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(54) **Title**: SOLID LIPID NANOPARTICLES ENTRAPPING HYDROPHILIC/ AMPHIPHILIC DRUG AND A PROCESS FOR PREPARING THE SAME

(57) **Abstract**: Solid lipid nanoparticles and the process thereof are disclosed. The solid lipid nanoparticles comprise: at least one lipid selected from the group consisting of glycerides and fatty acids, at least one hydrophilic drug or amphiphilic drug and at least one emulsifier.

SOLID LIPID NANOPARTICLES ENTRAPPING HYDROPHILIC/ AMPHIPHILIC DRUG AND A PROCESS FOR PREPARING THE SAME

FIELD OF THE DISCLOSURE

The present disclosure relates to entrapping hydrophilic or an amphiphilic drug within nanoparticles and a process for preparing the same.

Particularly, the present disclosure relates to solid lipid nanoparticles and a process for preparing the same.

BACKGROUND

Solid lipid nanoparticles (SLN) are generally spherical in shape with an average diameter between 10 to 1000 nanometers. Solid lipid nanoparticles possess a solid lipid core matrix that can solubilize lipophilic molecules. The lipid core is stabilized by surfactants (emulsifiers). The lipid typically includes triglycerides (e.g. tristearin), diglycerides (e.g. glycerol behenate), monoglycerides (e.g. glycerol monostearate), fatty acids (e.g. stearic acid), steroids (e.g. cholesterol) and waxes (e.g. cetyl palmitate). Various classes of emulsifiers (with respect to charge and molecular weight) have been used to stabilize the lipid dispersion. It has been found that the combination of emulsifiers might prevent particle agglomeration more efficiently (Mehnert et al, 2001 and Small, 1986).

Biological membrane lipids such as phospholipids, sphingomyelins, bile salts (sodium taurocholate) and sterols (cholesterol) are utilized as stabilizers. Biological lipids having minimum carrier cytotoxicity and the solid state of the lipid permit better controlled drug release due to increased mass transfer resistance (Manjunath et al., 2005).

Development of solid lipid nanoparticles is one of the emerging fields of nanotechnology with several potential applications in drug delivery, clinical medicine and research, as well as in other discipline. Due to their unique size-dependent properties, lipid nanoparticles offer the possibility to develop new therapeutics. The ability to incorporate drugs into nanocarriers offers a new prototype in drug delivery that could hold great promise for attaining the bioavailability enhancement along with controlled and site specific drug delivery.

Solid lipid nanoparticles have recently materialized as a novel approach to oral and parenteral drug delivery systems. SLNs combine the advantages of lipid emulsion and polymeric nanoparticle systems while overcoming the temporal and *in- vivo* stability

issues that troubles the conventional as well as polymeric nanoparticle drug delivery approaches (Mehnert et al, 2001).

Advantages of SLNs are the use of physiological lipids, the avoidance of organic solvents, a potential wide application spectrum (oral, dermal, intravenous) and the high pressure homogenization as an established production method. Additionally, improved bioavailability, protection of sensitive drug molecules from the outer environment (water and light) and even controlled release characteristics are claimed by incorporation of poorly water soluble drugs in the solid lipid matrix.

It has been proposed that SLNs combine numerous advantages over the other colloidal carriers i.e. feasibility of incorporating lipophilic and hydrophilic drugs, no biotoxicity of the carrier, avoidance of organic solvents, possibility of controlled drug release and drug targeting, increased drug stability and no problems with respect to large scale production.

The term "hydrophilic drug" is meant to refer to a drug that is readily soluble in water. Hydrophilic drugs generally have an aqueous solubility greater than about 10 g/liter.

Hydrophobic/lipophilic drug means a drug which lacks affinity for water or which do not readily dissolve in water.

Amphiphilic drugs are the drugs which tend to include hydrophobic and/or lipophilic regions.

The hydrophilic drugs or amphiphilic drugs have limited permeability across biological barriers such as blood brain barrier (BBB), as biological barriers are highly lipo-philic in nature. Further, as a result of limited permeability across biological barriers these drugs need to be administered in high dose on a daily basis which may lead to several side effects of varying severity. For the delivery of drug across biological barriers including the blood brain barrier and gut mucosal barrier, the drug candidate must be in a lipid soluble form. Contrary to this hydrophilic drugs or amphiphilic drugs face permeability related problems across biological barriers. Drug delivery systems which can in some way tailor the entry of the hydrophilic or amphiphilic drug candidates across the biological membranes like brain can form a more effective therapy especially for conditions where prolonged therapy is desired.

Some representative patent documents which disclose lipid nanoparticles are discussed herein below.

US Patent No. 5250236 discloses a process for preparing solid lipid microspheres in which a) a molten lipid, which may contain a drug, is contacted with a mixture consisting of water, a surfactant and possibly a co-surfactant heated to a temperature at least equal to the melting temperature of the lipid; b) the obtained micro-emulsion is dispersed in water of 2°C to 10 °C; c) the obtained lipid microsphere dispersion is washed with water by diafiltration and lyophilized. Later two steps make the process lengthy and complex. Furthermore it may lead to formation of aggregates which may not redisperse to give back the same particle size.

WO 2006/109317 discloses a process for the preparation of Poly DL-Lactide-coglycolide nanoparticles encapsulated with anti-tubercular drugs. The process includes (i) preparation of an aqueous solution of stable water soluble drugs in DW/NS/PBS, (ii) preparation of unstable drugs in DW/NS/PBS, (iii) preparation of a polymer and hydrophobic drug solution in an organic solvent, (iv) mixing separately the solutions of steps (i) and (ii) with that of step (iii) and sonicating under cold conditions, (v) adding the above emulsion to aqueous poly vinyl alcohol and resonicating under cold conditions, (vi) stirring the emulsion and centrifuging the same and (vii) washing the said particles, reconstituting the same and lyophilizing. The process disclosed in WO 2006/109317 uses an organic solvent (dichloromethane) for the preparation of nanoparticles. The presence of organic solvent may cause toxic effect in case of prolonged treatment, as is required for the anti-tubercular therapy.

US Patent No. 7611733 discloses solid lipid nanoparticles of antitumor platinum complexes and process for preparing the same. The process includes the following steps: a) first preparing a micro-emulsion by mixing a molten lipid, a surfactant, and optionally a co-surfactant and the platinum compound aqueous solution; b) preparing a solution by mixing a surfactant and optionally a co-surfactant in water, heating to complete solution, preferably at the same melting temperature as of the lipid used in step a) and adding a co-surfactant; c) dispersing the micro-emulsion obtained in step a) into the solution obtained in step b) obtaining a multiple micro-emulsion; d) dispersing the micro-emulsion obtained in step c) in aqueous medium at a temperature ranging from 0.5 °C to 4.0 °C obtaining a dispersion of solid lipid microspheres; e) washing with aqueous medium through ultra filtration to obtain lipid microspheres and lyophilizing, optionally in the presence of a bulking agent and of a cryoprotecting agent. The method disclosed in US

Patent No. 7611733 involves preparation of multiple micro-emulsions which renders the method complex.

The representative patent documents disclose complex methods of preparation of solid lipid nanoparticles. Further, the methods disclosed are not suitable for entrapment of hydrophilic or amphiphilic drugs and effective delivery of drug across biological barriers. In view of the above, there is envisaged in accordance with the present disclosure solid lipid nanoparticles having enhanced hydrophilic or amphiphilic drug entrapment efficiency and improved delivery through biological barriers.

OBJECTS:

Some of the objects of the present disclosure are as follows:

It is an object of the present disclosure to provide solid lipid nanoparitcles.

It is another object of the present disclosure to provide a simple process for preparing solid lipid nanoparticles.

It is yet another object of the present disclosure to provide a process which is capable of producing concentrated solid lipid nanoparticles without using lyophilization technique.

It is yet another object of the present disclosure to provide a simple process for preparing solid lipid nanoparticles using at least one lipid.

It is still another object of the present disclosure to provide solid lipid nanoparticles having improved drug entrapment efficiency.

It is still another object of the present disclosure to provide solid lipid nanoparticles with improved entrapment efficiency of hydrophilic / amphiphilic drugs.

It is a further object of the present disclosure to provide solid lipid nanoparticles which can effectively deliver the drug across biological barriers such as cerebral barrier, gut mucosal barrier and the like.

It is still another object of the present disclosure to provide solid lipid nanoparticles which are able to improve the pharmacokinetic behavior (viz C_{max}, AUC, clearance and $t_{1/2}$) of the drug entrapped therein.

It is still further object of the present disclosure to provide solid lipid nanoparticles which reduce the dosing frequency and dose dependent side effects.

SUMMARY

These and other objects of the present disclosure are to a great extent dealt in the disclosure.

In one aspect of the present disclosure there is provided a process for preparing solid lipid nanoparticles, said process comprising the following steps:

i) melting at least one lipid selected from the group consisting of glycerides and fatty acids;

ii) preparing an aqueous emulsifier mix by admixing at least one emulsifier and water followed by heating at a temperature atleast equal to the melting point of the selected lipid;

iii) adding at least one drug to the aqueous emulsifier mix to obtain a solution;

iv) adding the solution containing at least one drug to the lipid melt to obtain a microemulsion; and

v) preparing solid lipid nanoparticles having a particle size in the range of 10 nm to 1000 nm, preferably 20 nm to 150 nm from the microemulsion.

Typically, the method step of preparing solid lipid nanoparticles comprises dispersing the microemulsion in water maintained at a temperature ranging between 0 °C and 5 °C under continuous stirring/homogenizing to obtain solid lipid nanoparticles, wherein the ratio of microemulsion and water ranges between 1:1 and 1:4.9.

Alternatively, the method step of preparing the solid lipid nanoparticles comprises purging nitrogen through the microemulsion to obtain solid lipid nanoparticles.

Typically, the glyceride is at least one selected from the group consisting of mono-glycerides, di-glycerides and tri-glycerides.

In accordance with one of the embodiments of the present disclosure the glyceride is at least one selected from the group consisting of glyceryl behenate tricaprin, trilaurin, trimyristin, tripalmitin, tristearin, 1,2-dioctanoyl-*sn*-glycerol, 1,2-didecanoyl-*sn*-glycerol, 1,2-dilauroyl-*sn*-glycerol, 1,2-dimyristoyl-*sn*-glycerol, 1,2-dipalmitoyl-*sn*-glycerol, 1-palmitoyl-2-oleoyl-*sn*-glycerol, 1-stearoyl-2-linoleoyl-*sn*-glycerol, 1-stearoyl-2-arachidonoyl-*sn*-glycerol, 1-stearoyl-2-docosahexaenoyl-*sn*-glycerol, 1-oleoyl-2-acetyl-*sn*-glycerol, 1,2-di-O-phytanyl-*sn*-glycerol, 1,2-dipalmitoyl ethylene glycol, 1-2-dioleoyl ethylene glycol, glyceryl monostearate, behenoyl polyoxyl-8 glycerides, glyceryl palmitostearate, 1-O-hexadecyl-*sn*-glycerol, 1-O-hexadecyl-2-acetyl-*sn*-glycerol, 1-O-hexadecyl-2-O-methyl-*sn*-glycerol, 1,2-diacyl-3-O-(α-D-glucopyranosyl)-*sn*-glycerol, stearoyl macrogol-32 glycerides, stearoyl polyoxyl-32 glycerides, lauroyl macrogol-32 glycerides, lauroyl polyoxyl-32 glycerides, lauroyl macrogol-6 glycerides, lauroyl polyoxyl-6 glycerides, oleoyl macrogol-6 glycerides, oleoyl polyoxyl-6 glycerides, linoleoyl macrogol-6 glycerides, polyglyceryl-3 dioleate, glycerol monolinoleate, glyceryl monolinoleate, glycerol monooleates, diethylene glycol monoethyl ether, glyceryl dibehenate, glycerol distearate, glyceryl distearate, glyceryl dipalmitostearate and linoleoyl polyoxyl-6 glyceride.

Preferably, the glyceride is glyceryl behenate.

Typically, the fatty acid is selected from the group consisting of saturated C_4-C_{28} fatty acids and unsaturated C_4-C_{28} fatty acids.

Preferably, the fatty acid is stearic acid.

Typically, the proportion of the glyceride and the fatty acid is in the range of 1:0 to 1:20.

Alternatively, the proportion of the fatty acid and the glyceride is in the range of 1:0 to 1:20.

Typically, the emulsifier is at least one selected from the group consisting of anionic emulsifiers, cationic emulsifiers, non ionic emulsifiers or zwitterionic emulsifiers.

In accordance with one of the embodiment of the present disclosure the emulsifier is at least one selected from the group consisting of soy lecithin, egg lecithin, phosphatidylcholine; ethylene oxide copolymers, propylene oxide copolymers, poloxamers, sorbitan ethylene oxide/propylene oxide copolymers, polysorbate 20, polysorbate 60, polysorbate 80, sorbitan esters, span 20, span 40, span 60, span 80,

alkyllaryl polyether alcohol polymers, tyloxapol, bile salts, cholate, glycocholate, taurocholate, taurodeoxycholate, gemini surfactants and alcohols.

Typically, the drug is at least one selected from the group consisting of hydrophilic drugs and amphiphilic drugs.

Typically, the hydrophilic drug is atleast one selected from the group consisting of isoniazid, rifampicin, pyrazinamide, ethambutol, streptomycin, acyclovir, acetyl cysteine, acetylcholine chloride, alatrofloxacin, alendronate, amantadine hydrochloride, ambenomium, amifostine, amiloride hydrochloride, aminocaproic acid, amphiphilicin B, atenolol, atracurium besylate, atropine, azithromycin, aztreonam, bacitracin, becalermin, belladona, bepridil hydrochloride, bleomycin sulfate, calcitonin, calcitonin salmon, carboplatin, capecitabine, capreomycin sulfate, cefamandole nafate, cefazolin sodium, cefepime hydrochloride, cefixime, cefonicid sodium, cefoperazone, cefotetan disodium, cefotoxime, cefoxitin sodium, ceftizoxime, ceftriaxone, cefuroxime axetil, cephalexin, cephapirin sodium, chrionic gonadotropin, cidofovir, cisplatin, cladribine, clidinium bromide, clindamycin, ciprofloxacin, clondronate, colistimethate sodium, deforoxamine, denileukin diftitox, desmopressin, diatrizoate megluamine, dicyclomine, didanosine, dirithromycin, dopamine hydrochloride, dornase alpha doxacurium chloride, doxorubicin, editronate disodium, elanaprilat, enkephalin, enoxacin, enoxaprin sodium, ephedrine, epinephrine, erythromycin, esmol hydrochloride, famiciclovir, fludarabine, fluoxetine, ganciclovir, gentamycin, glucagon, glycopyrolate, heparin sodium, indinavir sulfate, insulin, lamivudine, leucovorin calcium, leuprolide acetate, levofloxacin, lincomycin, lobucavir, lomefloxacin, loracarbef, mannitol, methotrexate, methscopolamine, metformin hydrochloride, metroprolol, mezocillin sodium, mivacurium chloride, nedocromil sodium, neostigmine bromide, neostigmine methyl sulfate, neutontin, norfloxacin, octreotide acetate, ofloxacin, olpadronate, oxytocin, pamidronate disodium, pancuronium bromide, paroxetine, pefloxacin, pentamindine isethionate, pentostatin, pentoxifylline, periciclovir, pentagastrin, phentolamine mesylate, phenylalanine, physostigmine salicylate, piperacillin sodium, polymixin B sulfate, pralidoxine chloride, pramlintide, pregabalin, propofenone, propenthaline bromide, pyridostigmine bromide, residronate, ribavarin, rimantadine hydrochloride, salmetrol xinafoate, sincalide, solatol, somatostatin, sparfloxacin, spectinomycin, stavudine, streptozocin, suxamethonium chloride, tacrine hydrochloride, terbutaline sulfate, thiopbeta ticarcillin, tiludronate, timolol, trandolapril, trimetrexate gluconate, trospectinomycin, trovafloxacin, tubocurarine chloride, valaciclovir, valsartan, vasopressin, vecoronium bromide,

vinblastin, vincristine, vinorelbine, warfarin sodium, zalcitabine, zanamavir, zolandronate and zidovudine.

Typically, the amphiphilic drug is at least one selected from the group consisting of amphiphilicin B, bupivacaine, ropivacaine, prilocaine, mepivacaine, tetrocaine, etidocaine, morphine, fentanyl, alfentanil and sulfentanil.

Typically, the drug is a compound containing at least one hydroxyl group or at least one hydrophilic linkage.

Typically, the method step of dispersing the micro emulsion in water is carried out by continuous stirring/homogenizing at 4,000 to 12,000 rpm, preferably 5,000 to 7,000 rpm for a time period ranging between 20 minutes and 2 hours.

In another aspect of the present disclosure there is provided solid lipid nanoparticles prepared by the process of the present disclosure, said solid lipid nanoparticles comprising:

i) at least one lipid selected from the group consisting of glycerides and fatty acids;

ii) at least one drug selected from the group consisting of hydrophilic drugs and amphiphilic drugs; and

iii) at least one emulsifier.

Typically, the amount of drug is in the range of about 0.001% to about 99% with respect to the mass of the lipid.

Typically, the particle size of the solid lipid nanoparticles ranges between 10 to 1000 nm, preferably between 20-150 nm.

BRIEF DESCRIPTION OF ACCOMPANYING DRAWING:

The disclosure will now be described with reference to accompanying drawing.

Figure 1 illustrates comparative *In-vitro* drug release.

DETAIL DESCRIPTION OF THE DISCLOSURE

The present disclosure provides solid lipid nanoparticles (SLNs) containing drug selected from the group consisting of hydrophilic drugs and amphiphilic drugs.

The solid lipid nanoparticles with a particle size in the range of 10 to 1000 nm, preferably between 20-150 nm prepared in accordance with the present disclosure can bypass reticulo-endothelial system (RES) detection and limit metabolic elimination of the entrapped drug. The active ingredient (hydrophilic drug or amphiphilic drug) entrapped in solid lipid nanoparticles (having a particle size less than 200 nm) effectively cross the biological barriers such as cerebral barrier and gut mucosal barrier.

The enhanced targetability, permeability and bioavailability of hydrophilic drug or amphiphilic drug across the biological barriers including blood brain barrier and gut mucosal barrier are achieved as a result of solid lipid nanoparticles having a particle size below 200 nm.

The solid lipid nanoparticles prepared in accordance with the present disclosure in turn reduce the dose and the dosing frequency of hydrophilic drugs or amphiphilic drugs. Furthermore, the solid lipid nanoparticles prepared in accordance with the present disclosure provides sustained / controlled therapeutic effect with reduced side effects.

In one aspect of the present disclosure there is provided a process for preparing solid lipid nanoparticles.

In the first step, the selected lipid or lipid mixture is heated to melt. The lipid is selected from the group consisting glycerides and fatty acids.

The glyceride used to prepare the lipid melt in accordance with present disclosure is selected from the group consisting of mono-glycerides, di-glycerides and tri-glycerides.

In accordance with the present disclosure the glyceride used include but is not limited to glyceryl behenate, tricaprin, trilaurin, trimyristin, tripalmitin, tristearin, 1,2-dioctanoyl-*sn*-glycerol, 1,2-didecanoyl-*sn*-glycerol, 1,2-dilauroyl-*sn*-glycerol, 1,2-dimyristoyl-*sn*-glycerol, 1,2-dipalmitoyl-*sn*-glycerol, 1-palmitoyl-2-oleoyl-*sn*-glycerol, 1-stearoyl-2-linoleoyl-*sn*-glycerol, 1-stearoyl-2-arachidonoyl-*sn*-glycerol, 1-stearoyl-2-docosahexaenoyl-*sn*-glycerol, 1-oleoyl-2-acetyl-*sn*-glycerol, 1,2-di-O-phytanyl-*sn*-glycerol, 1,2-dipalmitoyl ethylene glycol, 1-2-dioleoyl ethylene glycol, glyceryl monostearate, behenoyl polyoxyl-8 glycerides, glyceryl palmitostearate, 1-O-hexadecyl-*sn*-glycerol, 1-O-hexadecyl-2-acetyl-*sn*-glycerol, 1-O-hexadecyl-2-O-methyl-*sn*-glycerol,

1,2-diacyl-3-O-(α-D-glucopyranosyl)-*sn*-glycerol, stearoyl macrogol-32 glycerides, stearoyl polyoxyl-32 glycerides, lauroyl macrogol-32 glycerides, lauroyl polyoxyl-32 glycerides, lauroyl macrogol-6 glycerides, lauroyl polyoxyl-6 glycerides, oleoyl macrogol-6 glycerides, oleoyl polyoxyl-6 glycerides, linoleoyl macrogol-6 glycerides, polyglyceryl-3 dioleate, glycerol monolinoleate, glyceryl monolinoleate, glycerol monooleates, diethylene glycol monoethyl ether, glyceryl dibehenate, glycerol distearate, glyceryl distearate, glyceryl dipalmitostearate and linoleoyl polyoxyl-6 glyceride.

Amongst the various glycerides, glyceryl behenate is preferred in accordance with present disclosure.

In accordance with the present disclosure the fatty acid used to prepare a lipid melt is selected from the group consisting of saturated C_4-C_{28} fatty acids and unsaturated C_4-C_{28} fatty acids. In accordance with the present disclosure, one of the preferred fatty acids is stearic acid.

During the preparation of a lipid melt, the proportion of the glyceride and the fatty acid is maintained in between 1:0 to 1:20 and vice versa.

In the second step, an aqueous emulsifier mix is prepared separately by admixing an emulsifier and water followed by heating at a temperature at least equal to the melting point of the selected lipid.

The emulsifier used for the preparation of emulsifier mix in accordance with the present disclosure include anionic emulsifiers, cationic emulsifiers, non ionic emulsifiers or zwitterionic emulsifiers which include but is not limited to soy lecithin, egg lecithin, phosphatidylcholine; ethylene oxide copolymers, propylene oxide copolymers, poloxamers, sorbitan ethylene oxide/propylene oxide copolymers, polysorbate 20, polysorbate 60, polysorbate 80, sorbitan esters, span 20, span 40, span 60, span 80, alkyllaryl polyether alcohol polymers, tyloxapol, bile salts, cholate, glycocholate, taurocholate, taurodeoxycholate, gemini surfactants, alcohols and the like.

In the third step, a dispersion containing at least one drug is obtained by adding at least one drug to the aqueous emulsifier mix obtained in the second step.

The drug is at least one selected from the group consisting of hydrophilic drugs and amphiphilic drugs.

The hydrophilic drug includes but is not limited to isoniazid, rifampicin, pyrazinamide, ethambutol, streptomycin, acyclovir; acetyl cysteine, acetylcholine chloride, alatrofloxacin, alendronate, amantadine hydrochloride, ambenomium, amifostine, amiloride hydrochloride, aminocaproic acid, amphiphilicin B, atenolol, atracurium besylate, atropine, azithromycin, aztreonam, bacitracin, becalermin, belladona, bepridil hydrochloride, bleomycin sulfate, calcitonin, calcitonin salmon, carboplatin, capecitabine, capreomycin sulfate, cefamandole nafate, cefazolin sodium, cefepime hydrochloride, cefixime, cefonicid sodium, cefoperazone, cefotetan disodium, cefotoxime, cefoxitin sodium, ceftizoxime, ceftriaxone, cefuroxime axetil, cephalexin, cephapirin sodium, chrionic gonadotropin, cidofovir, cisplatin, cladribine, clidinium bromide, clindamycin, ciprofloxacin, clondronate, colistimethate sodium, deforoxamine, denileukin diftitox, desmopressin, diatrizoate megluamine, dicyclomine, didanosine, dirithromycin, dopamine hydrochloride, dornase alpha doxacurium chloride, doxorubicin, editronate disodium, elanaprilat, enkephalin, enoxacin, enoxaprin sodium, ephedrine, epinephrine, erythromycin, esmol hydrochloride, famiciclovir, fludarabine, fluoxetine, ganciclovir, gentamycin, glucagon, glycopyrolate, heparin sodium, indinavir sulfate, insulin, lamivudine, leucovorin calcium, leuprolide acetate, levofloxacin, lincomycin, lobucavir, lomefloxacin, loracarbef, mannitol, methotrexate, methscopolamine, metformin hydrochloride, metroprolol, mezocillin sodium, mivacurium chloride, nedocromil sodium, neostigmine bromide, neostigmine methyl sulfate, neutontin, norfloxacin, octreotide acetate, ofloxacin, olpadronate, oxytocin, pamidronate disodium, pancuronium bromide, paroxetine, pefloxacin, pentamindine isethionate, pentostatin, pentoxifylline, periciclovir, pentagastrin, phentolamine mesylate, phenylalanine, physostigmine salicylate, piperacillin sodium, polymixin B sulfate, pralidoxine chloride, pramlintide, pregabalin, propofenone, propenthaline bromide, pyridostigmine bromide, residronate, ribavarin, rimantadine hydrochloride, salmetrol xinafoate, sincalide, solatol, somatostatin, sparfloxacin, spectinomycin, stavudine, streptozocin, suxamethonium chloride, tacrine hydrochloride, terbutaline sulfate, thiopbeta ticarcillin, tiludronate, timolol, trandolapril, trimetrexate gluconate, trospectinomycin, trovafloxacin, tubocurarine chloride, valaciclovir, valsartan, vasopressin, vecoronium bromide, vinblastin, vincristine, vinorelbine, warfarin sodium, zalcitabine, zanamavir, zolandronate, zidovudine and the like.

The amphiphilic drug includes but is not limited to amphiphilicin B, bupivacaine, ropivacaine, prilocaine, mepivacaine, tetrocaine, etidocaine, morphine, fentanyl, alfentanil, sulfentanil and the like.

The solid lipid nanoparticles of the present disclosure contain a drug which contains at least one hydroxyl group or at least one hydrophilic linkage.

In the fourth step, a micro emulsion is obtained by adding the solution containing at least one drug obtained in the third step to the lipid melt obtained in the first.

Finally, solid lipid nanoparticles are prepared from the microemulsion.

The microemulsion obtained in the fourth step is dispersed in water maintained at a temperature ranging between 0 °C and 5 °C under continuous stirring/homogenizing. The stirring is carried out at 4,000 to 12,000 rpm for a time period between 20 minutes to 2 hours. The stirring is preferably carried out at 5,000 to 7,000 rpm. The ratio of microemulsion and water ranges between 1:1 and 1:4.9.

The alternative way of obtaining solid lipid nanoparticles from the microemulsion is by purging nitrogen through the microemulsion.

The solid lipid nanoparticles prepared in accordance with the present disclosure have a particle size in the range of 10 nm to 1000nm.

The preferred particle size of the solid lipid nanoparticles is in the range of 20 nm to 150 nm.

In another aspect of the present disclosure there is provided solid lipid nanoparticles prepared by the process of present disclosure; said solid lipid nanoparticles comprising:

i) at least lipid selected from the group consisting of glycerides and fatty acids;

ii) at least one drug selected from the group consisting of hydrophilic drugs and amphiphilic drugs; and

iii) at least one emulsifier.

The amount of drug is in the range of about 0.001% to about 99%.

The particle size of the solid lipid nanoparticles prepared by the process of present disclosure is in the range of 10 to 1000 nm, preferably between 20-150 nm.

The present disclosure is further described in light of the following examples which are set forth for illustration purpose only and not to be construed for limiting the scope of the disclosure.

Example 1: Procedure for preparing the solid lipid nanoparticles containing hydrophilic drug

531.0 gm of polysorbate 80 and 7.9 gm of soy lecithin (phospholipon 90H) 374.0 ml of water were taken in a beaker and heated to 90 °C to obtain an emulsifier mix.
Separately, 79.2 gm of glyceryl behenate was heated at 90 °C to obtain a lipid melt. 7.9 gm of Isoniazid was added as hydrophilic drug to the emulsifier mix at 90 °C to obtain an aqueous solution containing isoniazid. Then, the solution containing isoniazid was added to lipid melt, under continuous magnetic stirring at 90 °C to obtain a clear microemulsion. The 1000.0 gm of microemulsion thus formed, was transferred into 1000.0 gm of cold water maintained at 2°C under continuous mechanical stirring/homogenizing at 5,000 rpm for a time period of about 30 minutes using a Wise Tis Homogenizer.

Example 2: Procedure for preparing the solid lipid nanoparticles containing hydrophilic drug

531.0 gm of polysorbate 80 and 7.9 gm of soy lecithin (phospholipon 90H) 374.0 ml of water were taken in a beaker and heated to 90 °C to obtain an emulsifier mix.
Separately, 79.2 gm of stearic acid was heated at 90 °C to obtain a lipid melt. 7.9 gm of Isoniazid was added as hydrophilic drug to the emulsifier mix at 90 °C to obtain an aqueous solution containing isoniazid. Then, the solution containing isoniazid was added to lipid melt, under continuous magnetic stirring at 90 °C to obtain a clear microemulsion. The 1000.0 gm of microemulsion thus formed, was transferred into 1000.0 gm of cold water maintained at 2°C under continuous mechanical stirring/homogenizing at 5,000 rpm for a time period of about 30 minutes using a Wise Tis Homogenizer.

Example 3: Procedure for preparing the solid lipid nanoparticles containing hydrophilic drug

531.0 gm of polysorbate 80 and 7.9 gm of soy lecithin (phospholipon 90H) 374.0 ml of water were taken in a beaker and heated to 90 °C to obtain an emulsifier mix.
63.36 gm of glyceryl behenate and 15.84 gm of stearic acid were heated at 90 °C to obtain a lipid melt. 7.9 gm of Isoniazid was added as hydrophilic drug to the emulsifier mix at 90 °C to obtain an aqueous solution containing isoniazid. Then, the solution containing isoniazid was added to lipid melt, under continuous magnetic stirring at 90 °C to obtain a clear microemulsion.

The 1000.0 gm of microemulsion thus formed, was transferred into 1000.0 gm of cold water maintained at 2°C under continuous mechanical stirring/homogenizing at 5,000 rpm for a time period of about 30 minutes using a Wise Tis Homogenizer.

Example 4: Procedure for preparing the solid lipid nanoparticles containing hydrophilic drug

531.0 gm of polysorbate 80 and 7.9 gm of soy lecithin (phospholipon 90H) 374.0 ml of water were taken in a beaker and heated to 90 °C to obtain an emulsifier mix.

Separately,63.36 gm of glyceryl behenate and 15.84 gm of stearic acid was heated at 90 °C to obtain a lipid melt. 7.9 gm of Isoniazid was added as hydrophilic drug to the emulsifier mix at 90 °C to obtain an aqueous solution containing isoniazid. Then, the solution containing isoniazid was added to lipid melt, under continuous magnetic stirring at 90 °C to obtain a clear microemulsion. The dry nitrogen gas was purged through the microemulsion for 2 hours to obtain the solid lipid nanoparticles.

The solid lipid nanoparticles prepared in accordance with the present disclosure were evaluated for various parameters such as total drug content, entrapment efficiency, particle size, zeta potential, poly dispersity index, *in-vitro* release (figure 1). The results are provided in Table 1.

Determination of particle size, poly dispersity index and zeta potential:

Solid lipid nanoparticles of Example 1 were evaluated for particle size, poly dispersity index and zeta potential using Beckman Coulter Delsa(TM) Nano Zeta Potential and Submicron Particle Size Analyzer wherein triple distilled water (TDW) was used as a diluting medium.

Determination of total drug content (TDC) and entrapment efficiency (EE):

Total amount of drug per unit volume present in the solid lipid nanoparticles (SLNs) was determined by disrupting 0.1 ml of the solid lipid nano-particle dispersion with 5 ml of chloroform and methanol (1:1). The amount of isoniazid was determined by HPLC. Each experiment was performed in triplicate. A similarly processed blank solid lipid nano-particle sample was taken as control. The total drug content was determined by using the equation given below.

$$TDC = \frac{C\text{alculated amount of drug / ml of SLN dispersion}}{\text{Total amount of drug added / ml of SLN dispersion}} \times 100$$

Entrapment efficiency was determined by analyzing the clear supernatant obtained by centrifuging the developed solid lipid nanoparticle dispersions at 8.02 Lac g for 2 h at 4°C using Beckman Coulter Ultracentrifuge (L100 K and 100 Ti rotor). The entrapment efficiency was calculated as follows:

$$EE = \frac{TDC - D_f}{TDC} x100$$

Where D_f = amount of drug in clear supernatant fluid

The results are provided herein below.

Table No. 1: Determination of particle size (D), poly dispersity index (PDI), total drug content (TDC), entrapment efficiency (EE) and zeta potential of the solid lipid nano particles containing isoniazid drug.

Sr. No.	Solid Lipid Nanoparticle Composition	D (nm)	PDI	TDC (%)	EE (%)	Zeta potential (mV)
1	Glyceryl behenate + isoniazid	120±0.70	0.281	94.215±0.781	69.013±0.675	- 0.101
2	Stearic acid + isoniazid	116±4.04	0.113	92.232±2.070	77.600±1.961	- 0.609
3	glyceryl behenate, stearic acid + isoniazid (Present disclosure)	113±4.00	0.207	92.520±1.351	84.032±1.103	- 0.069
4	glyceryl behenate, stearic acid + isoniazid (Present disclosure) *liquid nitrogen purging	128±3.12	0.287	98.251±2.561	82.413±2.113	-0.066

Conclusion:

Solid lipid nanoparticles prepared in accordance with the present disclosure exhibited a small particle size below 120 nm. The total drug content was found to be greater than 92% w/v. Further, the poly dispersity index for all the developed solid lipid nanoparticles was found to be below 0.3 which indicates a narrow particle size distribution.

The entrapment efficiency for solid lipid nanoparticles of combination of glyceryl behenate and stearic acid was found to be 84.032±1.103% in comparison to 77.600±1.961% and 69.013±0.675% for solid lipid nanoparticles of glyceryl behenate and solid lipid nanoparticles of stearic acid, respectively.

From these results it is clear that the solid lipid nanoparticles prepared using a combination of lipids or a lipid and a fatty acid prepared in accordance with the present disclosure possesses enhanced entrapment efficiency for hydrophilic and/or amphiphilic drugs.

***In vitro* drug release of isoniazid**

In vitro release of isoniazid was determined in phosphate buffer of pH 6.8 by dialysis bag method using dialysis membrane with a molecular weight cut off of 12000-14000 Da. An accurate volume (1 ml) of solid lipid nanoparticles dispersion containing 2.6 mg of isoniazid (as per the calculated Total Drug Content) was placed inside the dialysis bag, tied at both the ends and dipped in the dissolution medium (80 ml). Stirring was maintained at 100 rpm, using magnetic bead, at 37±0.2 °C. Two milliliters aliquots were withdrawn at pre-set time intervals (0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24 h) and replaced by an equal volume of fresh dissolution medium. After suitable dilution, the samples were analyzed by HPLC. Corrected concentration of isoniazid was calculated in the test samples using the regression equation of the calibration curve. The results are show in Table No. 2 and Figure 1(Free drug *, SLN of stearic acid**, SLN of glyceryl behenate*** and SLN of combination of glyceryl behenate and stearic acid****)

Table No. 2: *In-vitro* drug release of isoniazid from Solid Lipid Nanoparticles of the present disclosure.

Time (hrs)	Isoniazid solution ± SD	Glyceryl behenate solid lipid nano particles ± SD	Stearic acid solid lipid nano particles ± SD	Solid lipid nano particles containing glyceryl behenate and stearic acid ± SD (Present disclosure)
0	0.000 ± 0.00	0 ± 0.000	0 ± 0.000	0 ± 0.000
0.25	36.870 ± 0.489	18.749 ± 1.234	33.198 ± 0.856	22.427 ± 1.131
0.5	43.839 ± 1.747	26.095 ± 0.245	41.023 ± 0.849	29.446 ± 1.326
1	63.347 ± 0.036	32.773 ± 0.647	43.949 ± 2.481	45.758 ± 0.044
2	80.265 ± 1.734	37.602 ± 1.495	51.518 ± 1.921	55.450 ± 3.432
3	88.253 ± 1.963	40.473 ± 0.826	56.602 ± 0.513	60.132 ± 1.114
4	93.694 ± 1.073	46.068 ± 1.016	58.756 ± 1.786	68.430 ± 1.031
5	99.876 ± 0.696	56.002 ± 1.561	65.512 ± 0.357	74.824 ± 3.751
8		60.167 ± 1.192	67.172 ± 0.523	76.110 ± 2.904
12		60.536 ± 0.268	72.024 ± 0.536	77.566 ± 1.137
24		65.086 ± 0.964	79.967 ± 0.457	86.756 ± 1.006

Conclusion:

The drug release study was carried out for all the three developed solid lipid nanoparticles (solid lipid nanoparticles of glyceryl behenate, solid lipid nanoparticles of stearic acid and solid lipid nanoparticles of combination of glyceryl behenate and stearic acid). *In vitro* drug release showed tri-phasic behavior comprising an initial release of free drug (the free drug was not removed from the prepared solid lipid nanoparticle dispersions which were loaded into the dialysis bag), this phase could be due to the very small solid lipid nanoparticles (SLNs) present in the dispersion, across the dialysis membrane followed by a hump and a fast release probably corresponding to the release of drug present in the outer surfactant coat of the SLNs, considering that isoniazid is a hydrophilic drug. This was followed by a delayed release phase starting approximately at 5 h showing almost a controlled release. The release kinetics followed peppas model ($r^2 \geq$ 0.9) and 65.086 %, 79.967% and 86.756% of the drug was released in a period of 24 h

for solid lipid nanoparticles of glyceryl behenate, , solid lipid nanoparticles of stearic acid and solid lipid nanoparticles of combination of glyceryl behenate and stearic acid respectively.

Determination of drug concentration in plasma, brain, liver and kidney:

Two groups each containing forty two female Wistar rats were formed and named as Group A and Group B. Both the groups of rats were kept at uniform atmospheric conditions. The Wistar rats of Group A were given isoniazid (free drug) by an oral route while rats of Group B were given solid lipid nanoparticles of combination of glyceryl behenate and stearic acid containing isoniazid drug by an oral route. Drug concentration in the samples was determined using HPLC. The plasma samples were collected before sacrificing the rats. Six Wistar rats were sacrificed per time point. The details are mentioned below.

Preparation of plasma samples:

To 150-µL aliquot of plasma, a 300 µL of the deproteinizing agent (methanol) was added to precipitate the proteins which was then vortexed for 2 minutes. The samples were centrifuged at 15000 rpm for 10 minutes at 4 °C. The supernatant was then collected and an equal volume of water was added to the clear supernatant. The samples were filtered (0.20 µm nylon filters) and injected into the HPLC system.

Preparation of tissue samples (Brain, Liver and Kidney)

20 % aqueous tissue homogenates were prepared in cold 150 mM KCl. The homogenates were then centrifuged at 15000 rpm for 10 minutes at 4 °C to obtain clear tissue homogenates. To 150-µL aliquot of the clear tissue homogenates, a 300 µL of the deproteinizing agent (methanol) was added to obtain a dispersion which was vortexed for 2 minutes. The samples were then centrifuged at 15000 rpm for 10 minutes at 4 °C. The supernatant was then collected and an equal volume of water was added to the clear supernatant. The samples were then filtered (0.20 µm nylon filters) and were injected into the HPLC system.

Chromatographic conditions

The HPLC system consisted of Waters Alliance (Waters Corporation, MA, USA) equipped with a Waters e-2695 ALLIANCE separation module comprising of a solvent

(quaternary gradient mode), auto injector, column oven and a 4 channel in line degasser, a sample management system (sample heater cooler) and a 2998 PDA detector. Chromatographic separation was performed using symmetry C18 column at 254 nm. Data acquisition was performed by the Empower 2® software. The mobile phase consisted of a mixture phosphate buffer of pH 6.8 and methanol (85:15). The mobile phase was delivered at a flow rate of 0.9 ml/min and the detection of isoniazid (INH) was carried out at 254 nm. The injection volume was 20 µL and the analysis was performed at 30°C (sample 10°C).

The results are tabulated herein Table No. 3 to Table No. 6.

Table No. 3: Comparative analysis of concentration of isoniazid in plasma.

Parameters	**Solid Lipid Nano particles containing isoniazid (Present disclosure)**	**Isoniazid solution**
Extra vascular Time (h)	**Mean ± SD**	**Mean ± SD**
0	0 ± 0.00	0 ± 0.00
0.25	13014.79 ± 1932.75	5351.42 ± 1981.22
1	11139.28 ± 687.20	6477.62 ± 1868.80
2	11063.4 ± 2036.22	8052.65 ± 1352.64
4	11525.91 ± 520.62	4089.01 ± 873.92
6	9474.25 ± 700.37	2226.6 ± 49.97
8	8113.77 ± 344.78	1350.29 ± 807.46
12	6684.95 ± 592.66	482.51 ± 351.32
Dose (mg/kg)	25	25
C_{max} (ng/mL)	13014.79	8052.65
T_{max} (h)	0.25	2
AUC_{last} (ng/mL*h)	112348.3	37072.6
AUC_{extra} (ng/mL*h)	117827.67	1890.61
AUC_{tot} (ng/mL*h)	230175.97	38963.21
$\%AUC_{extra}$	51.19	4.85

Table No. 4: Comparative analysis of concentration of isoniazid in brain (cerebral permeability)

Parameters	Solid Lipid Nano particles containing isoniazid (Present disclosure)	Isoniazid solution
Extra vascular Time (h)	Mean ± SD	Mean ± SD
0	0 ± .00	0 ± 0.00
0.25	2208.25 ± 148.95	1323.45 ± 508.72
1	2065.36 ± 41.19	1211.17 ± 263.01
2	2541.98 ± 107.47	1480.22 ± 152.18
4	1868.27 ± 513.45	824.3 ± 292.50
6	1653.1 ± 207.65	1230.37 ± 416.38
8	1319.33 ± 179.71	592.28 ± 85.41
12	1406.92 ± 129.51	789.97 ± 72.68
Dose (mg/kg)	25	25
C_{max} (ng/mL)	2541.98	1480.22
T_{max} (h)	2	2
AUC_{last} (ng/mL*h)	20486.81	11266.63
AUC_{extra} (ng/mL*h)	24136.42	13349.82
AUC_{tot} (ng/mL*h)	44623.24	24616.45
$\%AUC_{extra}$	54.09	54.23

Table No. 5: Comparative analysis of concentration isoniazid in liver

Parameters	Solid Lipid Nano particles containing isoniazid (Present disclosure)	Isoniazid solution
Extra vascular Time (h)	Mean ± SD	Mean ± SD
0	0 ± 0.00	0 ± 0.00
0.25	2180.76 ± 251.34	2846.79 ± 354.61
1	1977.96 ± 135.39	2063.58 ± 108.92
2	2068.71 ± 319.85	1270.07 ± 216.25
4	1927.87 ± 247.27	1569.83 ± 418.61
6	1526.31 ± 235.05	1620.32 ± 403.91
8	1869.03 ± 202.50	1317.85 ± 339.88
12	1549.62 ± 202.12	1269.99 ± 91.11
Dose (mg/kg)	25	25
C_{max} (ng/mL)	2180.76	2846.79
T_{max} (h)	0.25	0.25
AUC_{last} (ng/mL*h)	21500.4	17949.27
AUC_{extra} (ng/mL*h)	58847.89	40825.2
AUC_{tot} (ng/mL*h)	80348.29	58774.47
$\%AUC_{extra}$	73.24	69.46

Table No. 6: Comparative analysis of concentration of isoniazid in kidney

Parameters	Solid Lipid Nano particles containing isoniazid (Present disclosure)	Isoniazid solution
Extra vascular Time (h)	Mean ± SD	Mean ± SD
0	0 ± 0.00	0 ± 0.00
0.25	17498.64 ± 2316.51	18526.35 ± 2372.84
1	17475.41 ± 6233.36	11613.3 ± 2474.31
2	16277.92 ± 1174.47	15211.59 ± 3308.07
4	11586.89 ± 4119.49	14109.52 ± 1688.51
6	6314.97 ± 946.15	10034.1 ± 3388.93
8	8904.48 ± 1447.66	8514.74 ± 1372.49
12	8143.91 ± 814.45	14544.48 ± 1489.75
Dose (mg/kg)	25	25
C_{max} (ng/mL)	17498.64	18526.35
T_{max} (h)	0.25	0.25
AUC_{last} (ng/mL*h)	126437.1	-
AUC_{extra} (ng/mL*h)	102616.1	81808.11
AUC_{tot} (ng/mL*h)	229053.2	180364.8
$\%AUC_{extra}$	44.8	45.36
Lz (1/h)	NA	0.1

Conclusion:

From the results as shown in Table No. 3 and Table No. 4 it is found that:

- The solid lipid nanoparticles with a particle size in the range of 10 to 1000 nm, preferably between 20-150 nm prepared in accordance with the present disclosure provide enhanced concentration of hydrophilic drug or amphiphilic drug in plasma.

- The solid lipid nanoparticles prepared in accordance with the present disclosure improves cerebral permeability of hydrophilic drug or amphiphilic drug entrapped therein.

TECHNICAL ADVANCEMENT AND ECONOMIC SIGNIFICANCE

The solid lipid nanoparticles prepared in accordance with the present disclosure enhance the bioavailability of the hydrophilic drug or amphiphilic drug as a result of improved permeability across the biological barriers. The improved bioavailability reduces dose, dosing frequency and dose dependent side effects of the drug.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

The use of the expression "at least" or "at least one" suggests the use of one or more elements or ingredients or quantities, as the use may be in the embodiment of the invention to achieve one or more of the desired objects or results.

The numerical values mentioned for the various physical parameters, dimensions or quantities are only approximations and it is envisaged that the values higher/lower than the numerical values assigned to the parameters, dimensions or quantities fall within the scope of the invention, unless there is a statement in the specification specific to the contrary.
The applicant craves leave to submit further clinical data.

While considerable emphasis has been placed herein on the particular features of this disclosure, it will be appreciated that various modifications can be made, and that many changes can be made in the preferred embodiments without departing from the principle of the disclosure. These and other modifications in the nature of the disclosure or the preferred embodiments will be apparent to those skilled in the art from the disclosure herein, whereby it is to be distinctly understood that the foregoing descriptive matter is to be interpreted merely as illustrative of the disclosure and not as a limitation.

Claims:

1. A process for preparing solid lipid nanoparticles, said process comprising the following steps:

   i) melting at least one lipid selected from the group consisting of glycerides and fatty acids;

   ii) preparing an aqueous emulsifier mix by admixing at least one emulsifier and water followed by heating at a temperature atleast equal to the melting point of the selected lipid;

   iii) adding at least one drug to the aqueous emulsifier mix to obtain a dispersion;

   iv) adding the dispersion containing at least one drug to the lipid melt to obtain a microemulsion; and

   v) preparing solid lipid nanoparticles having a particle size in the range of 10 nm to 1000 nm, preferably 20 nm to 150 nm from the microemulsion.

2. The process claimed in claim 1, wherein the method step of preparing solid lipid nanoparticles comprises dispersing the microemulsion in water maintained at a temperature ranging between 0 °C and 5 °C under continuous stirring/homogenizing to obtain solid lipid nanoparticles, wherein the ratio of microemulsion and water ranges between 1:1 and 1:4.9.

3. The process claimed in claim 1, wherein the method step of preparing the solid lipid nanoparticles comprises purging nitrogen through the microemulsion to obtain solid lipid nanoparticles.

4. The process claimed in claim 1, wherein the glyceride is at least one selected from the group consisting of mono-glycerides, di-glycerides and tri-glycerides.

5. The process claimed in claim 1, wherein the glyceride is at least one selected from the group consisting of glyceryl behenate tricaprin, trilaurin, trimyristin, tripalmitin, tristearin, 1,2-dioctanoyl-*sn*-glycerol, 1,2-didecanoyl-*sn*-glycerol, 1,2-dilauroyl-*sn*-glycerol, 1,2-dimyristoyl-*sn*-glycerol, 1,2-dipalmitoyl-*sn*-glycerol, 1-palmitoyl-2-oleoyl-*sn*-glycerol, 1-stearoyl-2-linoleoyl-*sn*-glycerol, 1-stearoyl-2-arachidonoyl-*sn*-glycerol, 1-stearoyl-2-docosahexaenoyl-*sn*-glycerol, 1-oleoyl-2-acetyl-*sn*-glycerol,

1,2-di-O-phytanyl-*sn*-glycerol, 1,2-dipalmitoyl ethylene glycol, 1-2-dioleoyl ethylene glycol, glyceryl monostearate, behenoyl polyoxyl-8 glycerides, glyceryl palmitostearate, 1-O-hexadecyl-*sn*-glycerol, 1-O-hexadecyl-2-acetyl-*sn*-glycerol, 1-O-hexadecyl-2-O-methyl-*sn*-glycerol, 1,2-diacyl-3-O-(α-D-glucopyranosyl)-*sn*-glycerol, stearoyl macrogol-32 glycerides, stearoyl polyoxyl-32 glycerides, lauroyl macrogol-32 glycerides, lauroyl polyoxyl-32 glycerides, lauroyl macrogol-6 glycerides, lauroyl polyoxyl-6 glycerides, oleoyl macrogol-6 glycerides, oleoyl polyoxyl-6 glycerides, linoleoyl macrogol-6 glycerides, polyglyceryl-3 dioleate, glycerol monolinoleate, glyceryl monolinoleate, glycerol monooleates, diethylene glycol monoethyl ether, glyceryl dibehenate, glycerol distearate, glyceryl distearate, glyceryl dipalmitostearate and linoleoyl polyoxyl-6 glyceride, preferably, glyceryl behenate.

6. The process claimed in claim 1, wherein the fatty acid is selected from the group consisting of saturated C_4-C_{28} fatty acids and unsaturated C_4-C_{28} fatty acids.

7. The process claimed in claim 1, wherein the fatty acid is stearic acid.

8. The process claimed in claim 1, wherein the proportion of the glyceride and the fatty acid is in the range of 1:0 to 1:20.

9. The process claimed in claim 1, wherein the proportion of the fatty acid and the glyceride is in the range of 1:0 to 1:20.

10. The process claimed in claim 1, wherein the emulsifier is at least one selected from the group consisting of anionic emulsifiers, cationic emulsifiers, non ionic emulsifiers or zwitterionic emulsifiers.

11. The process claimed in claim 1, wherein the emulsifier is at least one selected from the group consisting of soy lecithin, egg lecithin, phosphatidylcholine; ethylene oxide copolymers, propylene oxide copolymers, poloxamers, sorbitan ethylene oxide/propylene oxide copolymers, polysorbate 20, polysorbate 60, polysorbate 80, sorbitan esters, span 20, span 40, span 60, span 80, alkyllaryl polyether alcohol polymers, tyloxapol, bile salts, cholate, glycocholate, taurocholate, taurodeoxycholate, gemini surfactants and alcohols.

12. The process claimed in claim 1, wherein the drug is at least one selected from the group consisting of hydrophilic drugs and amphiphilic drugs.

13. The process claimed in claim 1, wherein the drug is at least one hydrophilic drug selected from the group consisting of isoniazid, rifampicin, pyrazinamide, ethambutol, streptomycin, acyclovir, acetyl cysteine, acetylcholine chloride, alatrofloxacin, alendronate, amantadine hydrochloride, ambenomium, amifostine, amiloride hydrochloride, aminocaproic acid, amphiphilicin B, atenolol, atracurium besylate, atropine, azithromycin, aztreonam, bacitracin, becalermin, belladona, bepridil hydrochloride, bleomycin sulfate, calcitonin, calcitonin salmon, carboplatin, capecitabine, capreomycin sulfate, cefamandole nafate, cefazolin sodium, cefepime hydrochloride, cefixime, cefonicid sodium, cefoperazone, cefotetan disodium, cefotoxime, cefoxitin sodium, ceftizoxime, ceftriaxone, cefuroxime axetil, cephalexin, cephapirin sodium, chrionic gonadotropin, cidofovir, cisplatin, cladribine, clidinium bromide, clindamycin, ciprofloxacin, clondronate, colistimethate sodium, deforoxamine, denileukin diftitox, desmopressin, diatrizoate megluamine, dicyclomine, didanosine, dirithromycin, dopamine hydrochloride, dornase alpha doxacurium chloride, doxorubicin, editronate disodium, elanaprilat, enkephalin, enoxacin, enoxaprin sodium, ephedrine, epinephrine, erythromycin, esmol hydrochloride, famiciclovir, fludarabine, fluoxetine, ganciclovir, gentamycin, glucagon, glycopyrolate, heparin sodium, indinavir sulfate, insulin, lamivudine, leucovorin calcium, leuprolide acetate, levofloxacin, lincomycin, lobucavir, lomefloxacin, loracarbef, mannitol, methotrexate, methscopolamine, metformin hydrochloride, metroprolol, mezocillin sodium, mivacurium chloride, nedocromil sodium, neostigmine bromide, neostigmine methyl sulfate, neutontin, norfloxacin, octreotide acetate, ofloxacin, olpadronate, oxytocin, pamidronate disodium, pancuronium bromide, paroxetine, pefloxacin, pentamindine isethionate, pentostatin, pentoxifylline, periciclovir, pentagastrin, phentolamine mesylate, phenylalanine, physostigmine salicylate, piperacillin sodium, polymixin B sulfate, pralidoxine chloride, pramlintide, pregabalin, propofenone, propenthaline bromide, pyridostigmine bromide, residronate, ribavarin, rimantadine hydrochloride, salmetrol xinafoate, sincalide, solatol, somatostatin, sparfloxacin, spectinomycin, stavudine, streptozocin, suxamethonium chloride, tacrine hydrochloride, terbutaline sulfate, thiopbeta ticarcillin, tiludronate, timolol, trandolapril, trimetrexate gluconate, trospectinomycin, trovafloxacin, tubocurarine chloride, valaciclovir, valsartan, vasopressin, vecoronium bromide, vinblastin, vincristine, vinorelbine, warfarin sodium, zalcitabine, zanamavir, zolandronate and zidovudine.

14. The process claimed in claim 1, wherein the drug is at least one amphiphilic drug selected from the group consisting of amphiphilicin B, bupivacaine, ropivacaine, prilocaine, mepivacaine, tetrocaine, etidocaine, morphine, fentanyl, alfentanil and sulfentanil.

15. The process claimed in claim 1, wherein the drug is at least one drug containing at least one hydroxyl group or at least one hydrophilic linkage.

16. The process claimed in claim 2, wherein the method step of dispersing the micro emulsion in water is carried out by continuous stirring/homogenizing at 4,000 to 12,000 rpm, preferably 5,000 to 7,000 rpm for a time period ranging between 20 minutes and 2 hours.

17. Solid lipid nanoparticles prepared by the process as claimed in any of the preceding claims, said solid lipid nanoparticles comprising:

i) at least one lipid selected from the group consisting of glycerides and fatty acids;

ii) at least one drug selected from the group consisting of hydrophilic drugs and amphiphilic drugs; and

iii) at least one emulsifier.

18. The solid lipid nanoparticles claimed in claim 17, wherein the glyceride is at least one selected from the group consisting of mono-glycerides, di-glycerides and tri-glycerides.

19. The solid lipid nanoparticles claimed in claim 17, wherein the glyceride is at least one selected from the group consisting of glyceryl behenate, tricaprin, trilaurin, trimyristin, tripalmitin, tristearin, 1,2-dioctanoyl-*sn*-glycerol, 1,2-didecanoyl-*sn*-glycerol, 1,2-dilauroyl-*sn*-glycerol, 1,2-dimyristoyl-*sn*-glycerol, 1,2-dipalmitoyl-*sn*-glycerol, 1-palmitoyl-2-oleoyl-*sn*-glycerol, 1-stearoyl-2-linoleoyl-*sn*-glycerol, 1-stearoyl-2-arachidonoyl-*sn*-glycerol, 1-stearoyl-2-docosahexaenoyl-*sn*-glycerol, 1-oleoyl-2-acetyl-*sn*-glycerol, 1,2-di-O-phytanyl-*sn*-glycerol, 1,2-dipalmitoyl ethylene glycol, 1-2-dioleoyl ethylene glycol, glyceryl monostearate, behenoyl polyoxyl-8 glycerides, glyceryl palmitostearate, 1-O-hexadecyl-*sn*-glycerol, 1-O-hexadecyl-2-acetyl-*sn*-glycerol, 1-O-hexadecyl-2-O-methyl-*sn*-glycerol, 1,2-diacyl-3-O-(α-D-glucopyranosyl)-*sn*-glycerol, stearoyl macrogol-32 glycerides, stearoyl polyoxyl-32

glycerides, lauroyl macrogol-32 glycerides, lauroyl polyoxyl-32 glycerides, lauroyl macrogol-6 glycerides, lauroyl polyoxyl-6 glycerides, oleoyl macrogol-6 glycerides, oleoyl polyoxyl-6 glycerides, linoleoyl macrogol-6 glycerides, polyglyceryl-3 dioleate, glycerol monolinoleate, glyceryl monolinoleate, glycerol monooleates, diethylene glycol monoethyl ether, glyceryl dibehenate, glycerol distearate, glyceryl distearate, glyceryl dipalmitostearate and linoleoyl polyoxyl-6 glyceride, preferably, glyceryl behenate.

20. The solid lipid nanoparticles claimed in claim 17, wherein the fatty acid is at least one selected from the group consisting of saturated C_4-C_{28} fatty acids and unsaturated C_4-C_{28} fatty acids.

21. The solid lipid nanoparticles claimed in claim 17, wherein the fatty acid is stearic acid.

22. The process claimed in claim 17, wherein the proportion of the glyceride and the fatty acid is in the range of 1:0 to 1:20.

23. The process claimed in claim 17, wherein the proportion of the fatty acid and the glyceride is in the range of 1:0 to 1:20.

24. The solid lipid nanoparticles claimed in claim 17, wherein the drug is at least one hydrophilic drug selected from the group consisting of isoniazid, rifampicin, pyrazinamide, ethambutol, streptomycin, acyclovir; acetyl cysteine, acetylcholine chloride, alatrofloxacin, alendronate, amantadine hydrochloride, ambenomium, amifostine, amiloride hydrochloride, aminocaproic acid, amphiphilicin B, atenolol, atracurium besylate, atropine, azithromycin, aztreonam, bacitracin, becalermin, belladona, bepridil hydrochloride, bleomycin sulfate, calcitonin, calcitonin salmon, carboplatin, capecitabine, capreomycin sulfate, cefamandole nafate, cefazolin sodium, cefepime hydrochloride, cefixime, cefonicid sodium, cefoperazone, cefotetan disodium, cefotoxime, cefoxitin sodium, ceftizoxime, ceftriaxone, cefuroxime axetil, cephalexin, cephapirin sodium, chrionic gonadotropin, cidofovir, cisplatin, cladribine, clidinium bromide, clindamycin, ciprofloxacin, clondronate, colistimethate sodium, deforoxamine, denileukin diftitox, desmopressin, diatrizoate megluamine, dicyclomine, didanosine, dirithromycin, dopamine hydrochloride, dornase alpha doxacurium chloride, doxorubicin, editronate disodium, elanaprilat, enkephalin, enoxacin, enoxaprin sodium, ephedrine, epinephrine, erythromycin,

esmol hydrochloride, famiciclovir, fludarabine, fluoxetine, ganciclovir, gentamycin, glucagon, glycopyrolate, heparin sodium, indinavir sulfate, insulin, lamivudine, leucovorin calcium, leuprolide acetate, levofloxacin, lincomycin, lobucavir, lomefloxacin, loracarbef, mannitol, methotrexate, methscopolamine, metformin hydrochloride, metroprolol, mezocillin sodium, mivacurium chloride, nedocromil sodium, neostigmine bromide, neostigmine methyl sulfate, neutontin, norfloxacin, octreotide acetate, ofloxacin, olpadronate, oxytocin, pamidronate disodium, pancuronium bromide, paroxetine, pefloxacin, pentamindine isethionate, pentostatin, pentoxifylline, pericíclovir, pentagastrin, phentolamine mesylate, phenylalanine, physostigmine salicylate, piperacillin sodium, polymixin B sulfate, pralidoxine chloride, pramlintide, pregabalin, propofenone, propenthaline bromide, pyridostigmine bromide, residronate, ribavarin, rimantadine hydrochloride, salmetrol xinafoate, sincalide, solatol, somatostatin, sparfloxacin, spectinomycin, stavudine, streptozocin, suxamethonium chloride, tacrine hydrochloride, terbutaline sulfate, thiopbeta ticarcillin, tiludronate, timolol, trandolapril, trimetrexate gluconate, trospectinomycin, trovafloxacin, tubocurarine chloride, valaciclovir, valsartan, vasopressin, vecoronium bromide, vinblastin, vincristine, vinorelbine, warfarin sodium, zalcitabine, zanamavir, zolandronate and zidovudine.

25. The solid lipid nanoparticles claimed in claim 17, wherein the drug is at least one amphiphilic drug selected from the group consisting of amphiphilicin B, bupivacaine, ropivacaine, prilocaine, mepivacaine, tetrocaine, etidocaine, morphine, fentanyl, alfentanil and sulfentanil.

26. The solid lipid nanoparticles claimed in claim 17, wherein the amount of drug is in the range of about 0.001% to about 99% with respect to the mass of the lipid.

27. The solid lipid nanoparticles claimed in claim 17, wherein the drug is at least one drug containing at least one hydroxyl group or at least one hydrophilic linkage.

28. The solid lipid nanoparticles claimed in any of the claims 17 to 27, wherein the particle size of the solid lipid nanoparticles ranges between 10 to 1000 nm, preferably between 20-150 nm.

29. The solid lipid nanoparticles claimed in claim 17, wherein the emulsifier is at least one selected from the group consisting of anionic emulsifiers, cationic emulsifiers, non ionic emulsifiers or zwitterionic emulsifiers.

30. The solid lipid nanoparticles claimed in claim 17, wherein the emulsifier is at least one selected from the group consisting of soy lecithin, egg lecithin, phosphatidylcholine; ethylene oxide copolymers, propylene oxide copolymers, poloxamers, sorbitan ethylene oxide/propylene oxide copolymers, polysorbate 20, polysorbate 60, polysorbate 80, sorbitan esters, span 20, span 40, span 60, span 80, alkyllaryl polyether alcohol polymers, tyloxapol, bile salts, cholate, glycocholate, taurocholate, taurodeoxycholate, gemini surfactants and alcohols.

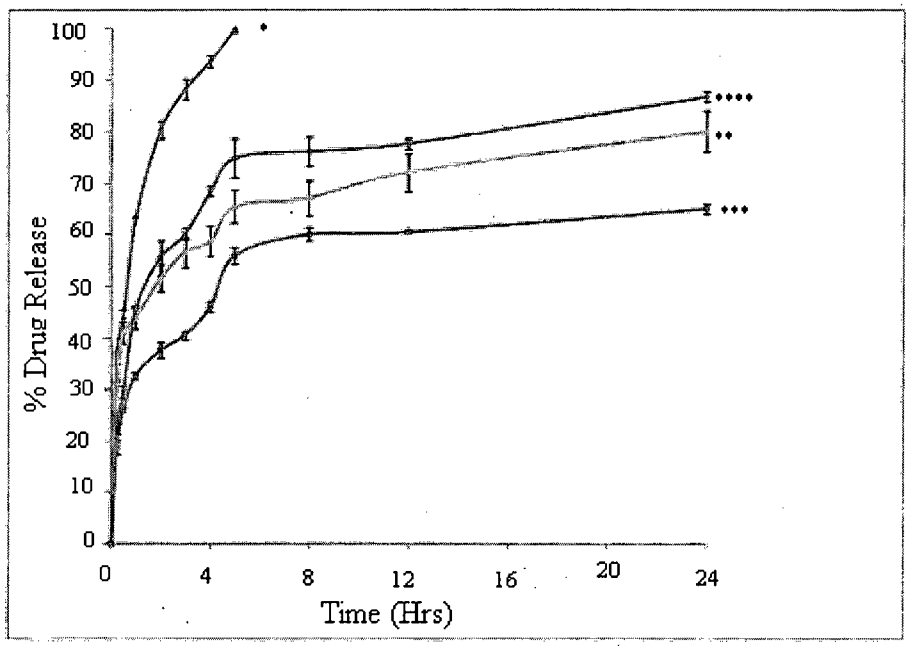
100
90
80
70
60
50
40
30
20
10
0
% Drug Release
0
4
8
12
16
20
24
Time (Hrs)
*
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FIG. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2012/000154

A. CLASSIFICATION OF SUBJECT MATTER

A61K9/127(2006.01)i

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)

IPC:A61K, A61P

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)

DATABASE:WPI,EPODOC,CNPAT,CNABS,CPRSABS,MOABS,HKABS,TWABS,DWPI,SIPOABS,CPEA,JPABS, CNTXT,EPTXT,USTXT,WOTXT,CNKI,CA,EMBASE,MEDLINE

KEYWORDS:solid?,lipid?,liposome?,nanoparticle?,particle?,grain?,glyceride?,emuls+,fuse,fusing,melt+, liquate,tektite,molten, fatty,acid

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN1621029A(UNIV SOUTHEAST), 1 Jun.2005(01.06.2005) See claims 1-3,	1-30
X	CN1490055A(UNIV SOUTHEAST), 21 Apr.2004(21.04.2004) See examples	1-30

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim (S) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"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

"&"document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8.Oct. 2012(8.10.2012)	**08 Nov. 2012 (08.11.2012)**
Name and mailing address of the ISA/CN The State Intellectual Property Office, the P.R.China 6 Xitucheng Rd., Jimen Bridge, Haidian District, Beijing, China 100088 Facsimile No. 86-10-62019451	Authorized officer LI,Fengyun Telephone No. (86-10)62412204

Form PCT/ISA /210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IN2012/000154

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO2007000531A1(CNRS CENT NAT RECH SC), 04 Jan.2007(04.01.2007) see the whole document	1-30

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IN2012/000154

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
CN1621029A	01.06.2005	CN1269472C	16.08.2006
CN1490055A	21.04.2004	CN1194760C	30.03.2005
WO2007000531A2	04.01.2007	FR2885538A1	17.11.2006
		WO2007000531A3	08.03.2007