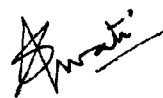


ABSTRACT

The present invention relates to a method for preparing acyclovir loaded topical solid-lipid nanoparticle (SLN) gel. The method comprises preparing an oil phase by using an antiviral drug, cholesterol, phosphatidyl choline and oleic acid. An aqueous phase is prepared by using surfactants such as Tween 80 and PEG 4000. A nano-emulsion is formed by mixing the oil phase and the aqueous phase at pre-determined conditions of pH and temperature. The method is a hot homogenization method. The present invention also relates to solid-lipid nanoparticles loaded with an antiviral drug, acyclovir. The acyclovir SLN formulation has the ability to avoid the systematic penetration through the skin.

FIG. 1



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CLAIMS

What is claimed is:

1. A hot homogenization method for synthesizing an acyclovir drug loaded solid-lipid nanoparticle gel comprises:
preparing an oil phase;
preparing an aqueous phase;
formulating a nanoemulsion, wherein the nanoemulsion consists a drug loaded solid-lipid nanoparticles, wherein the drug is acyclovir; and
incorporating the nanoemulsion into a hydrogel.
2. The method as claimed in claim 1, wherein the step of preparing the oil phase comprises:
mixing a drug, a phospholipid, a cholesterol and a fatty acid to form a mixture, wherein the drug is acyclovir, wherein the phospholipid is soya phosphatidyl choline (99%) and wherein the fatty acid is oleic acid;
dissolving the mixture in a pre-determined amount of a solution, wherein the solution consists organic solvents, wherein the organic solvents are chloroform and methanol mixed in a ratio of 1:1;
removing the organic solvents to obtain a drug embedded lipid layer, wherein the organic solvents are removed by using a Rota-evaporator used at a predetermined temperature and a pre-determined speed, wherein the pre-determined temperature is 80°C and the pre-determined speed is 30 rpm; and
heating the drug embedded lipid layer at a predetermined temperature to obtain the oil phase, wherein the predetermined temperature is 85°C.
3. The method as claimed in claim 1, wherein the step of preparing the aqueous phase comprises:
mixing a predetermined amount of Tween 80, a predetermined amount of polyethylene glycol 4000 and a predetermined amount of a double distilled water to get a mixture; and
heating the mixture at a predetermined temperature to form a hot aqueous phase, wherein the predetermined temperature is 85°C.

4. The method as claimed in claim 1, wherein the step of formulating the nanoemulsion comprises:
adding the aqueous phase to the oil phase to form a mixture;
homogenizing the mixture at a predetermined speed and for a predetermined time to form a coarse and a hot oil-in-water emulsion, wherein the predetermined speed is 2000 rpm and the predetermined time is 30 minutes;
ultrasonicated the coarse and the hot oil-in-water emulsion for 20 minutes to obtain the drug loaded solid-lipid nanoparticle; and
cooling the drug loaded solid-lipid nanoparticle at room temperature.
5. The method as claimed in claim 1, wherein the step of incorporating the nanoemulsion into the hydrogel comprises:
dispersing a predetermined amount of a gel in a mixture of a hot ultrapure water and glycerol, wherein the hydrogel is carbopol;
allowing the hydrogel to swell overnight;
dissolving the nanoemulsion into the hydrogel to form a mixture;
mixing the mixture in a high speed stirrer while adjusting a pH to form a topical gel, wherein the mixture is mixed at a speed of 1000 rpm for 5 minutes, and wherein the pH is adjusted to 7 by adding triethanol amine dropwise; and
storing the topical gel in a container at 4°C, wherein the gel includes acyclovir loaded solid-lipid nanoparticles.
6. A solid-lipid nanoparticle comprising:
a drug, wherein the drug is an antiviral drug, wherein the drug is acyclovir.
7. The solid-lipid nanoparticle as claimed in claim 6, wherein the solid lipid nanoparticle has a particle size distribution of 10.10 nm to 105.7 nm.
8. The solid-lipid nanoparticle as claimed in claim 6, wherein the solid-lipid nanoparticle has a particle size of 47.81 nm.
9. The solid-lipid nanoparticles as claimed in claim 6, wherein the solid-lipid nanoparticle has a uniform spherical shape.
10. A pharmaceutical formulation for a drug delivery comprises:

a plurality of solid-lipid nanoparticles loaded with a drug, wherein the drug is an antiviral drug, wherein the drug is acyclovir.

11. The formulation as claimed in claim 10, wherein the formulation is in the form of a gel.
12. The formulation as claimed in claim 10, wherein the formulation is a topical gel.
13. The formulation as claimed in claim 10, wherein the formulation is used in the treatment of topical viral disease.



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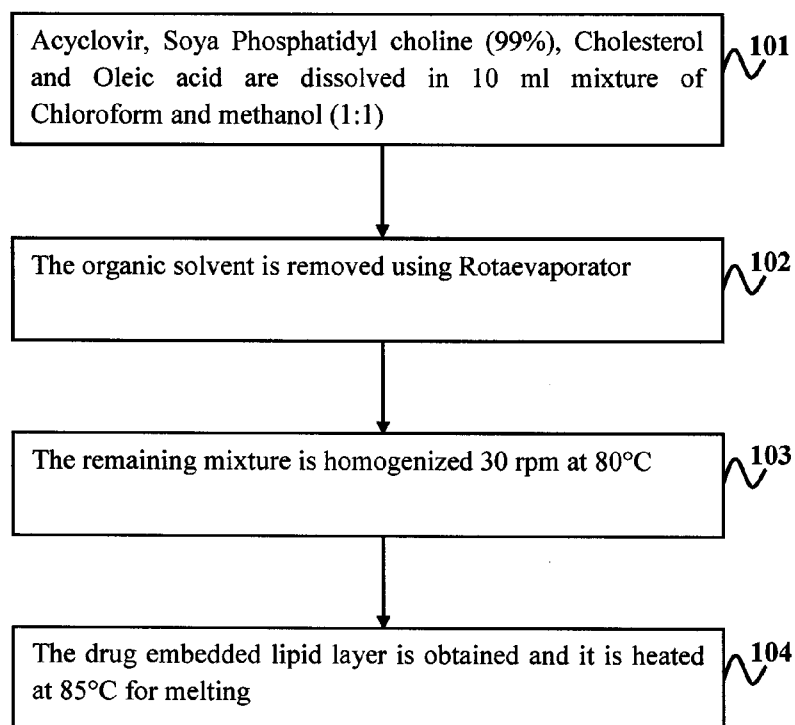
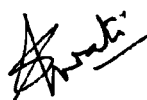


FIG. 1


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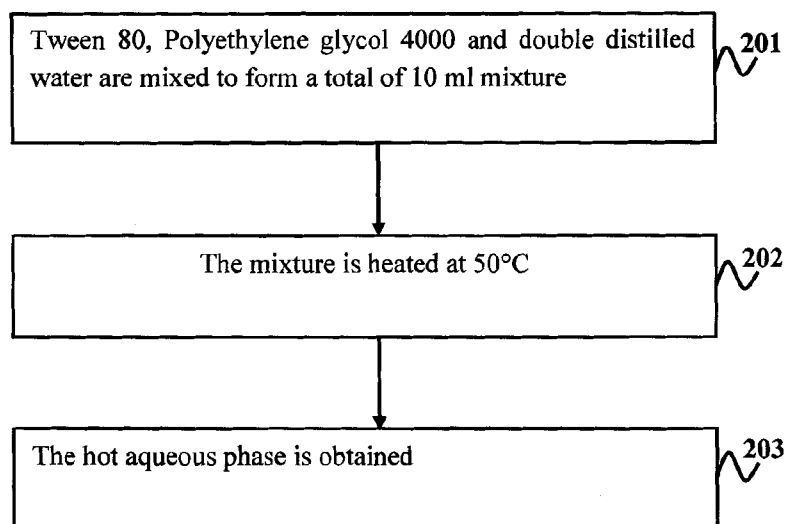


FIG.2

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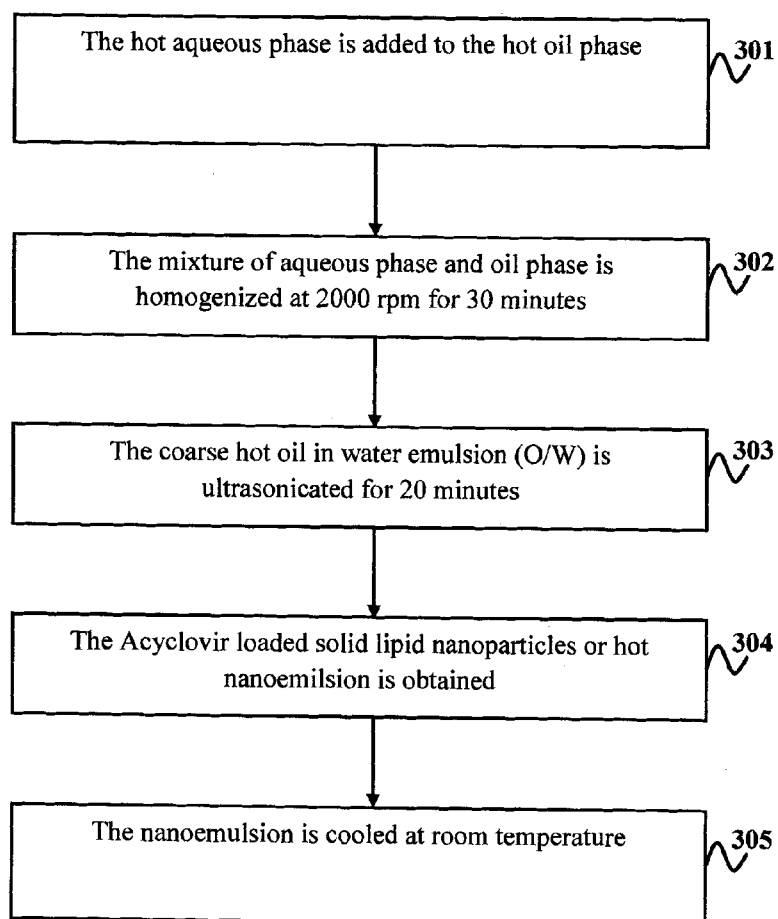
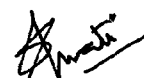


FIG.3


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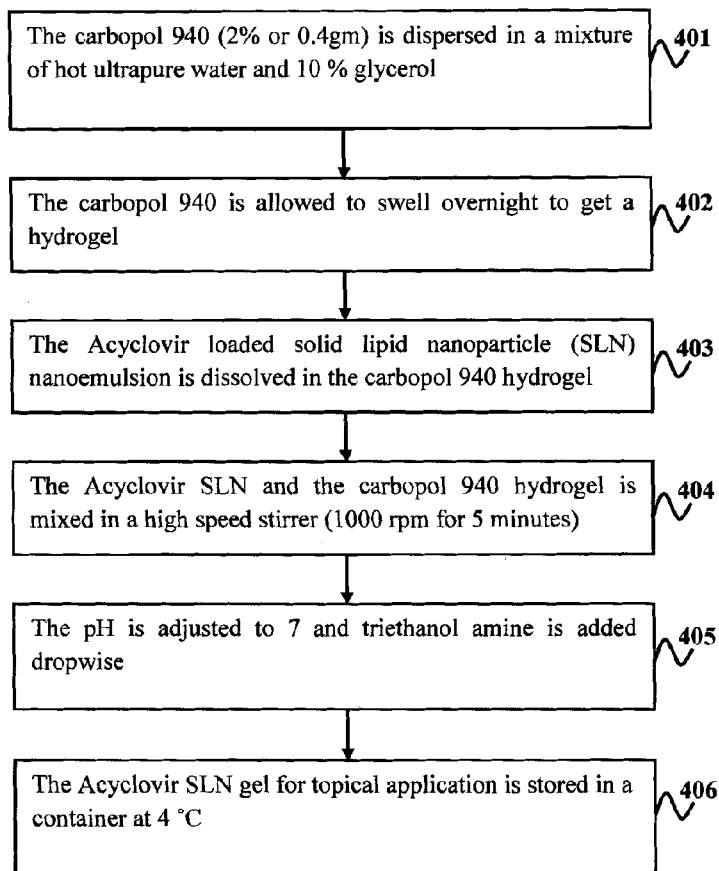
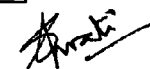


FIG.4


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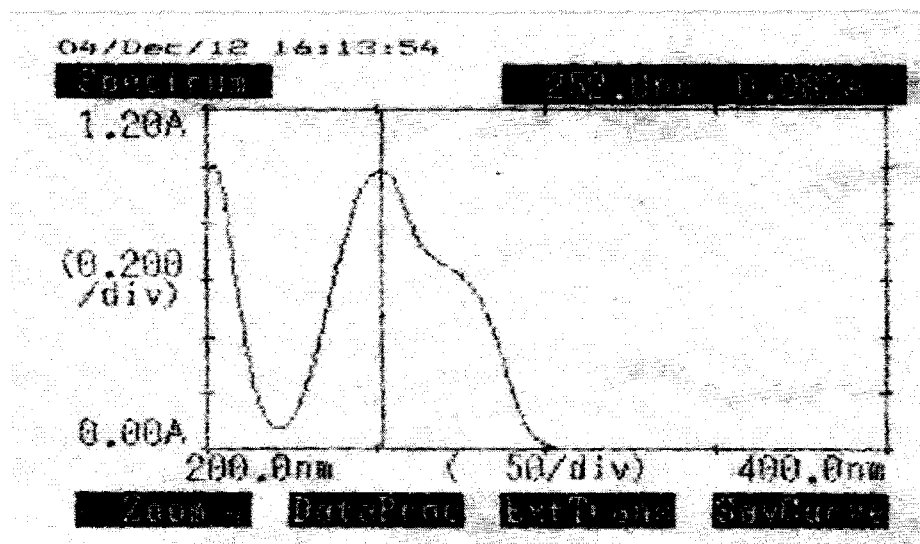


FIG.5A

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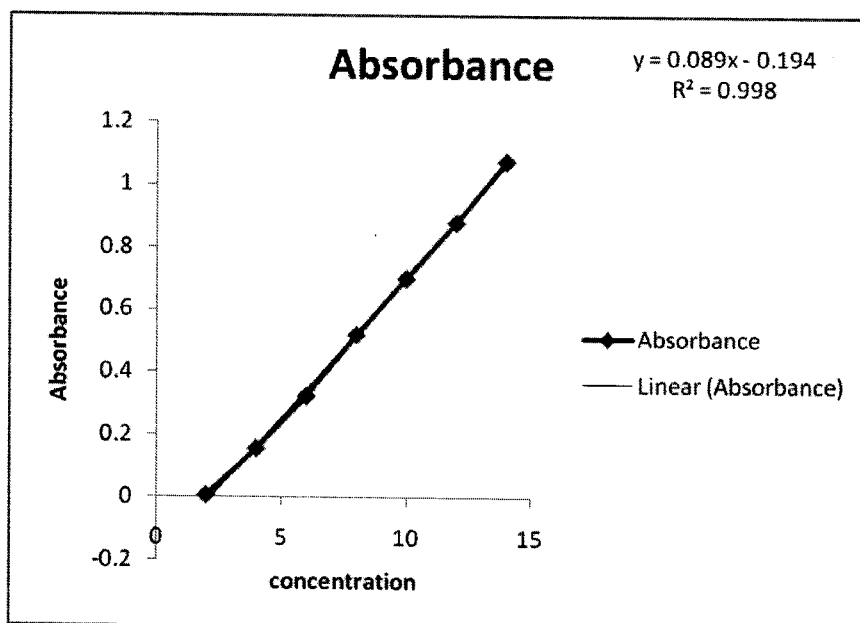


FIG.6


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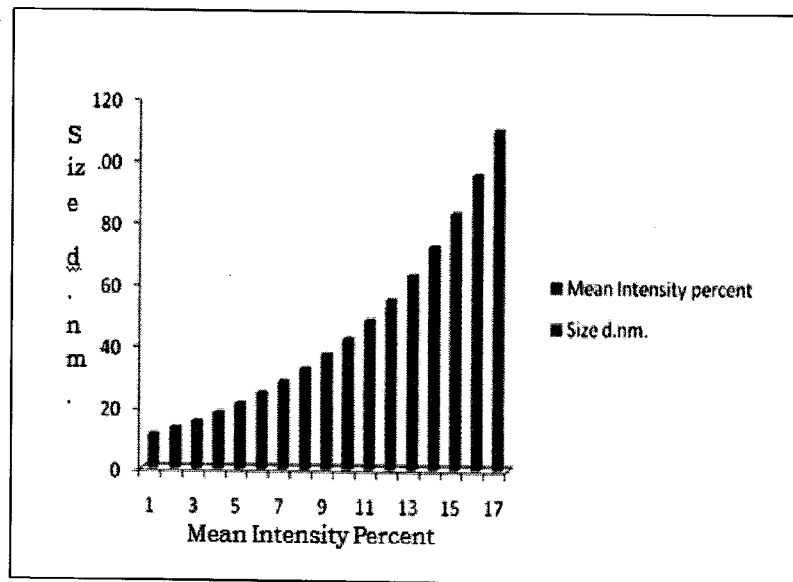


FIG.7A

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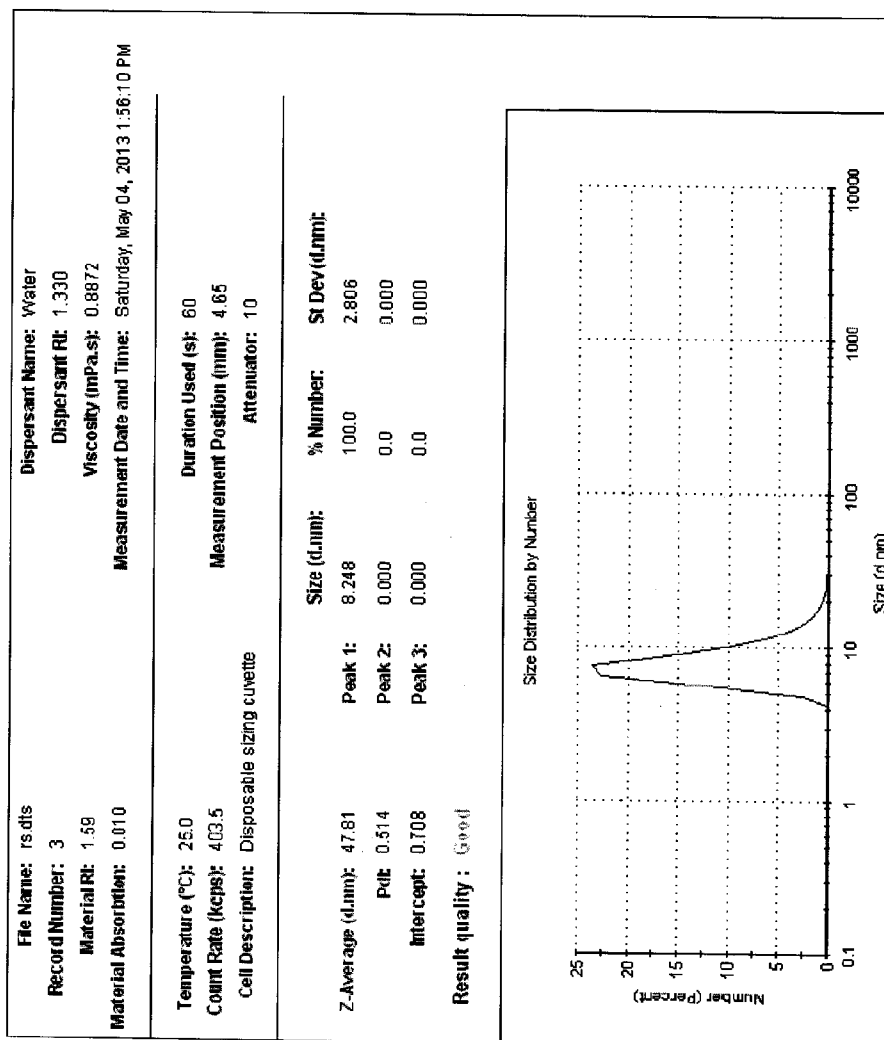


FIG.7B

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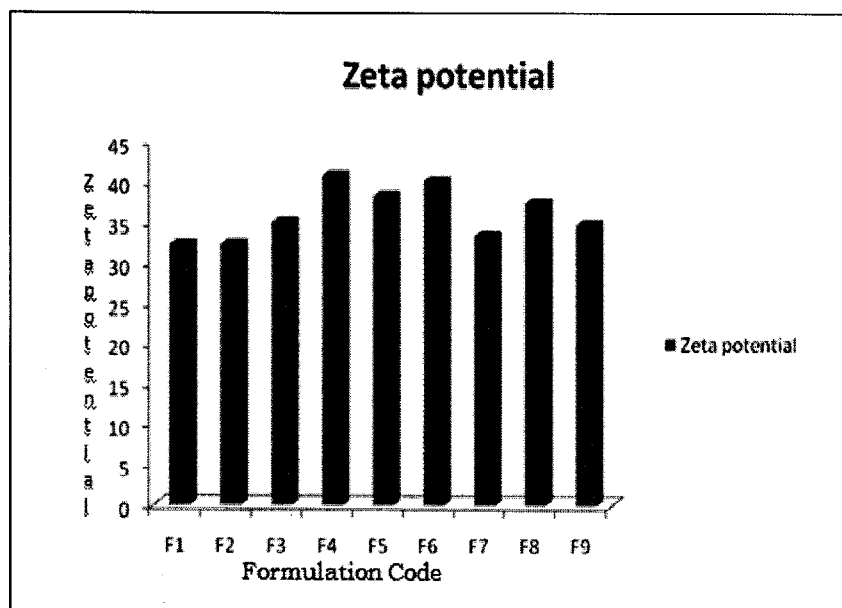


FIG.8A

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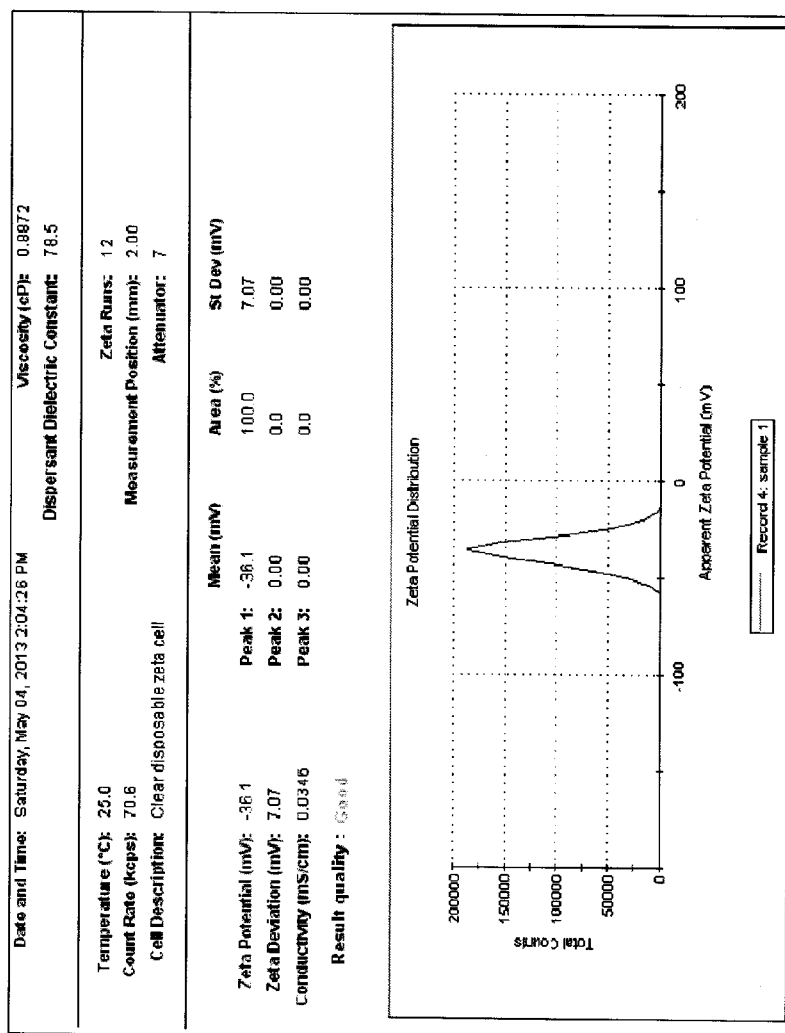


FIG.8B

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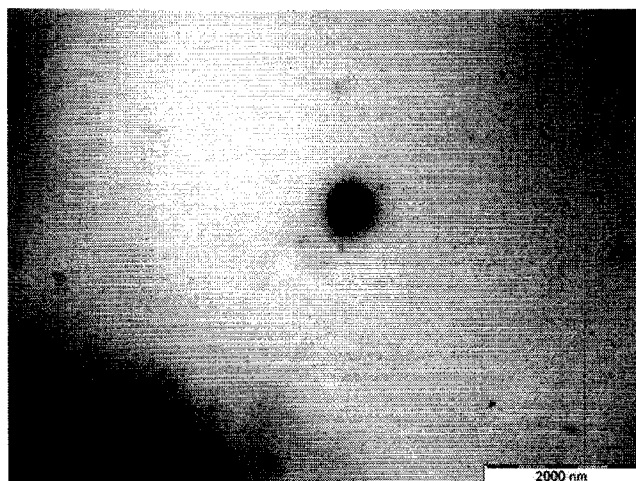
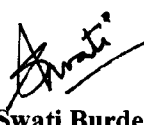


FIG. 9A



FIG.9B



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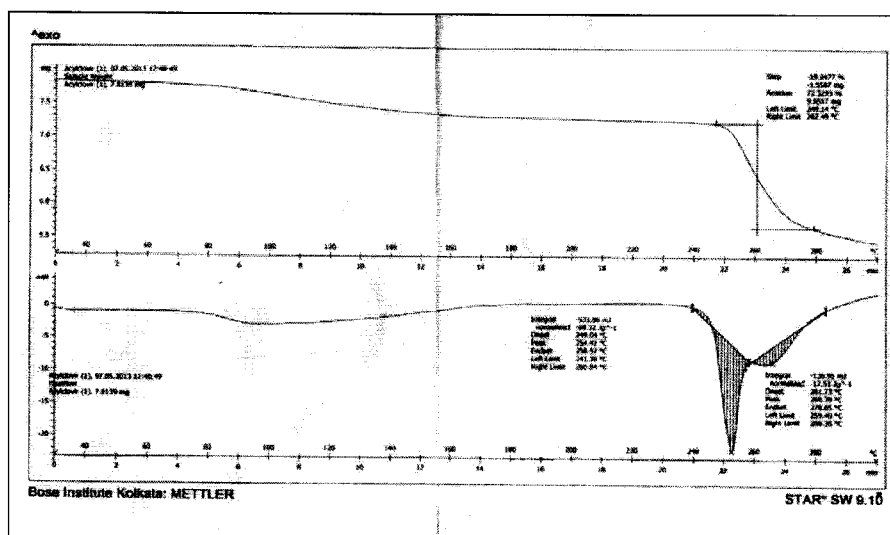


FIG.10A

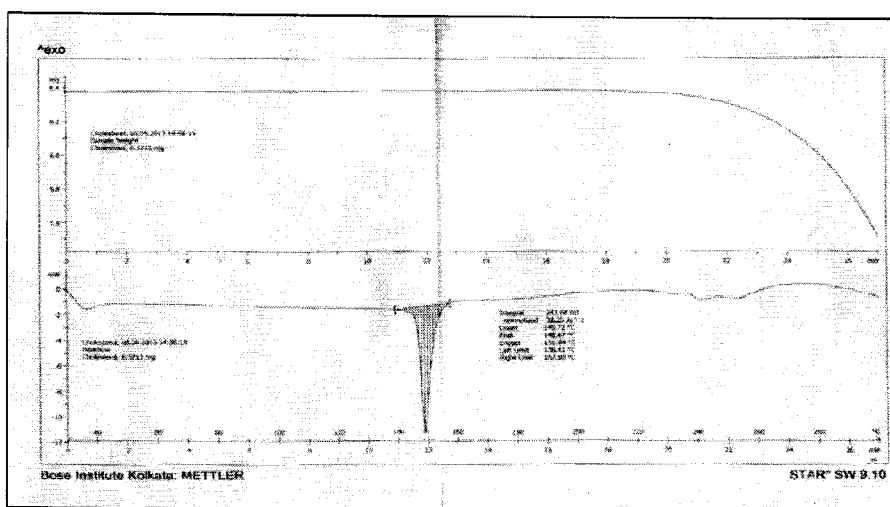


FIG.10B

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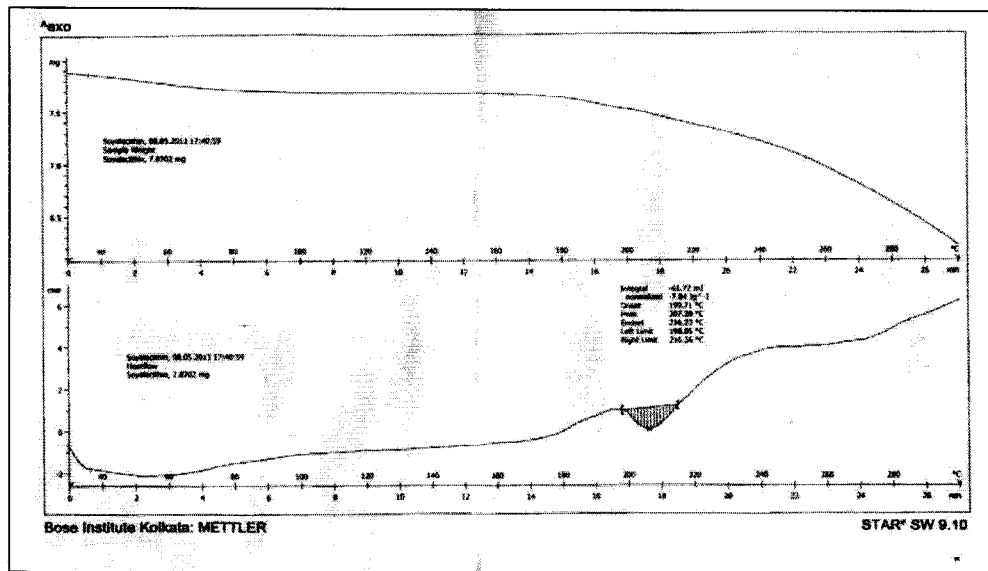


FIG.10C

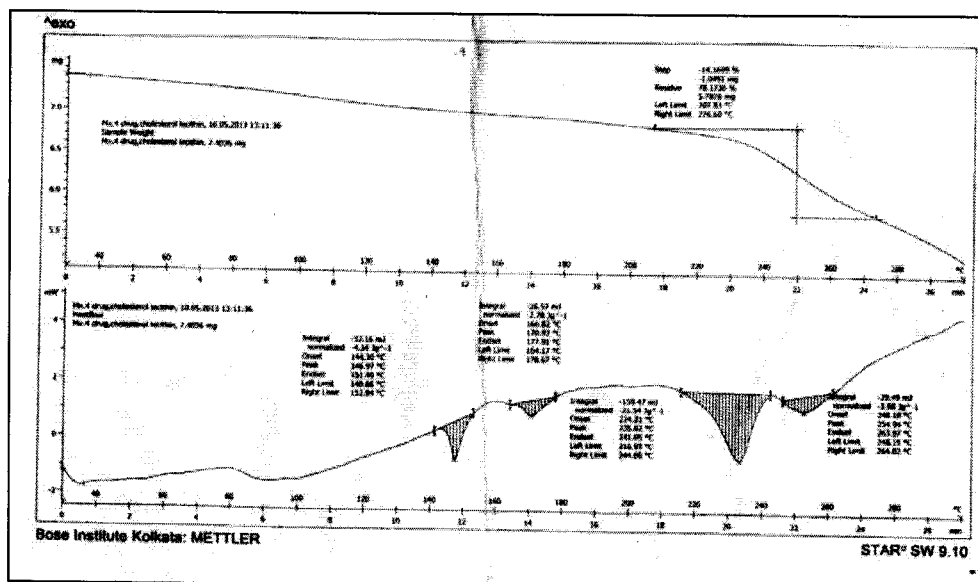


FIG.10D

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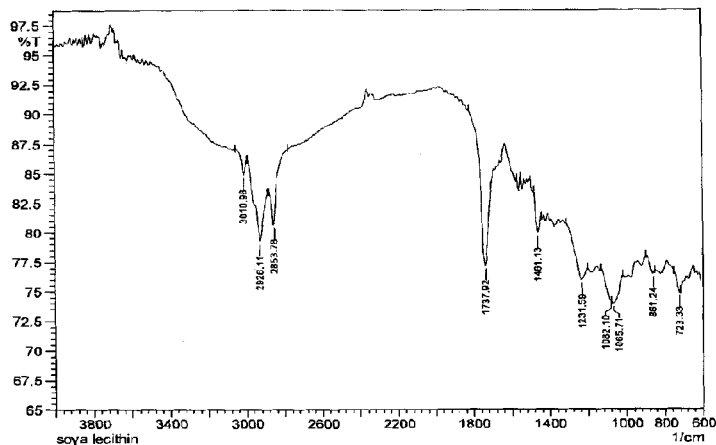


FIG.11A

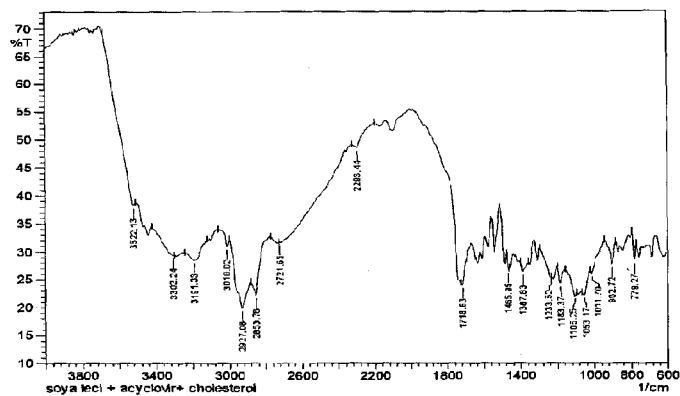


FIG.11B

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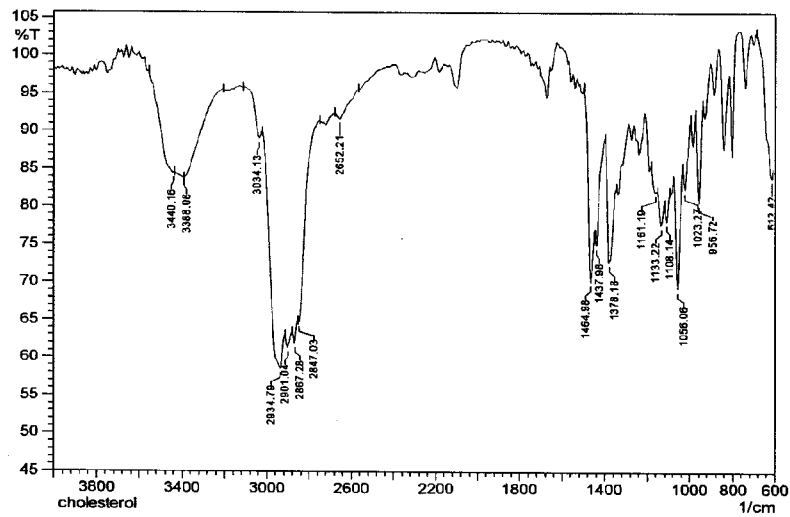


FIG.11C

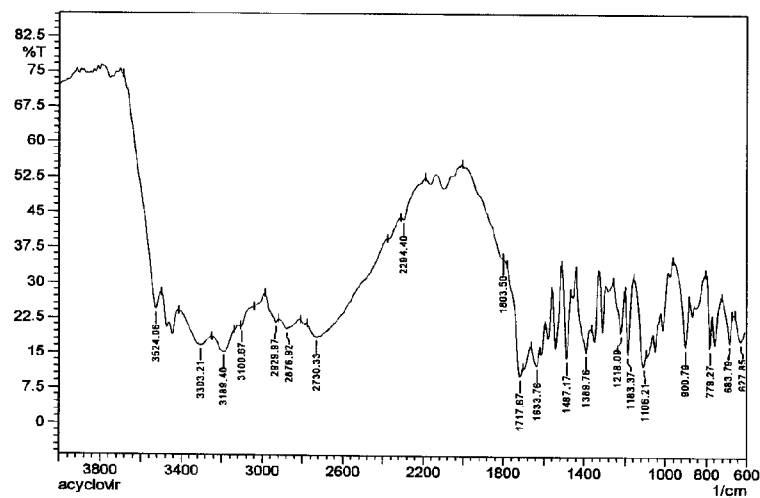


FIG.11D

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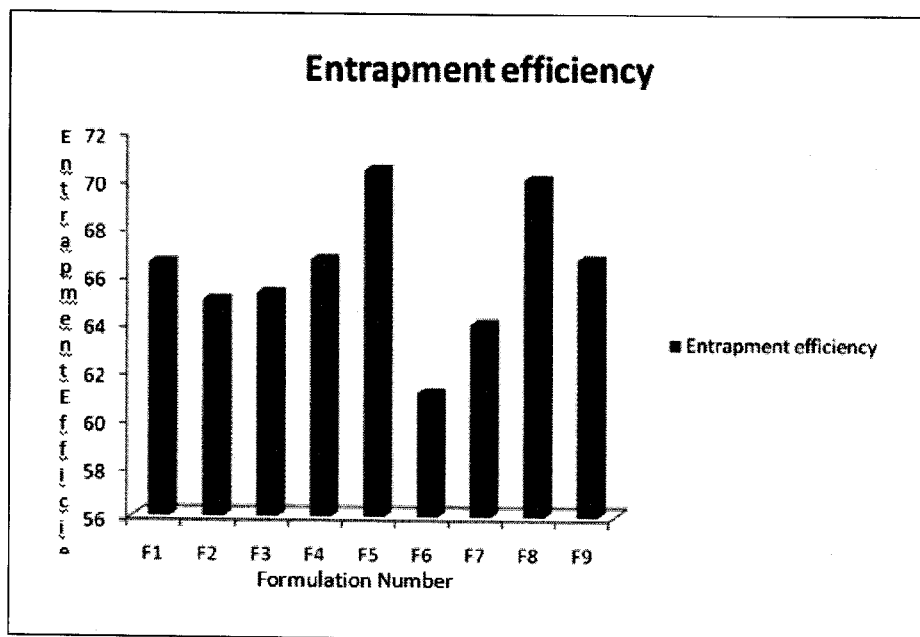


FIG.12


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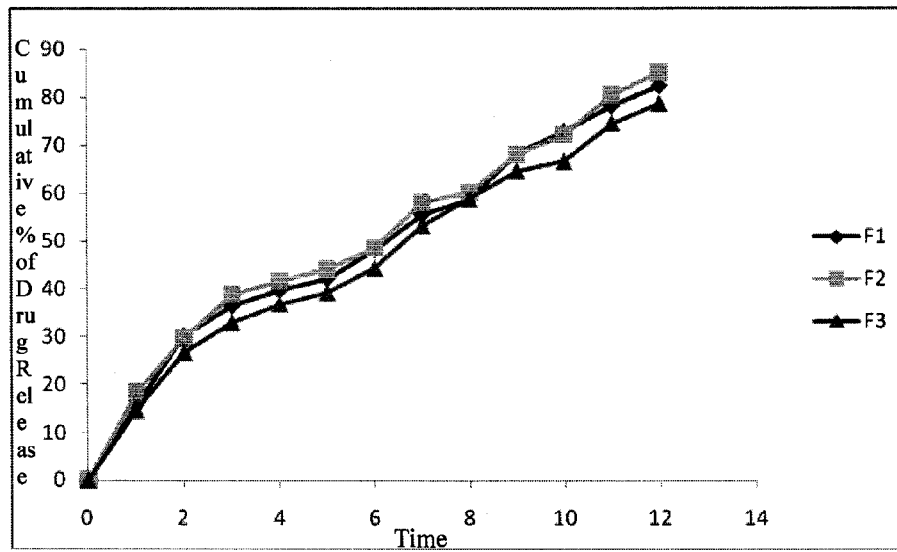


FIG.13A

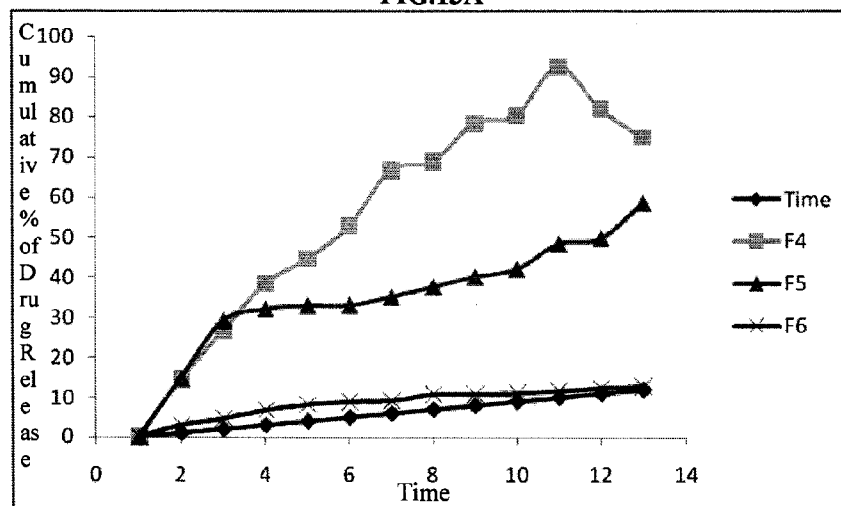


FIG.13B

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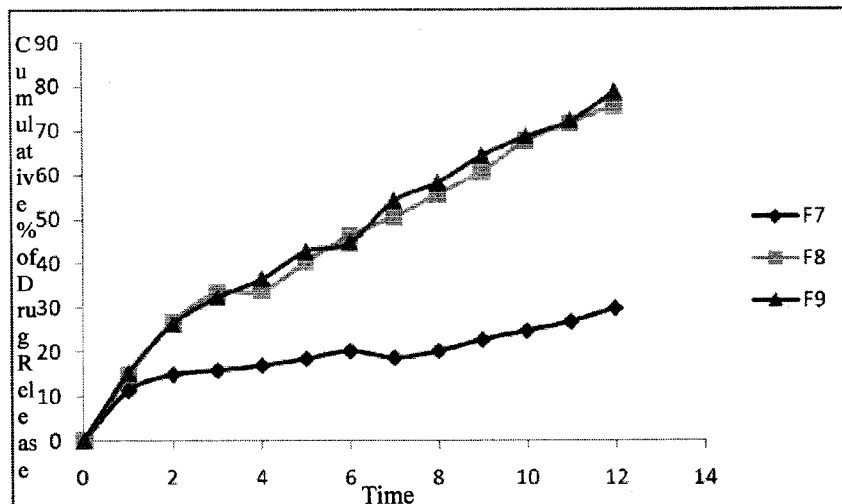


FIG.13C

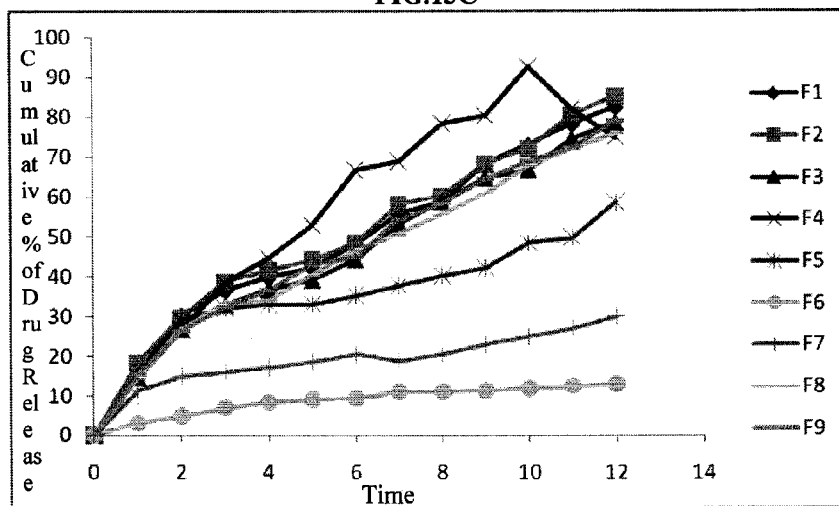


FIG.13D

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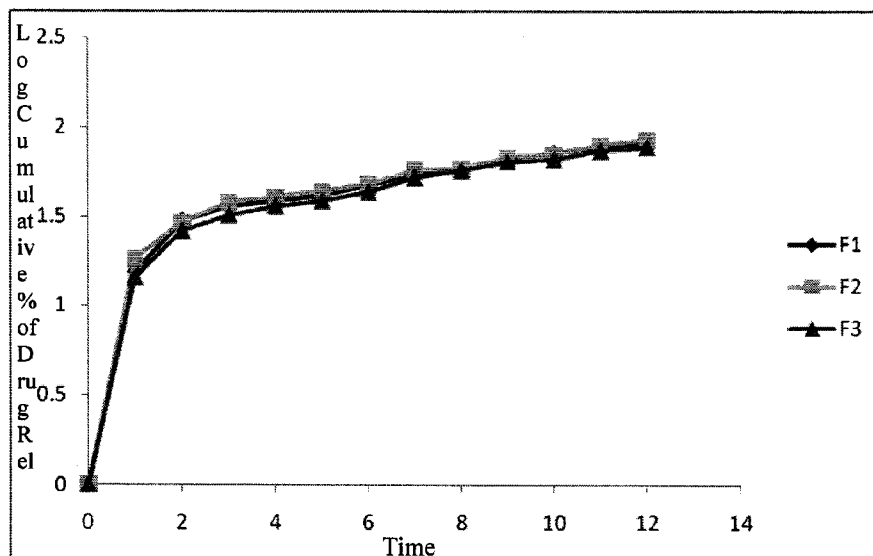


FIG.14A

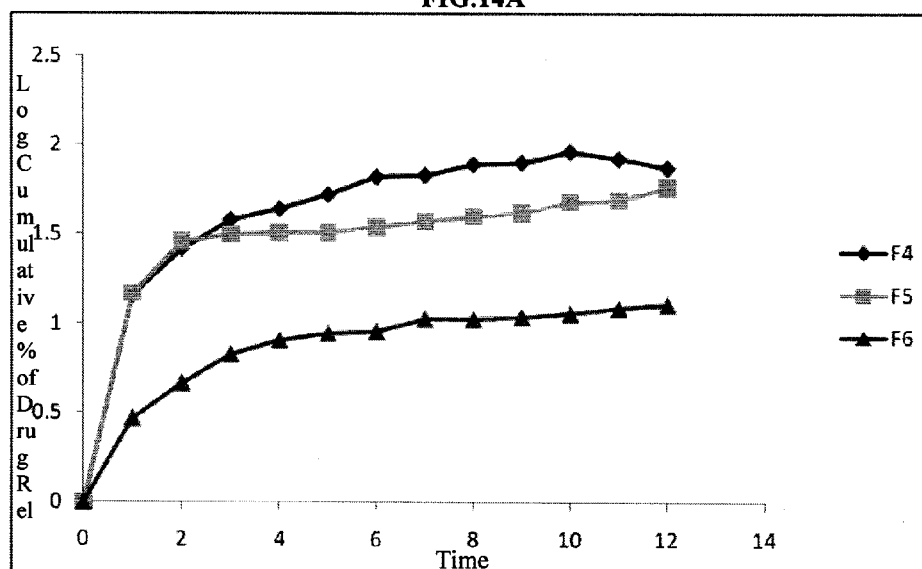


FIG.14B


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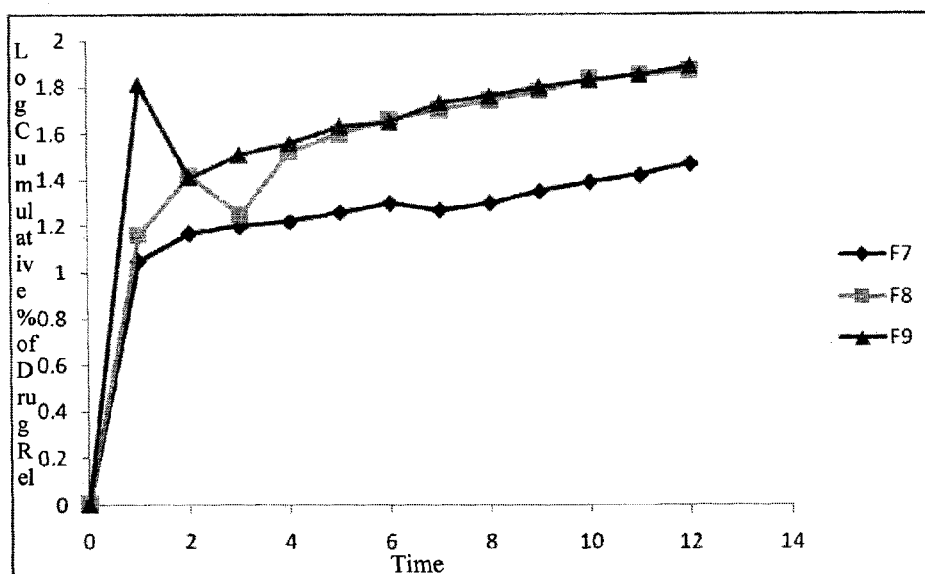


FIG.14C

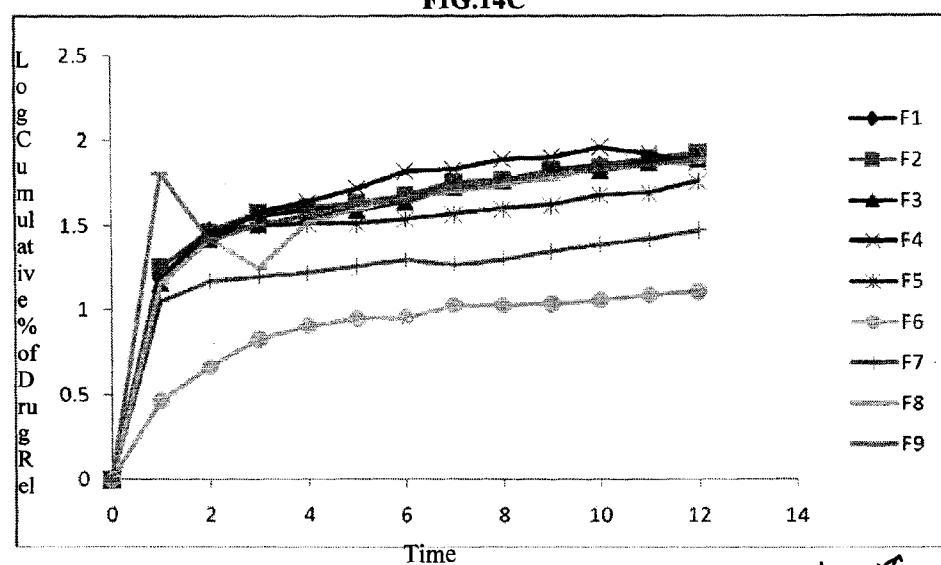


FIG.14D

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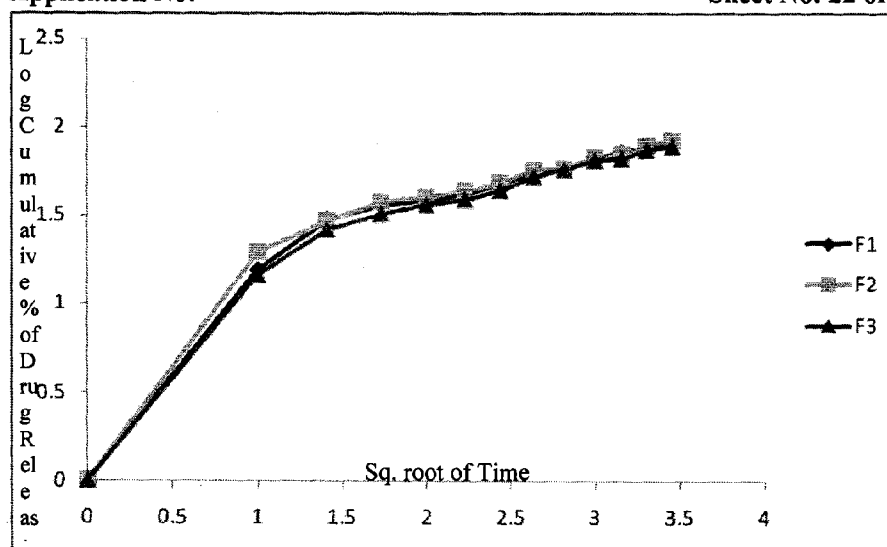


FIG.15A

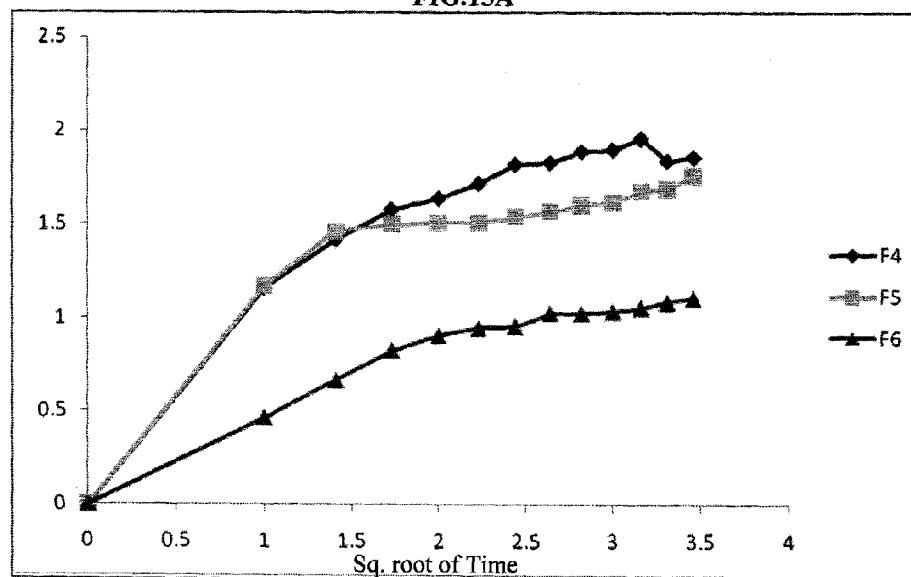


FIG.15B

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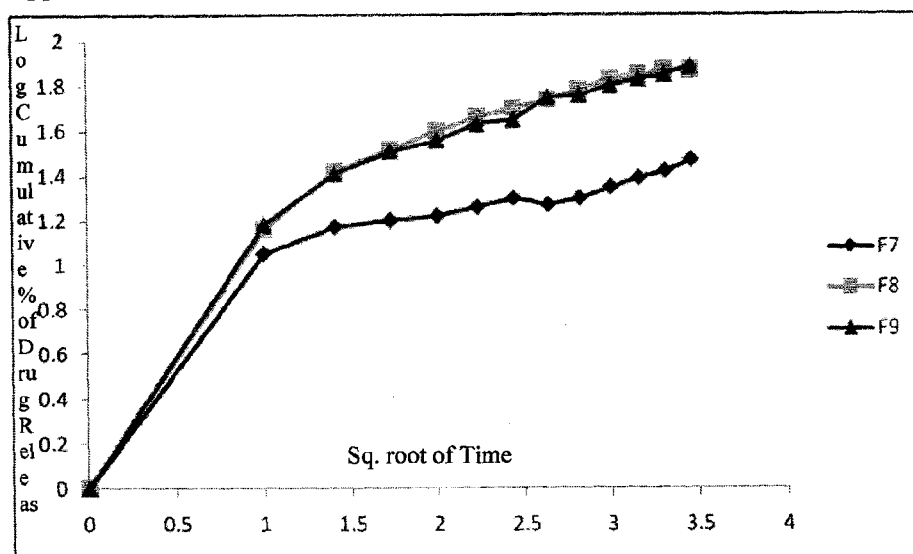


FIG.15C

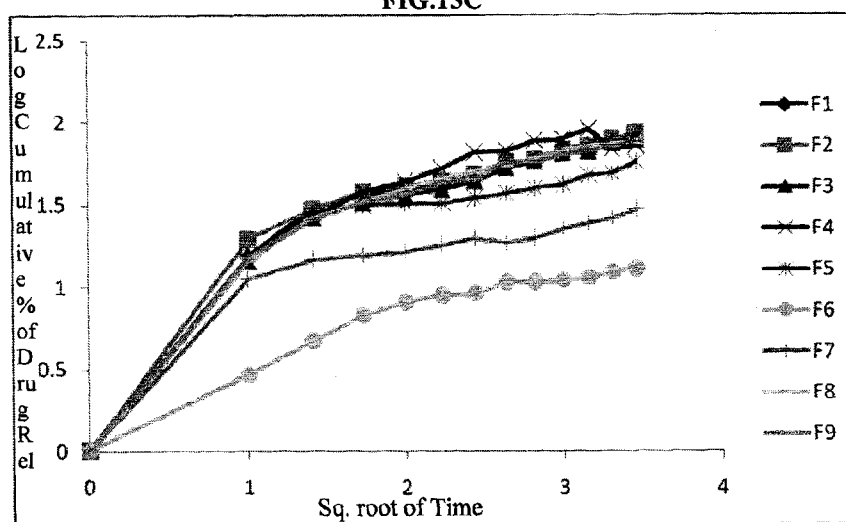


FIG.15D

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A) TECHNICAL FIELD

[0001] The present invention generally relates to the field of therapeutics and particularly relates to nano-medicine. The present invention more particularly relates to a method for preparing a pharmaceutical formulation comprising a drug loaded solid-lipid nanoparticles gel used for topical application. The present invention also relates to a pharmaceutical formulation for treating topical viral disease.

B) BACKGROUND OF THE INVENTION

[0002] The nanotechnology is the science of matter and material that deals with the small colloidal particles that are made of non-biodegradable and biodegradable polymers. The diameter of these particles varies and is less than 100 nm. The word “*nano*” is derived from the Latin word, which means dwarf (1nm= 10^{-9} m). The nano-medicine deals with comprehensive monitoring, controlling, constructing, repairing, defense and improving the human biological system at molecular level using engineered nanostructures and nano-devices. The pharmaceutical nanotechnology has provided more fine tuned diagnosis and focused treatment of diseases. The nanotechnology helps in the detection of antigens associated with the diseases such as cancer, diabetes mellitus, neurodegenerative disorders as well as detecting the virus associated with many infections.

[0003] The nanoparticles are defined as particles having a diameter less than 100 nm. The size of the nanoparticles enables increased bioavailability of drugs, dosage proportionality, decreased toxicity of drugs and smaller dosage form with stability. Earlier, non-biodegradable polymeric system was used as nanotechnology for drug delivery. But due to chronic toxicity and immunological responses towards the non-biodegradable polymers their use in therapeutics is not useful. Hence, the biodegradable polymers were used for the preparation of these nanoparticles. The polymeric nanoparticles are used to carry drugs or proteinaceous substances such as antigen or antibody inside the body to

the target site. The drugs are added during the preparation of the nanoparticles or to previously prepared nanoparticles. The nanoparticles are designed in various forms such as nanospheres, nanocapsules, nanocrystals or nano-particulates for carrying the therapeutic agents. The nanoparticles are classified as gold nanoparticles, magnetic nanoparticles, ceramic nanoparticles, protein nanoparticles and solid-lipid nanoparticles.

[0004] The Solid lipid nanoparticles (SLN) are a new generation of submicron sized lipid emulsions where the liquid lipid (oil) has been substituted with a solid lipid. SLNs offer unique properties such as smaller size, larger surface area, higher drug loading and the interaction of phases at the interfaces. The SLNs are useful for their potential in improving the performance of pharmaceuticals, nutraceuticals and other materials.

[0005] The solid-lipid nanoparticles have emerged as a variable substitute to liposomes as drug carriers. The successful implementation of nanoparticles for the drug delivery depends upon their ability to penetrate through the several anatomical barriers, sustained release of their content and their stability in the nanometer size. To overcome these limitations of polymeric nanoparticles, lipids have been put forward as an alternative carrier, particularly for lipophilic pharmaceuticals. These lipid nanoparticles are defined as “Solid lipid nanoparticles”.

[0006] The Solid lipid nanoparticles (SLN) possess a solid lipid core matrix that can solubilize lipophilic molecules. The core has a monolayer of phospholipid coating and contains the drug dispersed or dissolved in the lipid matrix. The lipid core is stabilized by surfactants or emulsions. The Solid lipid nanoparticles are in the range of submicron size such as 50-100 nm and are composed of physiologically tolerated lipid component which are in the solid state at room temperature.

[0007] The solid lipid nanoparticles have several potential applications such as drug delivery, clinical medicine and research and development of new therapeutics. The ability of SLN to incorporate drugs offers a new prototype in

the drug delivery that could hold great promise for attaining the bioavailability enhancement along with the controlled and site specific drug delivery.

[0008] The solid lipid nanoparticles (SLN) have many advantages such as good biocompatibility, lower toxicity, better delivery of lipophilic drugs, physical stability, smaller size, larger surface area, high drug loading capacity and the interaction of phases at the interfaces. Further the SLNs are attracting major attention as novel colloidal drug carrier for intravenous application. The SLNs are a promising sustained release and drug targeting system for lipophilic antiviral drugs. Further the SLNs have no bio-toxicity of the carrier wherein the lipophilic and the hydrophilic drugs can be incorporated easily.

[0009] There are several methods for the preparation of solid lipid nanoparticles. The “ultrasonication or high speed homogenization” method is used for SLN preparation but this method has certain disadvantages. There is potential metal contamination and physical instability like particle growth upon storage. Another method for preparing the SLN is the “solvent evaporation” method, but this method is extremely energy consuming. Also there are chances of biomolecular damage. Another method for SLN preparation is “microemulsion” based method. This method is labor intensive and leads to low nanoparticle concentrations. The “spray drying” method which is yet another method for SLN preparation leads to particle aggregation due to high temperature and leads to melting of the prepared nanoparticles.

[0010] Hence, there is a need for a method and system for preparing solid lipid nanoparticles which is non-damaging to the biomolecules and is not labor and energy-intensive. Moreover, there is a need for a method which provides high yield concentrations of the nanoparticles without damaging them.

C) OBJECTIVES OF THE INVENTION

[0011] The primary object of the present invention is to provide a method and system for preparing topical gel comprising solid-lipid nanoparticles.

[0012] Another object of the present invention is to provide a solid-lipid nanoparticle gel of an antiviral drug, wherein the antiviral drug is acyclovir.

[0013] Yet another object of the present invention is to provide a gel based pharmaceutical formulation made up of solid-lipid nanoparticles loaded with a drug called acyclovir for topical application over the skin.

[0014] Yet another object of the present invention is to provide a gel based formulation comprising solid-lipid nanoparticles for topical application having better permeation for the treatment of topical diseases.

[0015] Yet another object of the present invention is to provide a "hot homogenization method" for the preparation of a pharmaceutical formulation comprising topical solid-lipid nanoparticles loaded with a drug called acyclovir which is an antiviral drug.

[0016] Yet another object of the present invention is to provide a method of preparing solid-lipid nanoparticles using cholesterol and soya lecithin as a lipid phase and oleic acid and PEG 4000 as a surfactant phase.

[0017] Yet another object of the present invention is to provide solid-lipid nanoparticles with controlled and targeted drug release.

[0018] Yet another object of the present invention is to provide solid-lipid nanoparticles with ability to incorporate both hydrophilic and hydrophobic drugs.

[0019] Yet another object of the present invention is to provide solid-lipid nanoparticles which protect drug molecules from outer environment i.e. moisture, air, light, etc. and yet can be lyophilized.

[0020] These and other objects and advantages of the present invention will become readily apparent from the following detailed description taken in conjunction with the accompanying drawings.

D) SUMMARY OF THE INVENTION

[0021] The various embodiments of the present invention provide a method for preparing an acyclovir drug loaded solid-lipid nanoparticle gel. The embodiments herein also provide a pharmaceutical formulation for treating topical viral disease.

[0022] According to one embodiment of the present invention, a hot homogenization method for synthesizing an acyclovir drug loaded solid-lipid

nanoparticle gel is provided. The hot homogenization method for synthesizing an acyclovir drug loaded solid-lipid nanoparticle gel comprises preparing an oil phase, preparing an aqueous phase, formulating a nanoemulsion and incorporating the nanoemulsion into a hydrogel. The nanoemulsion consists a drug loaded solid-lipid nanoparticles. The drug is acyclovir.

[0023] The step of preparing the oil phase comprises mixing a drug, a phospholipid, a cholesterol and a fatty acid to form a mixture. The drug is acyclovir. The phospholipid is soya phosphatidyl choline (99%) and the fatty acid is oleic acid. The mixture is dissolved in a pre-determined amount of a solution. The solution consists of organic solvents. The organic solvents are chloroform and methanol mixed in a ratio of 1:1. The organic solvents are removed to obtain a drug embedded lipid layer. The organic solvents are removed by using a Rota-evaporator used at a predetermined temperature and a pre-determined speed. The pre-determined temperature is 80°C and the pre-determined speed is 30 rpm. The drug embedded lipid layer is heated at a predetermined temperature to obtain the oil phase. The predetermined temperature is 85°C.

[0024] The step of preparing the aqueous phase comprises mixing a predetermined amount of Tween 80, a predetermined amount of polyethylene glycol 4000 and a predetermined amount of a double distilled water to get a mixture. The mixture is heated at a predetermined temperature to form a hot aqueous phase. The predetermined temperature is 85°C.

[0025] The step of formulating the nanoemulsion comprises adding the aqueous phase to the oil phase to form a mixture. The mixture is homogenized at a predetermined speed and for a predetermined time to form a coarse and a hot oil-in-water emulsion. The predetermined speed is 2000 rpm and the predetermined time is 30 minutes. The coarse and the hot oil-in-water emulsion is ultrasonicated for 20 minutes to obtain the drug loaded solid-lipid nanoparticle. The drug loaded solid-lipid nanoparticle is cooled at room temperature.

[0026] The step of incorporating the nanoemulsion into the hydrogel comprises dispersing a predetermined amount of a gel in a mixture of a hot ultrapure water

and glycerol. The hydrogel is carbopol. The hydrogel is allowed to swell overnight. The nanoemulsion is dissolved into the hydrogel to form a mixture. The mixture is mixed in a high speed stirrer while adjusting a pH to form a topical gel. The mixture is mixed at a speed of 1000 rpm for 5 minutes and the pH is adjusted to 7 by adding triethanol amine drop wise. The topical gel is stored in a container at 4°C. The gel includes acyclovir loaded solid-lipid nanoparticles.

[0027] According to another embodiment of the present invention, a solid-lipid nanoparticle comprising a drug. The drug is embedded in the solid-lipid nanoparticle. The drug is an antiviral drug, and the drug is acyclovir. The solid lipid nanoparticle has a particle size distribution of 10.10 nm to 105.7 nm. The solid-lipid nanoparticle has a particle size of 47.81 nm. The solid-lipid nanoparticle has a uniform spherical shape.

[0028] According to another embodiment of the present invention, a pharmaceutical formulation for a drug delivery comprises a plurality of solid-lipid nanoparticles loaded with a drug. The drug is an antiviral drug and the drug is acyclovir. The formulation is in the form of a gel. The formulation is a topical gel. The formulation is used in the treatment of topical viral disease.

[0029] These and other aspects of the embodiments herein will be better appreciated and understood when considered in conjunction with the following description and the accompanying drawings. It should be understood, however, that the following descriptions, while indicating preferred embodiments and numerous specific details thereof, are given by way of illustration and not of limitation. Many changes and modifications may be made within the scope of the embodiments herein without departing from the spirit thereof, and the embodiments herein include all such modifications.

E) BRIEF DESCRIPTION OF THE DRAWINGS

[0030] The other objects, features and advantages will occur to those skilled in the art from the following description of the preferred embodiment and the accompanying drawings in which:

[0031] **FIG. 1** is a flow chart illustrating the various steps involved in the preparation of an oil phase used for the synthesis of the solid lipid nanoparticles, according to an embodiment of the present invention.

[0032] **FIG. 2** is a flow chart illustrating the various steps involved in the preparation of an aqueous phase used for the synthesis of the solid lipid nanoparticles, according to an embodiment of the present invention.

[0033] **FIG. 3** is a flowchart illustrating the various steps involved in the preparation of the nanoemulsion comprising the solid lipid nanoparticles loaded with the antiviral drug, according to an embodiment of the present invention.

[0034] **FIG.4** is a flow chart illustrating the various steps involved in the method of preparing the acyclovir loaded solid lipid nanoparticles gel for topical application, according to an embodiment of the present invention.

[0035] **FIG.5A** and **FIG.5B** are graphs illustrating the λ max of the drug sample, according to an embodiment of the present invention.

[0036] **FIG. 6** is a graph illustrating the standard calibration curve of the acyclovir drug at pH 7.4, according to an embodiment of the present invention.

[0037] **FIG. 7A** and **FIG. 7B** are graphs illustrating the particle size distribution of the acyclovir loaded solid lipid nanoparticles (SLN), according to an embodiment of the present invention.

[0038] **FIG. 8A** and **FIG.8B** are graphs illustrating the zeta potential of the acyclovir drug loaded solid lipid nanoparticles (SLN), according to an embodiment of the present invention.

[0039] **FIG. 9A** and **FIG. 9B** are Transmission Electron Microscopic (TEM) images of the acyclovir drug loaded solid lipid nanoparticles (SLN), according to an embodiment of the present invention.

[0040] **FIG. 10A** is a thermogram illustrating the Differential Scanning Calorimetry (DSC) result of the acyclovir drug for Acyclovir-polymer interaction in the solid lipid nanoparticles, according to an embodiment of the present invention.

[0041] **FIG. 10B** is a thermogram illustrating the Differential Scanning Calorimetry (DSC) result of the cholesterol for Acyclovir-polymer interaction in

the solid lipid nanoparticles, according to an embodiment of the present invention.

[0042] FIG. 10C is a thermogram illustrating the Differential Scanning Calorimetry (DSC) result of the soya lecithin for Acyclovir-polymer interaction in the solid lipid nanoparticles, according to an embodiment of the present invention.

[0043] FIG. 10D is a thermogram illustrating the Differential Scanning Calorimetry (DSC) result of the acyclovir drug, cholesterol and the soya lecithin for the Acyclovir-polymer interaction in the solid lipid nanoparticles, according to an embodiment of the present invention.

[0044] FIG. 11A is a graph illustrating the Fourier Transform Infra-Red Spectroscopy (FTIR) result of the soya lecithin for the Acyclovir-polymer interaction in the solid lipid nanoparticles, according to an embodiment of the present invention.

[0045] FIG. 11B is a graph illustrating the Fourier Transform Infra-Red Spectroscopy (FTIR) result of the acyclovir drug, cholesterol and the soya lecithin for Acyclovir-polymer interaction in the solid lipid nanoparticles, according to an embodiment of the present invention.

[0046] FIG. 11C is a graph illustrating the Fourier Transform Infra-Red Spectroscopy (FTIR) result of the cholesterol for the Acyclovir-polymer interaction in the solid lipid nanoparticles, according to an embodiment of the present invention.

[0047] FIG. 11D is a graph illustrating the Fourier Transform Infra-Red Spectroscopy (FTIR) result of the acyclovir drug for Acyclovir-polymer interaction in the solid lipid nanoparticles, according to an embodiment of the present invention.

[0048] FIG. 12 is a bar graph illustrating the drug entrapment efficiency of the solid lipid nanoparticles (SLN), according to an embodiment of the present invention.

[0049] **FIG.13A-13D** are the graphs illustrating the zero order release kinetics of the various formulations i.e. F1 to F3, F4 to F6, F7 to F9 and F1 to F9 respectively, according to one embodiment of the present invention.

[0050] **FIG.14A-14D** are the graphs illustrating the first order release kinetics of formulations F1 to F3, F4 to F6, F7 to F9 and F1 to F9 respectively, according to one embodiment of the present invention.

[0051] **FIG.15A-15D** are the graphs illustrating the Higuchi release kinetics of formulations F1 to F3, F4 to F6, F7 to F9 and F1 to F9 respectively, according to one embodiment of the present invention.

[0052] Although the specific features of the present invention are shown in some drawings and not in others. This is done for convenience only as each feature may be combined with any or all of the other features in accordance with the present invention.

F) DETAILED DESCRIPTION OF THE INVENTION

[0053] In the following detailed description, reference is made to the accompanying drawings that form a part hereof, and in which the specific embodiments that may be practiced is shown by way of illustration. These embodiments are described in sufficient detail to enable those skilled in the art to practice the embodiments and it is to be understood that the logical, mechanical and other changes may be made without departing from the scope of the embodiments. The following detailed description is therefore not to be taken in a limiting sense.

[0054] The various embodiments of the present invention provide a method for synthesizing the solid lipid nanoparticles (SLN) by a hot homogenization method. The method comprises an oil phase preparation, an aqueous phase preparation, a nanoemulsion preparation and an incorporation of a drug loaded SLN into a suitable hydrogel for topical use. The nanoemulsion comprises solid-lipid nanoparticles loaded with a suitable drug. According to an embodiment of the present invention, the suitable drug is an antiviral drug, wherein the drug preferably is acyclovir.

[0055] According to an embodiment of the present invention, the oil phase is synthesized by mixing a sufficient amount of a drug, one or more phospholipid, a sterol compound and one or more fatty acid to form a mixture. The mixture is dissolved in a solution of organic solvents. The organic solvents are evaporated and homogenized to form the oil phase. The oil phase comprises solid-lipid nanoparticles loaded with the drug.

[0056] According to an embodiment of the present invention, the drug is an antiviral drug, preferably the drug is acyclovir. The one or more phospholipid is lecithin or soya phosphatidyl choline, preferably soya phosphatidyl choline (99%). The sterol compound is cholesterol and one or more fatty acid is oleic acid.

[0057] According to an embodiment of the present invention, the aqueous phase is synthesized by mixing a sufficient amount of one or more surfactant and emulsifier and a compound of polyether with a sufficient amount of distilled water to form a mixture. The mixture is further heated at a predetermined temperature to form the aqueous phase. According to an embodiment of the present invention, the one or more surfactant and emulsifier is polysorbate 80 or Tween 80. The compound of polyether is polyethylene glycol 4000.

[0058] According to an embodiment of the present invention, the nanoemulsion is synthesized. The nanoemulsion comprises drug loaded solid-lipid nanoparticles. The drug loaded solid-lipid nanoparticles are acyclovir loaded solid-lipid nanoparticles. In the method of synthesizing the nanoemulsion, the aqueous phase is added to the oil phase to form a mixture. The mixture of the aqueous phase and the oil phase is homogenized for a predetermined time to obtain a coarse hot oil-in-water emulsion (o/w). The emulsion is ultrasonicated to obtain the acyclovir loaded solid lipid nanoparticles.

[0059] According to an embodiment of the present invention, the obtained acyclovir loaded SLNs are further incorporated into a suitable hydrogel for topical application. The hydrogel is preferably carbopol 940. A gel is dispersed in hot solution of ultrapure water and glycerol and allowed to swell overnight to form the hydrogel. The gel preferably is carbopol. The acyclovir loaded SLNs

are dissolved in the hydrogel and mixed in a high speed stirrer while adjusting the pH. The pH is adjusted to 7. The obtained acyclovir SLNs gel for topical application is further stored in a container at 4°C.

EXPERIMENTAL DETAILS

[0060] The solid lipid nanoparticles are evaluated for the parameters. The parameters are particle size and zeta potential, entrapment efficiency and drug loading, surface morphology, drug polymer interaction study, *invitro* skin permeation and stability study of acyclovir loaded SLNs of the present invention.

[0061] The average particle size and polydispersity index are measured by photon correlation spectroscopy at 25°C under a fixed angle of 90°C in disposable polystyrene cuvettes. The zeta potential is measured in folded capillary cells using the nano ZS90 Zetasizer. The measurements are performed in distilled water adjusted with a solution of 0.1 mmol/lit of sodium chloride to a conductivity of 50 us/cm at 25°C. The zeta potential values are calculated using the smoluchowski equation. The entrapment efficiency and the drug loading are calculated by the standard formulae. Further the surface morphology is analyzed by the Transmission Electron Microscope (TEM). The drug polymer interaction study is carried out by Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC) study. The in-vitro skin permeation study is carried out using hairless abdominal skin of wistar goat. The permeation rate of the antiviral drug acyclovir is calculated. The stability study of the acyclovir loaded SLNs is analyzed at 2-8°C for 3 months via clarity, particle size zeta potential and HPLC analysis of the acyclovir.

MATERIALS

[0062] Table 1 below illustrates the materials used in the preparation of the solid lipid nanoparticles, according to the embodiments of the present invention.

Table 1 showing the list of materials used in the synthesis of SLNs

Excipient Name	Excipients Type	Producer
Acyclovir	White, crystalline power	East-India

		Pharmaceuticals.
Soya Lecithin	RM 637 (1 X 100 GMS)	Hi-Media
Cholesterol	RM 335	Hi-Media
Oleic acid	Surfactant	Sigma chemicals Co. Ltd. (USA)
Glycerol monostearate		Loba chemic Pvt. Ltd.
Chloroform	L.R.	S.D. Finechem Ltd.
Methanol	L.R.	S.D. Fine Chem Ltd.
Tween 80	Surfactant	Sigma Chemicals Co. Ltd.
PEG 4000		Wuhan Xinhenglong Technology Co. Ltd.
Carbopol	940	Loba Chemic Pvt. Ltd.(Mumbai)
Pot Dihydrogen Phosphate	L.R.	

[0063] Table 2 below illustrates the instruments used in the preparation of the solid lipid nanoparticles, according to the embodiments of the present invention.

Table 2 showing the instrument requirements

Serial No.	Instruments	Company Name
1.	Electrical Balance	Sartorius GD103, U.S.A.
2.	Franz diffusion cell	Testing Instruments
3.	U.V. – Visible Spectrophotometer	Shimadzu – 1700, Japan
4.	Rotaevaporator	CR-2000 Cyberlab Corporation
5.	PH Meter	Sartorius, GD103, U.S.A

[0064] The acyclovir loaded solid-lipid nanoparticle (SLN) gel used for the topical application is synthesized by a “hot homogenization method”. In the hot homogenization method an oil phase and an aqueous phase are prepared. The oil

phase and the aqueous phase are mixed to get the SLNs. The various formulations are synthesized based on the different percentage of the components for the analysis.

[0065] Independent variables are :

X_1 = PC : CHOL X_2 = Sonication time.

[0066] Dependent variables are :

Y1 = Entrapment efficiency

Y2 = Particle Size

Y3 = Drug Release

[0067] Table 3 below illustrates the amount of variable as per the factorial design used for formulating the various formulations, according to the embodiments of the present invention.

Tables – 3: Amount of independent variables as per factorial design.

Independent variables	-1	0	+1
X1	2 : 1	1 : 1	3 : 1
X2	10	20	30

[0068] Table 4 below illustrates the list of composition of nine batches prepared, according to the embodiments herein.

Tables – 4: Lists of composition of nine batches of the formulation.

Formulation code	Variable X1 Ratio of PC : CHOL (mg)	Variable X2 Sonication time.
F1	3 : 1	30
F2	3 : 1	20
F3	3 : 1	10
F4	2 : 1	30
F5	1 : 1	20
F6	2 : 1	10
F7	1 : 1	30
F8	1 : 1	10

F9	2 :1	20
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[0069] Table 5 below illustrates the various formulations used for analyzing the efficacy of the prepared SLN gel, according to the embodiments of the present invention.

Table 5 showing the various formulations used for analysis

Serial No.	Drug (mg)	Cholesterol	Soya Lecithin	Oleic Acid	Tween 80	Carbopol 940 (w/v)	Sonication time
F1	50	3%	9%	21%	5%	2%	30
F2	50	3%	9%	21%	5%	2%	20
F3	50	3%	9%	21%	5%	2%	10
F4	50	3%	6%	21%	5%	2%	30
F5	50	3%	3%	21%	5%	2%	20
F6	50	3%	6%	21%	5%	2%	10
F7	50	3%	3%	21%	5%	2%	30
F8	50	3%	3%	21%	5%	2%	20
F9	50	3%	6%	21%	5%	2%	10

[0070] RESULTS AND ANALYSIS

[0071] **FIG. 1** is a flow chart illustrating the various steps involved in the preparation of the oil phase used for the synthesis of the solid-lipid nanoparticles, according to an embodiment of the present invention. With respect to **FIG. 1**, a predetermined amount of acyclovir, a predetermined amount of a soya phosphatidyl choline (99%), a predetermined amount of cholesterol and a predetermined amount of an oleic acid are dissolved in a 10 ml mixture of chloroform and methanol dissolved in a ration of 1:1 (**101**). The predetermined amount of acyclovir is 50 mg. The predetermined amount of soya phosphatidyl choline is 9% by weight. The predetermined amount of cholesterol is 3% by weight. The predetermined amount of the oleic acid is 21% by weight. The

organic solvents are further removed by using Rota-evaporator (102). The remaining mixture is homogenized at 30 rpm at 80°C (103). The drug embedded lipid bilayer is obtained and heated at 85°C for melting (104).

[0072] FIG. 2 is a flow chart illustrating the various steps involved in the preparation of the aqueous phase used for the synthesis of the solid lipid nanoparticles, according to an embodiment of the present invention. With respect to FIG. 2, a predetermined amount of Tween 80 and a predetermined amount of the polyethylene glycol 4000 are added to double distilled water and mixed to form a total of 10 ml mixture (201). The mixture is heated at 50°C (202) and the hot aqueous phase is obtained (203).

[0073] FIG. 3 is a flowchart illustrating the various steps involved in the preparation of the nanoemulsion comprising the solid-lipid nanoparticles, according to an embodiment of the present invention. With respect to FIG. 3, the prepared hot aqueous phase is added to the prepared hot oil phase (301). The mixture of the aqueous phase and the oil phase is homogenized at 2000 rpm for 30 minutes to obtain a coarse and hot oil-in-water emulsion (302). The coarse hot oil-in-water emulsion (o/w) is ultrasonicated for 20 minutes (303). The acyclovir loaded solid-lipid nanoparticles or the hot nanoemulsion is obtained (304). The nanoemulsion is cooled at a room temperature (305).

[0074] FIG. 4 is a flow chart illustrating the various steps involved in the method of preparing the acyclovir drug loaded solid-lipid nanoparticle gel used for topical application, according to an embodiment of the present invention. With respect to FIG. 4, the carbopol 940 (2% or 0.4 gm) is dispersed in a mixture of a hot ultrapure water and 10% glycerol (401). The carbopol 940 is allowed to swell overnight to get a hydrogel (402). The acyclovir loaded solid lipid nanoparticles (SLN) nanoemulsion is dissolved in the carbopol 940 hydrogel (403). The acyclovir SLN and the carbopol 940 hydrogel are mixed in a high speed stirrer at a speed of 1000 rpm for 5 minutes (404). The pH is adjusted to 7 by adding triethanol amine drop wise (405). The acyclovir SLN gel for topical application is stored in a container at 4°C (406).

[0075] The prepared solid-lipid nanoparticles (SLNs) are characterized for several parameters such as particle size and zeta potential, entrapment efficiency, drug loading, surface morphology, drug polymer interaction study and in vitro permeation study, stability study of acyclovir loaded SLN. The particle size is evaluated by photon correlation spectroscopy. The zeta potential is evaluated in folded capillary cells using the nano zs90 zetasizer. The entrapment efficiency and the drug loading are evaluated by UV-VIS spectrophotometer. The surface morphology is evaluated by Transmission Electron Microscope (TEM). Further the drug polymer interaction is evaluated by Fourier Transform Infrared Spectroscopy (FTIR) and the Differential Scanning Calorimetry (DSC). The *in-vitro* skin permeation is evaluated in the hairless abdominal skin of wistar goat by a standard protocol. The chemical and the physical stability of the acyclovir loaded SLN is evaluated at 2-8°C for 3 months by clarity, particle size, zeta potential and HPLC analysis of acyclovir.

[0076] FIG. 5A and FIG.5B are the graphs illustrating the λ max of the drug sample, according to an embodiment of the present invention. With respect to FIG. 5A and FIG. 5B, the drug sample is scanned in the range of 200 nm-400 nm. The scanning results illustrate the λ max is 252 nm which complies with the I.P standard of the individual monograph of the pseudoephedrine hydrochloride.

[0077] The UV spectrophotometric method is implemented for the analysis of the drug acyclovir using the double beam Shimadzu 1700 spectrophotometer. The phosphate buffer is prepared by weighing 1.36 gms of potassium dihydrogen phosphate and placing in a 1000 ml volumetric flask. Then adding a 1000 ml of distilled water and shaking well until potassium dihydrogen phosphate dissolves completely. Further the standard stock solution is prepared by weighing 10 mg of the standard sample of the acyclovir drug and dissolving in 10 ml distilled water to get a concentration of 1 mg/ml. Then 1 ml is taken from this solution and is dissolved in 10 ml of distilled water. The concentration of acyclovir in this second stock solution is 100 mg/ml. Further series of dilution prepared by transferring 0.2, 0.4, 0.6, 0.8, 1, 1.2 and 1.4 ml into a 10 ml volumetric flask is done and the volume is made upto 10 ml using the distilled water. The final

concentration of the acyclovir in these solutions is now 2, 4, 6, 8, 10, and 14 mcg/ml, respectively. Table 6 below illustrates the absorbance of acyclovir standard solutions, according to the embodiments of the present invention.

Table 6 showing the absorbance details of the prepared standard solutions of acyclovir

Sl. No.	Concentration ($\mu\text{g/ml}$)	Absorbance
1	2	0.008
2	4	0.156
3	6	0.325
4	8	0.521
5	10	0.702
6	12	0.88
7	14	1.075

[0078] The calibration curve of acyclovir drug is determined by taking the absorbance of the final diluted solution. This is measured in a U.V. spectrophotometer (Shimadzu 1700) at 252 nm against phosphate buffer at pH 7.4 as blank. The absorbance so obtained is tabulated and standard curve is plotted as shown in **FIG. 6**. **FIG. 6** is a graph illustrating the standard calibration curve of acyclovir drug at pH 7.4, according to an embodiment of the present invention. With respect to **FIG. 6**, the λ max is 252 nm at pH 7.4. It is observed that the relation between the drug concentration and the absorbance is linear and the curve obeys the Bee-Lamberts law in the concentration range of 2-20 $\mu\text{g/ml}$ of acyclovir. The regression co-efficient (R^2) value is found 0.998 with Y value of 0.089mat 0.194 intercept. This indicates the linearity of the method.

[0079] **FIG. 7A** and **FIG. 7B** is a graph illustrating the particle size distribution for the acyclovir drug loaded solid lipid nanoparticles (SLN), according to an embodiment of the present invention. With respect to **FIG. 7A** and **FIG. 7B**, the different acyclovir SLNs are respectively measured by Photon Correlation Microscopy. The Table 7 below illustrates the particle size of acyclovir SLNs, according to the embodiments of the present invention.

Table 7 showing the particle size distribution of acyclovir SLNs

Size d. nm.	Mean Intensity Percent
10.10	1.1
11.70	1.5
13.54	1.9
15.69	2.5
18.17	3.1
21.04	3.7
24.36	4.1
28.21	4.5
32.67	4.7
37.84	4.7
43.82	4.6
50.75	4.4
58.77	4.3
68.06	4.2
78.82	4.2
91.28	4.2
105.7	4.3

[0080] It was found that the average size of all the formulations is located between 10.10 nm to 105.7 nm. The acyclovir SLN has the most narrow size distribution. No significant difference of the particle size distribution is observed between acyclovir SLN and acyclovir free SLN. The incorporation of the acyclovir into the drug free SLN only resulted in a slight change of average size.

[0081] FIG. 8A and FIG. 8B are the graphs illustrating the zeta potential of the acyclovir loaded solid lipid nanoparticles (SLN), according to an embodiment of the present invention. With respect to FIG. 8A and FIG. 8B, the zeta potentials of all the formulations are about -36.1 mv. The measurements are performed in the distilled water adjusted with a solution of 0.1 mmol/lit sodium chloride to a conductivity of 50 μ s/cm at 25°C. The zeta potential values are calculated using

Smoluchowski equation. The incorporation of the acyclovir into the SLNs showed no significant influence on the zeta potentials of the nanoparticles. The use of surfactant Tween 80 provides a steric stability which helps in maintaining the stability of the SLNs. Table 8 below illustrates the zeta potential of the different formulations prepared, according to the embodiments of the present invention.

Table 8 showing the zeta potential of various formulations

Formulation Code	Zeta potential
F1	-32.32 $\pm$ 2.11
F2	-32.38 $\pm$ 2.34
F3	-35.1 $\pm$ 3.01
F4	-40.9 $\pm$ 3.88
F5	-38.36 $\pm$ 3.11
F6	-40.23 $\pm$ 3.26
F7	-33.41 $\pm$ 3.0
F8	-37.5 $\pm$ 3.2
F9	-34.79 $\pm$ 3.36

[0082] FIG. 9A and FIG. 9B are Transmission Electron Microscopic (TEM) images of the acyclovir drug loaded solid lipid nanoparticles (SLN), according to an embodiment of the present invention. With respect to FIG. 9A and FIG. 9B, it was observed that the acyclovir loaded solid lipid nanoparticles exhibit a spherical shape and have a narrow size distribution. The TEM images are obtained using a Technai G+2-20 TEM. The TEM is used to characterize the microstructure of the acyclovir loaded – SLNs. The SLNs are placed on a carbon-coated copper grid and then a drop of 1% phosphotungstic acid covered on SLNs. The superfluous phosphotungstic acid on SLN is wiped off by the filter paper.

[0083] In another analysis process, the Differential Scanning Calorimetry (DSC) analysis is performed. The DSC is performed on 5 mg samples. The samples are sealed in standard aluminum pans. The thermograms are obtained at a scanning rate of 20°C/min. Each sample is scanned between 0°C to 300°C. The DSC is

performed for the drug acyclovir along with the other excipients. The output of the DSC is a plot of heat flux (rate versus temperature) at a specified temperature rate. The temperature is elevated from 30°C to 300°C. A 10 mg sample is weighed and subjected for DSC and the thermogram obtained are shown in the figures 10A-10D.

[0084] **FIG. 10A** is a thermogram illustrating the Differential Scanning Calorimetry (DSC) result of the acyclovir drug for Acyclovir-polymer interaction in the solid lipid nanoparticles, according to an embodiment of the present invention.

[0085] **FIG. 10B** is a thermogram illustrating the Differential Scanning Calorimetry (DSC) result of the cholesterol for Acyclovir-polymer interaction in the solid lipid nanoparticles, according to an embodiment of the present invention.

[0086] **FIG. 10C** is a thermogram illustrating the Differential Scanning Calorimetry (DSC) result of the soya lecithin for Acyclovir-polymer interaction in the solid lipid nanoparticles, according to an embodiment of the present invention.

[0087] **FIG. 10D** is a thermogram illustrating the Differential Scanning Calorimetry (DSC) result of the acyclovir drug, the cholesterol and the soya lecithin for Acyclovir-polymer interaction in the solid lipid nanoparticles, according to an embodiment of the present invention. With respect to **FIG. 10A-FIG. 10D**, the acyclovir presents a sharp endothermic peak at 256.27°C corresponding to the melting point of the drug, slight variation in endothermic peak value (251.82°C) is observed of acyclovir and phosphatidyl choline physical mixture. The thermograms further illustrate that there is a slight interaction, which has no effect on the drug stability. It is confirmed from the DSC thermogram that the drug is in its amorphous state.

[0088] Further, the Fourier Transform Infra-Red Spectroscopy (FTIR) is used to ensure that no chemical interaction between the drugs and polymers has occurred. **FIG. 11A** is a graph illustrating the Fourier Transform Infra-red Spectroscopy (FTIR) result of the soya lecithin for Acyclovir-polymer

interaction in the solid lipid nanoparticles, according to an embodiment of the present invention.

[0089] FIG. 11B is a graph illustrating the Fourier Transform Infra-red Spectroscopy (FTIR) result of the drug acyclovir, the cholesterol and the soya lecithin for Acyclovir-polymer interaction in the solid lipid nanoparticles, according to an embodiment of the present invention.

[0090] FIG. 11C is a graph illustrating the Fourier Transform Infra-red Spectroscopy (FTIR) result of the cholesterol for Acyclovir-polymer interaction in the solid lipid nanoparticles, according to an embodiment of the present invention.

[0091] FIG. 11D is a graph illustrating the Fourier Transform Infra-red Spectroscopy (FTIR) result of the acyclovir drug for Acyclovir-polymer interaction in the solid lipid nanoparticles, according to an embodiment of the present invention. With respect to FIG. 11A –FIG. 11D, principle peaks of acyclovir drug has been observed. No significant interaction between the acyclovir, cholesterol and the soya lecithin is observed.

[0092] For the determination of the entrapment efficiency, the freeze dried SLNs are dissolved in methanol and 7.4 PBS under water bath at 65°C for 30 minutes. The SLNs in the PBS are cooled to room temperature to preferentially precipitate the lipid. The drug content in the supernatant after centrifugation (4000 rpm for 15 minutes) is measured at 252 nm against blank by UV-VIS spectrophotometer (Shimadzu 1800). The drug entrapment efficiencies are calculated from the following formulae:

$$\text{Percent Drug Entrapment Efficiency} = \frac{(\text{Weight of total} - \text{weight Drug of free drug})}{\text{Weight of drug Used for S.L.N. Preparation}} \times 100$$

[0093] The Table 9 below illustrates the entrapment efficiency of various formulations, according to an embodiment herein.

Table 9 showing the entrapment efficiency

Formulation Code	Entrapment efficiency
F1	66.56
F2	65
F3	65.3
F4	66.7
F5	70.45
F6	61.14
F7	64
F8	70.05
F9	66.7

[0094] FIG. 12 is a bar graph illustrating the drug entrapment efficiency of the solid lipid nanoparticles (SLN), according to an embodiment of the present invention. With respect to FIG. 12, it is observed that the entrapment efficiency of the formulation 5 (F5) is highest followed by the entrapment efficiency of formulation 8 (F8).

[0095] The in-vitro drug release and drug release kinetic studies are also performed. Table 10 below illustrates the in-vitro drug release of Acyclovir SLN, according to the embodiments of the present invention.

Table 10 showing the drug release profiles of various formulations

Time	Cumulative % of drug release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	15.7 ± 1.1	18.3 ± 2.4	14.6 ± 1.7	14.5 ± 1.3	14.9 ± 1.4	3.01 ± 0.2	11.3 ± 1.7	14.58 ± 1.5	15.3 ± 1.6
2	30.2 ± 1.4	29.7 ± 2.1	26.6 ± 2.2	26.6 ± 2.7	29.24 ± 2.6	4.7 ± 0.8	14.89 ± 1.8	26.8 ± 1.2	26.3 ± 1.2
3	36.4 ± 1.6	38.7 ± 2.4	32.8 ± 2.3	37.4 ± 2.6	32.9 ± 2.6	6.82 ± 0.4	15.85 ± 1.4	33.4 ± 1.2	32.4 ± 2.2
4	39.7 ± 1.3	41.6 ± 2.7	36.7 ± 2.6	44.6 ± 2.5	32.98 ± 2.3	8.27 ± 0.5	16.94 ± 1.1	33.84 ± 1.6	36.6 ± 2.9
5	42.1 ± 1.3	44.1 ± 2.7	39.1 ± 2.5	52.8 ± 2.5	33.08 ± 2.5	8.97 ± 0.7	18.45 ± 1.1	40.34 ± 2.2	42.8 ± 2.8

	1.1	2.5	5		7		1.6	.5	
6	48.2± 1.2	48.5± 2.2	44.2±2. 6	66.7±2.5	35.2±2.3	9.27±0.3	20.24± 1.3	46.4±2. 7	44.8±2.8
7	55.6± 1.3	58.1± 2.6	53.1±2. 3	68.9±2.5	37.78±2. 7	10.82±1. 4	18.67± 1.5	50.53±2 .3	54.3±2.8
8	57.8± 1.5	60.2± 2.8	58.7±2. 6	78.4±2.6	40.24±2. 2	10.92±1. 7	20.24± 1.7	55.7±2. 7	58.4±2.6
9	68.3± 2.2	68.1± 2.2	64.6±2. 4	80.3±2.6	42.18±2. 4	11.12±1. 2	22.81± 1.4	60.8±2. 1	64.5±2.5
10	72.8± 2.8	72.2± 2.3	66.6±2. 1	92.6±2.1	48.52±2. 8	11.73±13	24.78± 1.3	67.7±2. 3	68.8±2.4
11	78.2± 2.4	80.5± 2.5	74.5±2. 3	11.2±1.4	49.87±2. 7	12.4±1.1	26.86± 1.3	71.7±2. 6	72.3±2.4
12	82.4± 2.3	85.2± 2.6	78.6±2. 8	14.4±1.4	58.68±2. 2	12.89±1. 3	29.87± 1.3	75.7±2. 3	78.9±2.7

[0096] The study reveals that increase in the polymer (phosphatidyl choline) concentration resulted in the retarded drug release for longer period of time.

[0097] The skin permeation studies with the drug acyclovir containing the solid lipid nanoparticles formulation are carried out using hairless abdominal skin (3.5 cm square) of wister goat, employing the modified Franz diffusion cells. The animals are sacrificed after getting approval from the animal ethics committee. The hairs on the dorsal side of the animal are removed. After removing the hairs, the skin is rinsed with physiological saline and fat tissues are chopped carefully to get uniform thickness. The skin is clamped between the donor and the receptor chamber of the vertical diffusion cell where the receptor chamber is filled with freshly prepared mixture of saline and ethanol (7:3 V/V). The ethanol is used to solubilize the acyclovir drug. The diffusion cells are maintained at 37°C using a re-circulating water bath. The fluid is continuously centrifuged at 300 r.p.m. The SLN gel is placed uniformly on the dorsal side of the rat skin in the donor chamber.

[0098] At 1-4 hours, 0.5 ml of the sample is periodically replaced with the same amount of the saline and 95% ethanol. The permeation rate of the acyclovir

through the rat skin is calculated from the slope of the linear portion of the cumulative amount permeated per unit area versus time plot.

[0099] The kinetics and mechanism of drug release data is obtained from the dissolution study is fitted into zero order (time vs cum % drug release) shown in Table 2, Higuchi mechanism (square root of time vs cumulative percentage of drug released) shown in Table 5, Korsmeyer's Peppas (log time vs log cumulative percentage of drug release) shown in Table 6.

[00100] FIG.13A to FIG.13D are the graphs illustrating the zero order release kinetics of formulation F1 to F3, F4 to F6, F7 to F9 and F1 to F9 respectively, according to one embodiment of the present invention. With respect to FIG. 13A, the formulation F2 has highest cumulative percentage of drug release, followed by formulation F1 and formulation F3 respectively. With respect to FIG. 13B, the formulation F4 has highest cumulative percentage of drug release, followed by formulation F5 and formulation F6 respectively. With respect to FIG. 13C, the formulation F9 has the highest cumulative percentage of drug release, followed by formulation F8 and formulation F7, respectively. With respect to FIG. 13D, the formulation F4 has the highest cumulative percentage of drug release among all nine formulations.

[00101] Table 11 below illustrates the zero order release kinetics data for formulation F1 to F9. The parameters are time and cumulative percentage of the drug release, according to the embodiments of the present invention.

Table 11 showing the zero order release kinetics of various formulations

Time	Cumulative % of drug release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	15.7 ± 1.1	18.3± 2.4	14.6±1. 7	14.5±1. 3	14.9±1.4	3.01±0.2	11.3±1.7	14.58±1. 5	15.3±1. 6
2	30.2± 1.4	29.7± 2.1	26.6±2. 2	26.6±2. 7	29.24±2. 6	4.7±0.8	14.89±1. 8	26.8±1.2	26.3±1. 2
3	36.4± 1.6	38.7± 2.4	32.8±2. 3	37.4±2. 6	32.9±2.6	6.82±0.4	15.85±1. 4	33.4±1.2	32.4±2. 2
4	39.7±	41.6±	36.7±2.	44.6±2.	32.98±2.	8.27±0.5	16.94±1.	33.84±1.	36.6±2.

	1.3	2.7	6	5	3		1	6	9
5	42.1± 1.1	44.1± 2.5	39.1±2. 5	52.8±2. 5	33.08±2. 7	8.97±0.7	18.45±1. 6	40.34±2. 5	42.8±2. 8
6	48.2± 1.2	48.5± 2.2	44.2±2. 6	66.7±2. 5	35.2±2.3	9.27±0.3	20.24±1. 3	46.4±2.7	44.8±2. 8
7	55.6± 1.3	58.1± 2.6	53.1±2. 3	68.9±2. 5	37.78±2. 7	10.82±1. 4	18.67±1. 5	50.53±2. 3	54.3±2. 8
8	57.8± 1.5	60.2± 2.8	58.7±2. 6	78.4±2. 6	40.24±2. 2	10.92±1. 7	20.24±1. 7	55.7±2.7	58.4±2. 6
9	68.3± 2.2	68.1± 2.2	64.6±2. 4	80.3±2. 6	42.18±2. 4	11.12±1. 2	22.81±1. 4	60.8±2.1	64.5±2. 5
10	72.8± 2.8	72.2± 2.3	66.6±2. 1	92.6±2. 1	48.52±2. 8	11.73±1.3	24.78±1. 3	67.7±2.3	68.8±2. 4
11	78.2± 2.4	80.5± 2.5	74.5±2. 3	11.2±1. 4	49.87±2. 7	12.4±1.1	26.86±1. 3	71.7±2.6	72.3±2. 4
12	82.4± 2.3	85.2± 2.6	78.6±2. 8	14.4±1. 4	58.68±2. 2	12.89±1. 3	29.87±1. 3	75.7±2.3	78.9±2. 7

[00102] FIG.14A to FIG. 14D are the graphs illustrating the first order release kinetics of formulation F1 to F3, F4 to F6, F7 to F9 and F1 to F9 respectively, according to one embodiment of the present invention. With respect to FIG. 14A, the formulation F2 has highest cumulative percentage of drug release, followed by formulation F1 and formulation F3, respectively. With respect to FIG. 14B, the formulation F4 has highest cumulative percentage of drug release, followed by formulation F5, and formulation F6, respectively. With respect to FIG. 14C, the formulation F9 and formulation F8 have same highest cumulative percentage of drug release, followed by formulation F7. With respect to FIG. 14D, the formulation F4 has the highest cumulative percentage of drug release among all nine formulations. Table 12 below illustrates the first order release kinetics data for Formulation F1 to F9, according to the embodiments of the present invention. The parameters are time and cumulative percentage of the drug release.

Table 12 showing the cumulative drug release profiles of various formulations

Time	Log Cumulative % of drug release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	1.19 ± 1.1	1.26± 2.4	1.16±1. 7	1.16±1. 3	1.17±1.4	0.47±0.2	1.05±1.7	1.16±1.5	1.18±1. 6
2	1.48 ± 1.4	1.47± 2.1	1.42±2. 2	1.42 ±2.7	1.46±2.6	0.67±0.8	1.17±1.8	1.42±1.2	1.41±1. 2
3	1.56 ± 1.6	1.58± 2.4	1.51±2. 3	1.58±2. 6	1.50±2.6	0.83±0.4	1.20±1.4	1.52±1.2	1.51±2. 2
4	1.59 ± 1.3	1.61± 2.7	1.56±2. 6	1.64±2. 5	1.51±2.3	0.91±0.5	1.22±1.1	1.52±1.6	1.56±2. 9
5	1.62 ± 1.1	1.64± 2.5	1.59±2. 5	1.72±2. 5	1.51±2.7	0.95±0.7	1.26±1.6	1.60±2.5	1.63±2. 8
6	1.68 ± 1.2	1.68± 2.2	1.64±2. 6	1.82±2. 5	1.54±2.3	0.96±0.3	1.30±1.3	1.66±2.7	1.65±2. 8
7	1.74 ± 1.3	1.76± 2.6	1.72±2. 3	1.83±2. 5	1.57±2.7	1.03±1.4	1.27±1.5	1.70±2.3	1.75±2. 8
8	1.76 ± 1.5	1.77± 2.8	1.76±2. 6	1.89±2. 6	1.60±2.2	1.03±1.7	1.30±1.7	1.74±2.7	1.76±2. 6
9	1.83 ± 2.2	1.83± 2.2	1.81±2. 4	1.90±2. 6	1.62±2.4	1.04±1.2	1.35±1.4	1.78±2.1	1.80±2. 5
10	1.86 ± 2.8	1.85± 2.3	1.82±2. 1	1.96±2. 1	1.68±2.8	1.06±1.3	1.39±1.3	1.83±2.3	1.83±2. 4
11	1.89 ± 2.4	1.90± 2.5	1.87±2. 3	1.04±1. 4	1.69±2.7	1.09±1.1	1.42±1.3	1.85±2.6	1.85±2. 4
12	1.91 ± 2.3	1.93± 2.6	1.89±2. 8	1.158	1.76±2.2	1.11±1.3	1.47±1.3	1.87±2.3	1.89±2. 7

[00103] FIG.15A to FIG. 15D are the graphs illustrating the Higuchi release kinetics of formulation F1 to F3, F4 to F6, F7 to F9 and F1 to F9 respectively, according to one embodiment of the present invention. With respect to FIG. 15A, the formulation F2 has the highest cumulative percentage of drug release, followed by formulation F1 and formulation F3, respectively. With

respect to **FIG. 15B**, the formulation F4 has the highest cumulative percentage of drug release, followed by formulation F5 and formulation F6, respectively. With respect to **FIG. 15C**, the formulation F9 and formulation F8 have same highest cumulative percentage of drug release, followed by formulation F7. With respect to **FIG. 15D**, the formulation F4 has the highest cumulative percentage of drug release among all nine formulations. Table 13 below illustrates the first order release kinetics data for formulation F1 to F9, according to the embodiments the present invention. The parameters are time and cumulative percentage of the drug release.

Table 13 showing the first order release kinetics data of various formulations

Sq. root of Time	Log Cumulative % of drug release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	1.19 ± 1.1	1.26± 2.4	1.16±1. 7	1.16±1. 3	1.17±1. 4	0.47±0.2	1.05±1.7	1.16±1.5	1.18±1. 6
1.41	1.48± 1.4	1.47± 2.1	1.42±2. 2	1.42 ±2.7	1.46±2. 6	0.67±0.8	1.17±1.8	1.42±1.2	1.41±1. 2
1.73	1.56± 1.6	1.58± 2.4	1.51±2. 3	1.58±2. 6	1.50±2. 6	0.83±0.4	1.20±1.4	1.52±1.2	1.51±2. 2
2	1.59± 1.3	1.61± 2.7	1.56±2. 6	1.64±2. 5	1.51±2. 3	0.91±0.5	1.22±1.1	1.52±1.6	1.56±2. 9
2.23	1.62± 1.1	1.64± 2.5	1.59±2. 5	1.72±2. 5	1.51±2. 7	0.95±0.7	1.26±1.6	1.60±2.5	1.63±2. 8
2.44	1.68± 1.2	1.68± 2.2	1.64±2. 6	1.82±2. 5	1.54±2. 3	0.96±0.3	1.30±1.3	1.66±2.7	1.65±2. 8
2.64	1.74± 1.3	1.76± 2.6	1.72±2. 3	1.83±2. 5	1.57±2. 7	1.03±1.4	1.27±1.5	1.70±2.3	1.75±2. 8
2.82	1.76± 1.5	1.77± 2.8	1.76±2. 6	1.89±2. 6	1.60±2. 2	1.03±1.7	1.30±1.7	1.74±2.7	1.76±2. 6
3	1.83±	1.83± 2.2	1.81±2.	1.90±2.	1.62±2.	1.04±1.2	1.35±1.4	1.78±2.1	1.80±2.

	2.2		4	6	4				5
3.16	1.86± 2.8	1.85± 2.3	1.82±2. 1	1.96±2. 1	1. 68±2.8	1.06±13	1.39±1.3	1.83±2.3	1.83±2. 4
3.31	1.89± 2.4	1.90± 2.5	1.87±2. 3	1.04±1. 4	1.69±2. 7	1.09±1.1	1.42±1.3	1.85±2.6	1.85±2. 4
3.46	1.91± 2.3	1.93± 2.6	1.89±2. 8		1.76±2. 2	1.11±1.3	1.47±1.3	1.87±2.3	1.89±2. 7

[00104] The chemical and the physical stabilities of the acyclovir-SLN is evaluated at 2-8°C for 3 months. The clarity, the particle size, the zeta potential and the HPLC analysis of the topical gel is analyzed. The topical gel is stable under all conditions. Further the particle size is found to be 47.81 nm. The zeta potential of the topical gel is found to be -36.1. The shape of the Acyclovir loaded solid lipid nanoparticle is uniform and spherical in nature. The entrapment efficiency of the topical gel is in the range of 75%-95%.

[00105] The foregoing description of the specific embodiments will so fully reveal the general nature of the embodiments herein that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments.

[00106] It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. Therefore, while the embodiments herein have been described in terms of preferred embodiments, those skilled in the art will recognize that the embodiments herein can be practiced with modification within the spirit and scope of the appended claims.