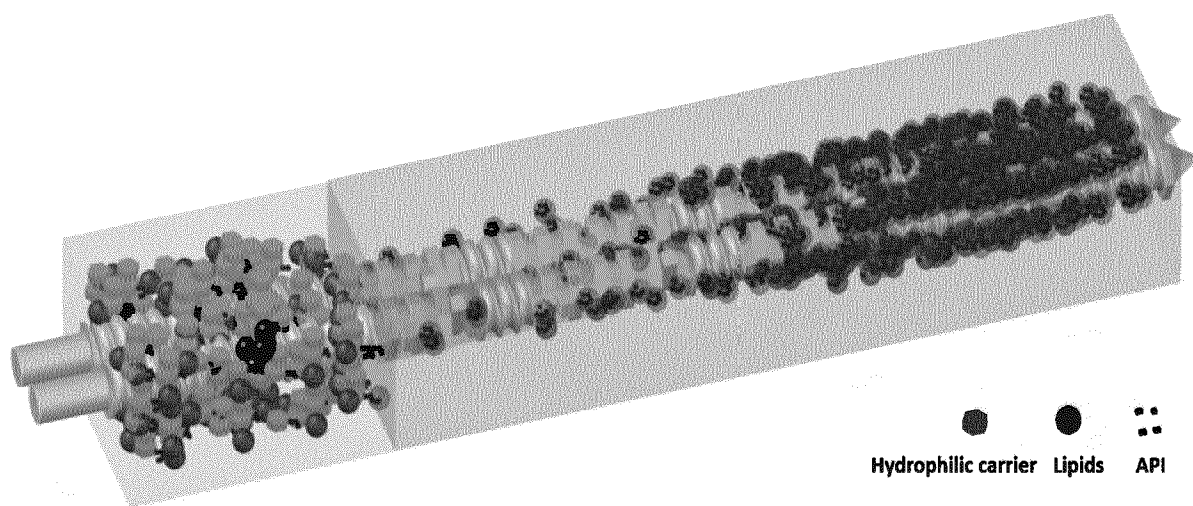


Figure 3

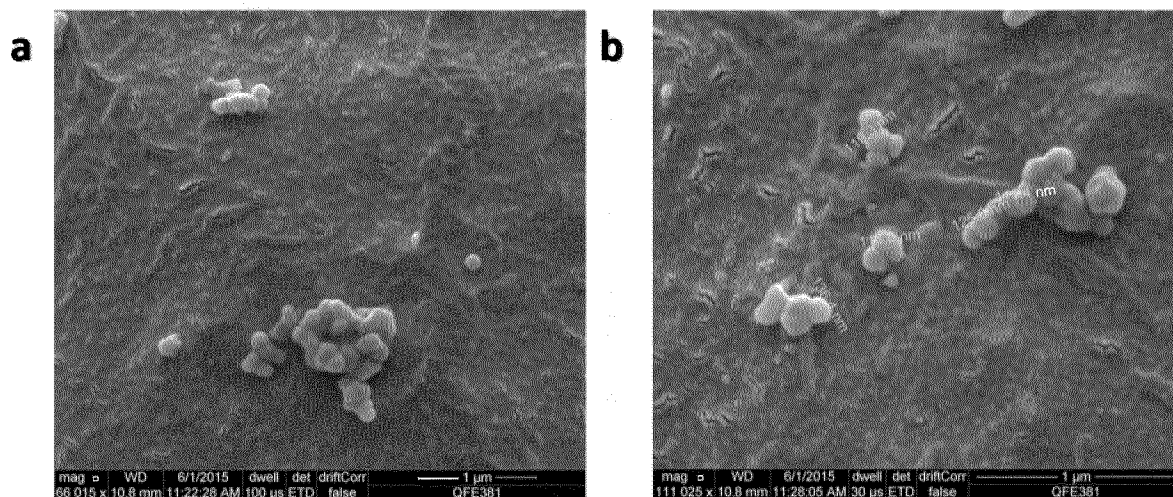


Figure 4

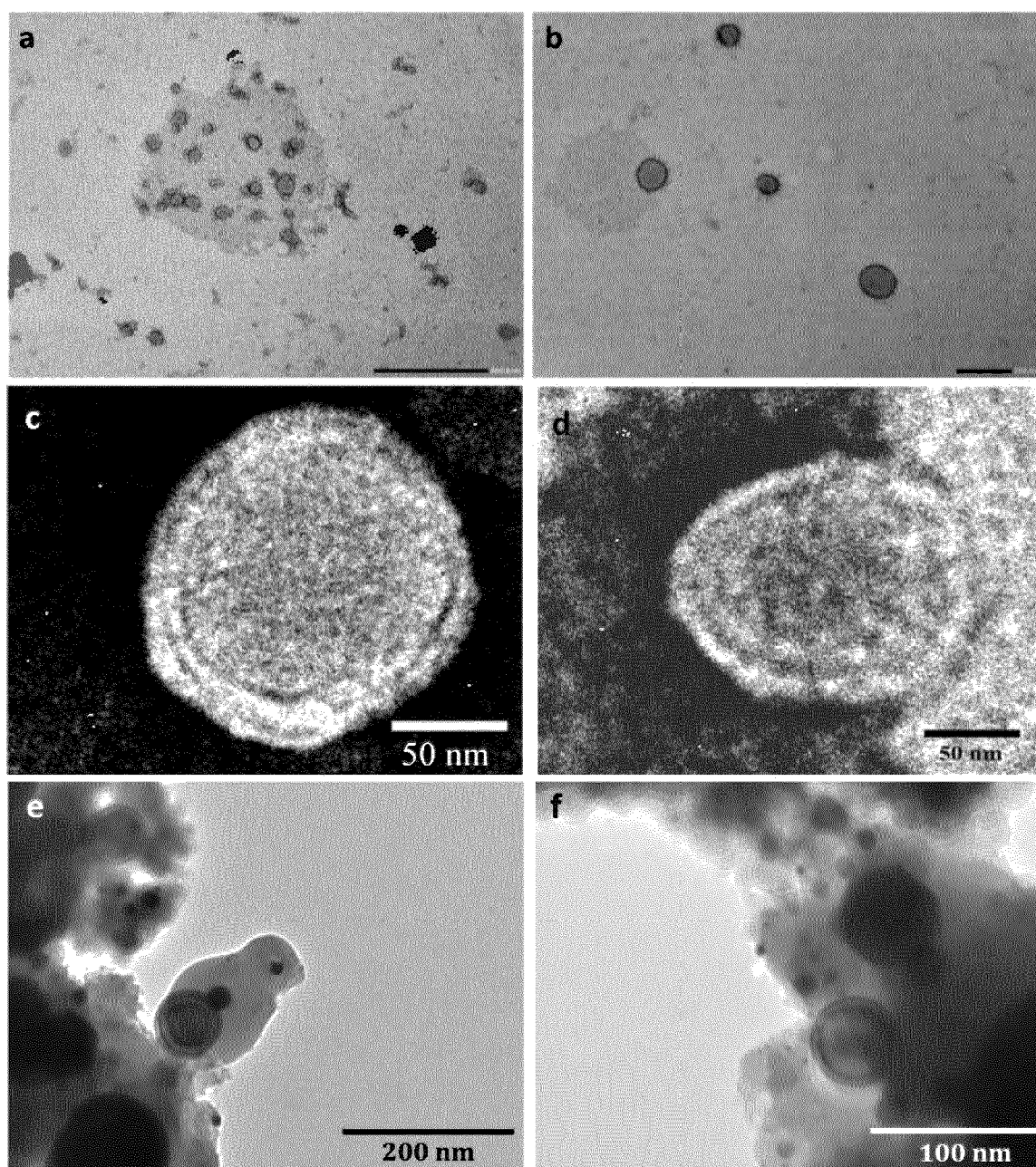


Figure 5

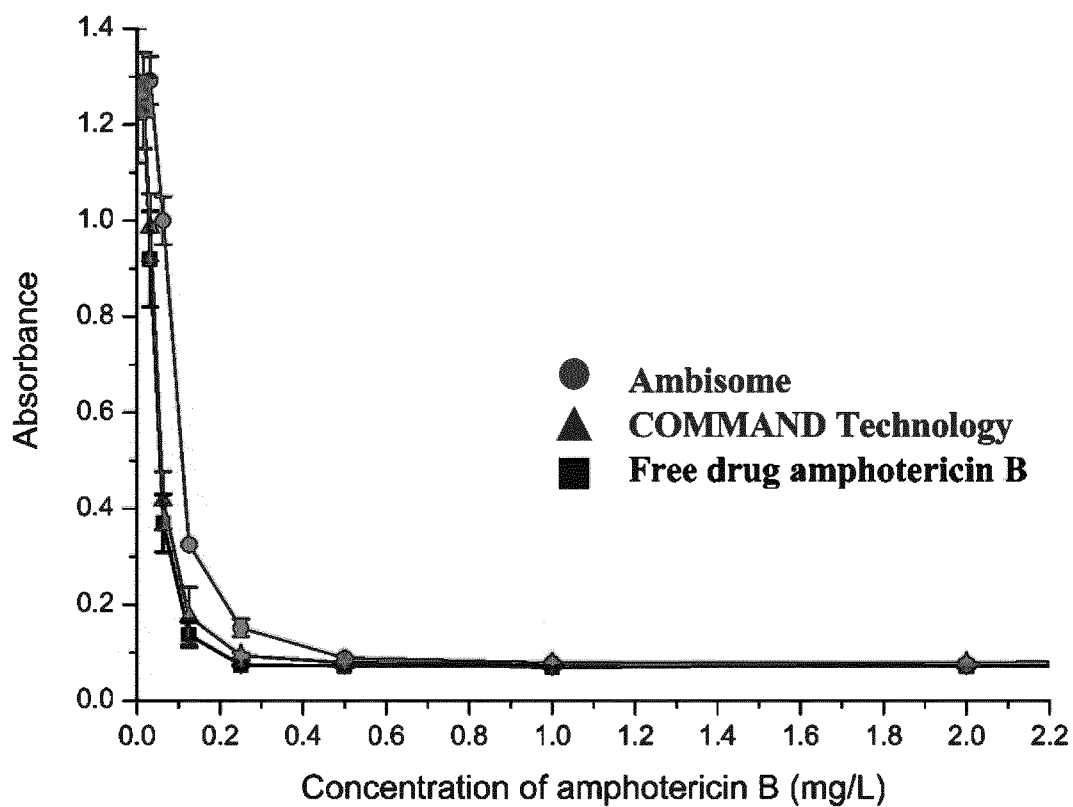


Figure 6

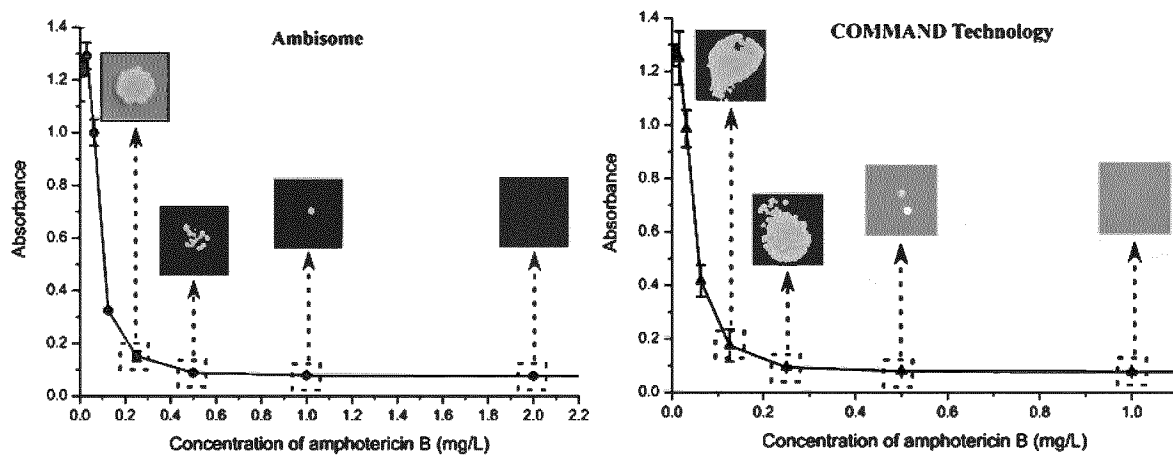


Figure 7

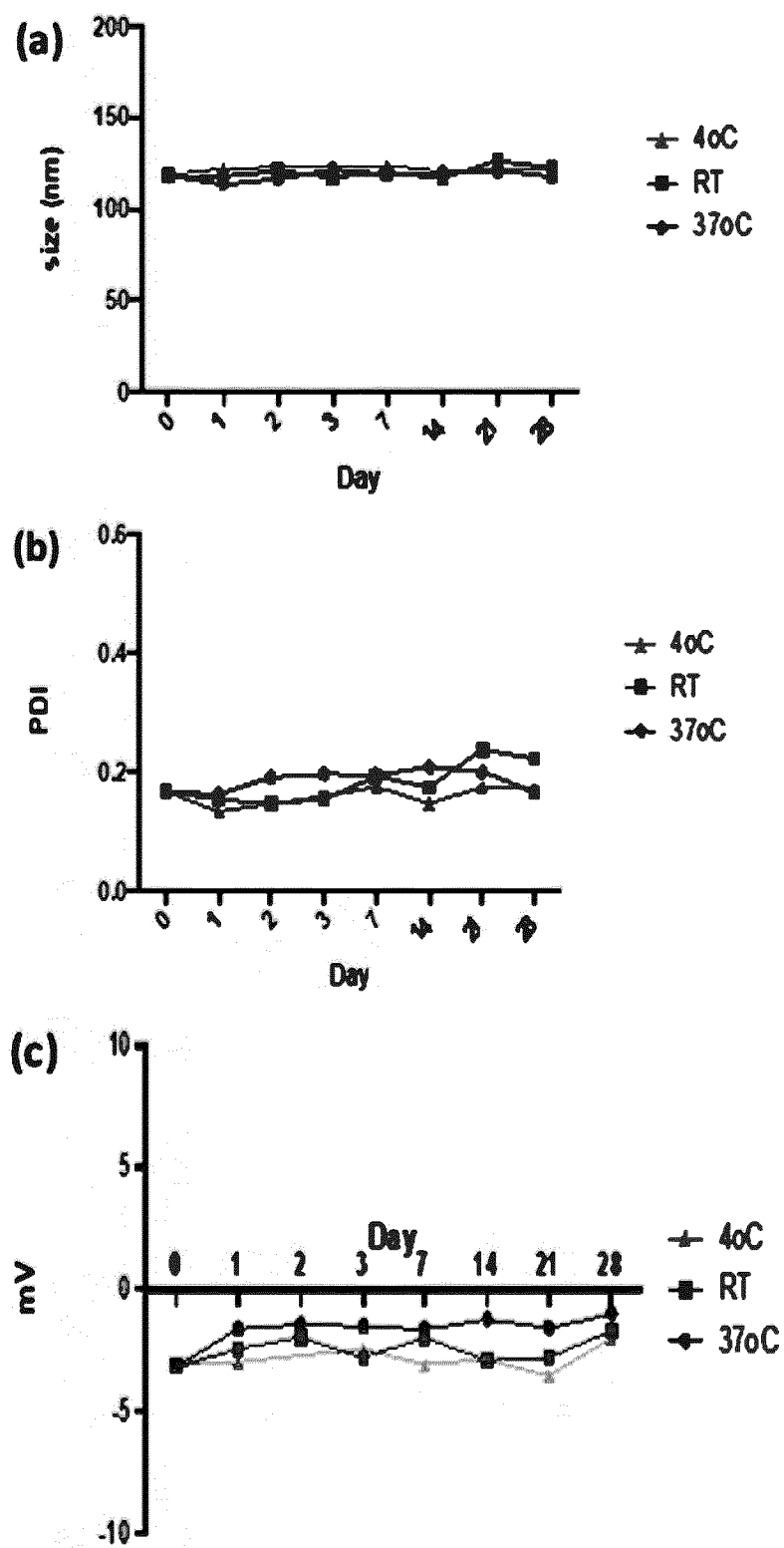


Figure 8

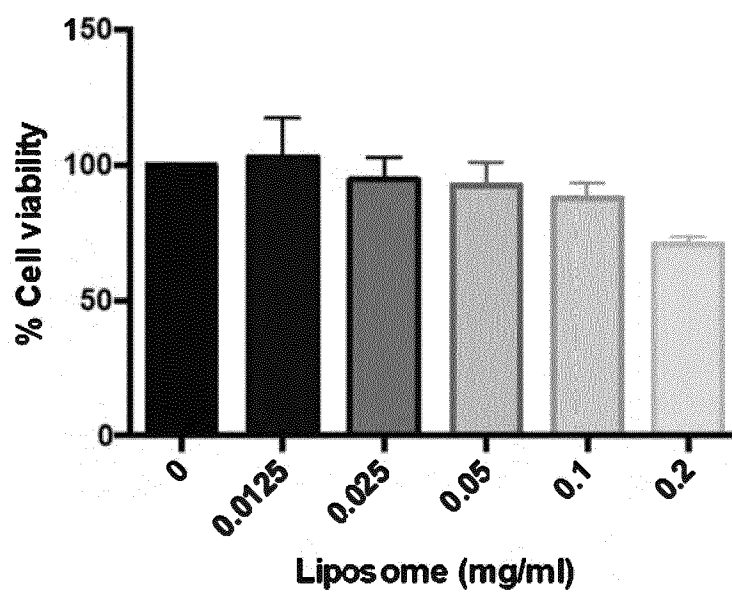


Figure 9

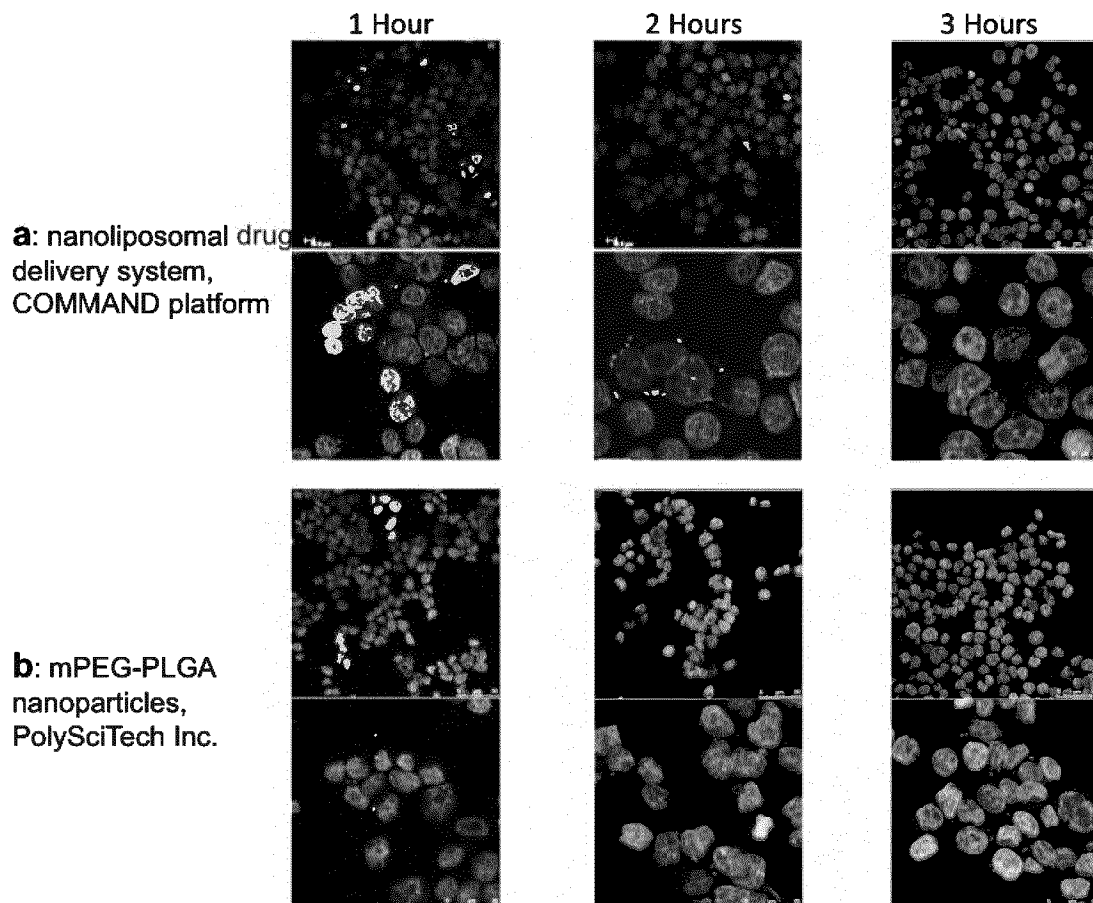


Figure 10

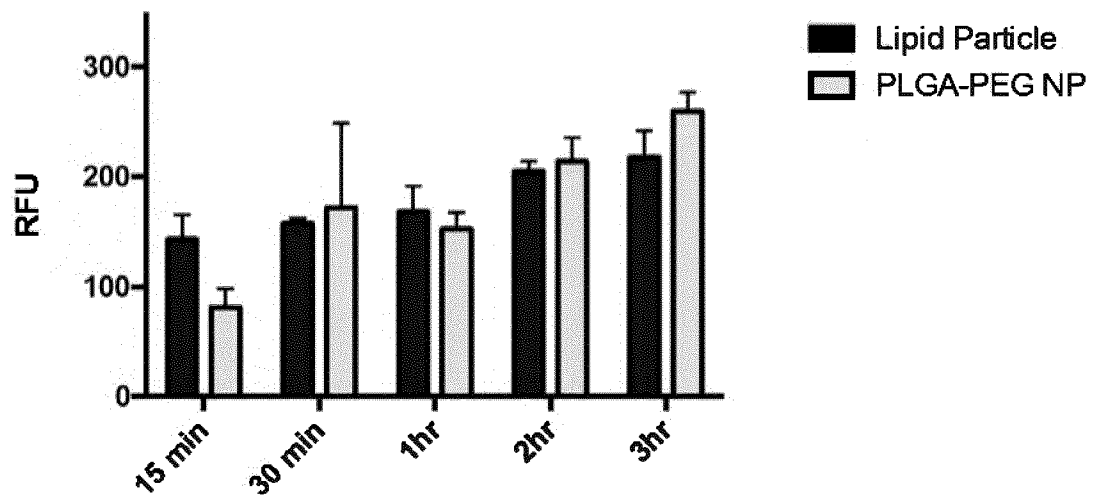


Figure 11

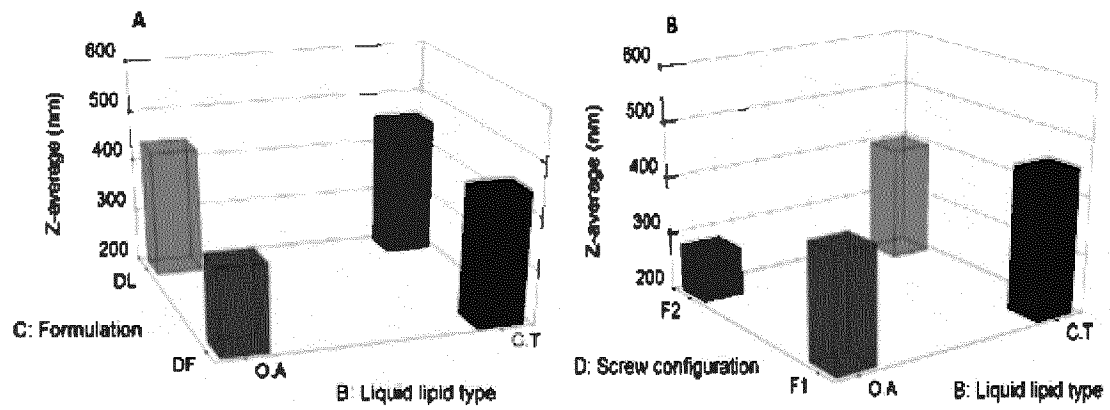


Figure 12

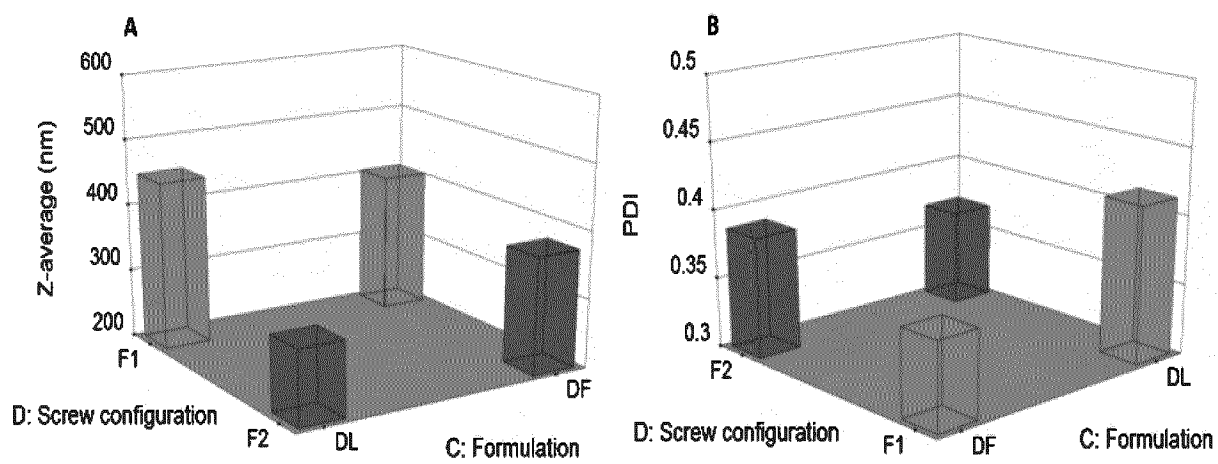


Figure 13

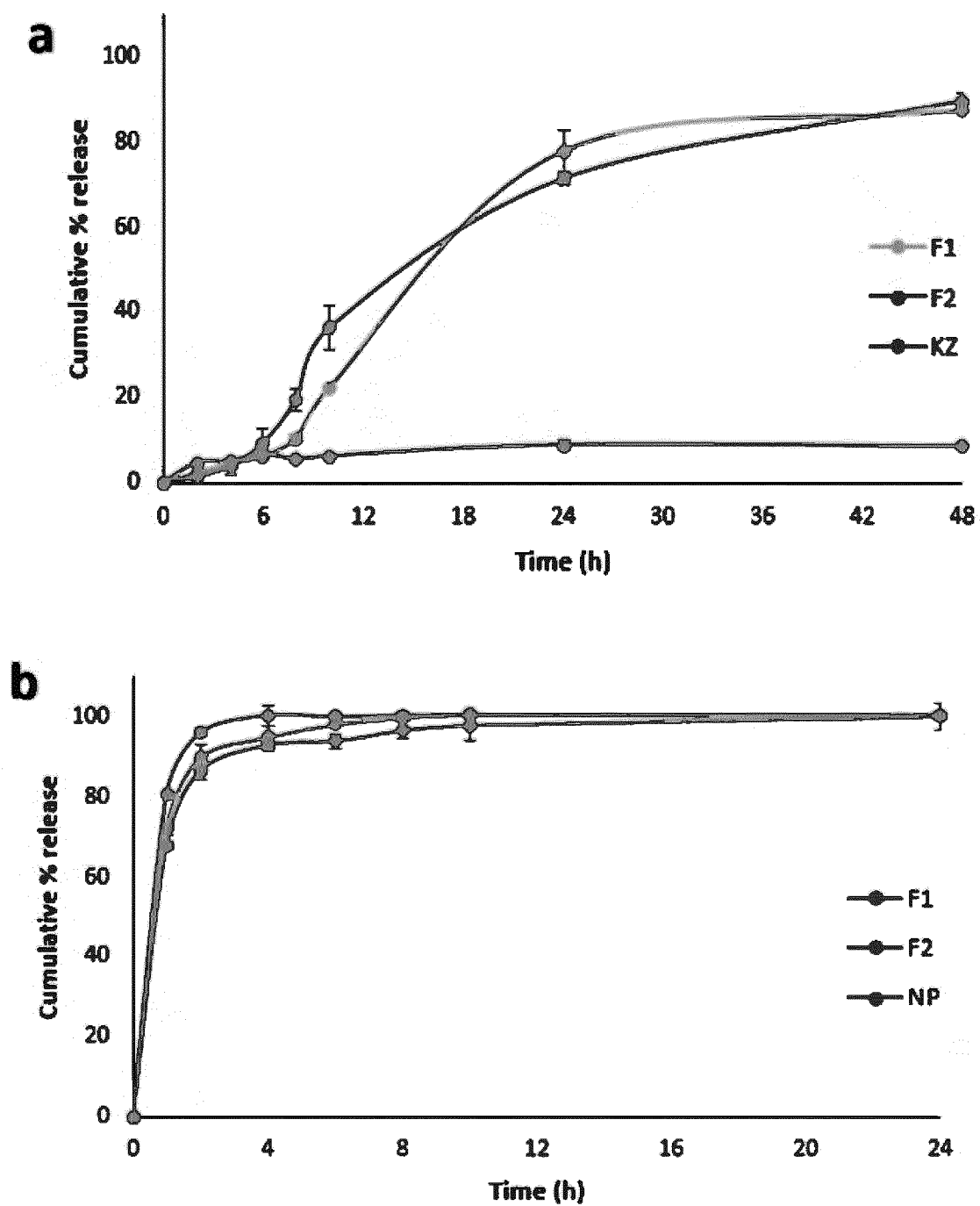


Figure 14

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2020/050682

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K9/51
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, CHEM ABS Data, EMBASE, WPI Data, BIOSIS, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/125132 A2 (MPEX PHARMACEUTICALS INC [US]; SURBER MARK W [US] ET AL.) 23 November 2006 (2006-11-23) paragraph [00242]	1-13,56
X	WO 2004/069180 A2 (SMITHKLINE BEECHAM CORP [US]; PATEL KAMLESH H [US] ET AL.) 19 August 2004 (2004-08-19) example 1	1-13,56
A	WO 00/57853 A2 (SCHERING AG [DE]; HUELSMANN STEFAN [DE]; BACKENSFELD THOMAS [DE]) 5 October 2000 (2000-10-05) examples 1-6	1-56



Further documents are listed in the continuation of Box C.



See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

25 March 2020

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2020/050682

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