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(54) Title: CARRIER BASED NANOgel FORMULATION FOR SKIN TARGETING.

(57) Abstract: The invention comprises a lipid nano carrier based nanogel formulation of an active ingredient and method of their preparation. The formulation is specifically useful for dermal delivery or topical application of drugs, which may be a hydrophilic drug including 5-fluorouracil (5-FU) and acyclovir, or a hydrophobic drug such as isotretinoin. The invention is illustrated by a lipid nano carrier based nanogel formulation of 5-FU. A lipid nano carrier based nanogel formulation comprising 5-FU in a concentration of 0.001% to 10% w/w. A method of preparing lipid nano carrier based nanogel formulation of an active ingredient comprises the steps of: preparation of drug loaded vesicular lipid carrier dispersion, wherein the surface of vesicles of the lipid carrier contains a surfactant, sonication the drug loaded vesicular lipid carrier dispersion of step (a.) to prepare a lipid nano carrier dispersion in nanometric size range, concentrating the lipid nano carrier dispersion in nanometric range by steps of subjecting the lipid nano carrier dispersion in nanometric size range of step (b.) either to (i) rotary evaporation, or(ii) freeze drying with or without a cryoprotectant, preparation of hydrophilic gel; and mixing of lipid nano carrier dispersion of step (c.) with hydrophilic gel of step (b) to form a lipid nano carrier based nanogel formulation.
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TITLE
CARRIER BASED NANOGEL FORMULATION FOR SKIN TARGETING.

TECHNICAL FIELD
The present invention describes lipid nano carrier based nanogel formulation for skin targeting to achieve enhanced topical delivery. Commonly used drug for skin cancer 5-Fluorouracil (5-FU) has been illustrated to increase the local bioavailability of a drug. The inventions also relates to nanogel formulation comprising lipid, surfactant, drug, polymer and pharmaceutically acceptable ingredients. The present invention also provides a process for preparation of said lipid nano carrier based nanogel formulation.

BACKGROUND OF INVENTION

Topical chemotherapy is used to treat non-melanoma skin cancer that is limited to the top layer of skin. It is also used to treat actinic keratoses (AK), lesions that can develop on skin that has received years of sun exposure. Formulations used in current topical chemotherapy for skin cancer are conventional formulation like ointment, cream and gel. These formulations act only as topical vehicle and do not facilitate the skin permeation of drug. Present topical chemotherapy has been associated with poor skin permeation, poor reach to disease tissue, poor retention at target site so that some part of the drug gets absorbed systemically into blood circulation and skin irritation potential (10-29% patient have pain, itching, burning and inflammation), low therapeutic index (TI) and non-specificity leads to development of several dose dependent side effects, inability to achieve therapeutic concentrations at the target site, systemic side effects,
nonspecific cytotoxicity to critical normal tissues, development of resistance, poor intracellular penetration and problems associated with formulations of the drugs. Thus, there is a need for effective delivery systems that not only acts as a formulation aid but also alter the bio-distribution of drugs in such a way so that a greater fraction of the dose reaches the target site and improves the local bioavailability of the drug, has lesser irritation potential and does not get absorbed systemically into blood circulation. One specific objective is to develop a 5-FU nanogel formulation that shall have high local bioavailability with reduced skin irritation potential and will increase the therapeutic effectiveness. However, it is also an objective to develop an improved composition and improved system of topical application of Active agents in general for delivery across the skin.

As indicated in the study by Smith et al. [Stacy Smith MD1, Dan Piacquadio MD1, Vera Morhenn MD, Deborah Atkin MD, Richard Fitzpatrick MD, Short Incubation Photodynamic Therapy (PDT) versus 5-FU in treating Actinic Keratoses, J Drugs Dermatol 2003; 2:6;629-635)] the advantages of 5-FU treatment are that large areas can be treated at one time and that this therapy also targets incipient lesions. However, the disadvantages include the length of treatment, significant inflammatory reactions, and concerns that the drug may not reach deep or hyperkeratotic lesions. It is also an objective of this invention to overcome the limitations identified by Smith et al.
Singh et al (US6670335) disclosed a topical formulation containing fluorouracil, comprising: (i) an oil-in-water emulsion; and (ii) dispersed within the oil-in-water emulsion, (a) fluorouracil; and (b) fluorouracil-impregnated porous microparticles, where the formulation has a total fluorouracil content that the sum of the fluorouracil in (ii)(a) and the fluorouracil in the fluorouracil-impregnated porous microparticles of (ii)(b) of from about 0.01% to about 10% by weight and a free fluorouracil content that is the fluorouracil in (ii)(a) of from 20% to 50% by weight of the total fluorouracil content. However, the microparticles are rigid and act only as vehicle and hence, shall have limited skin penetration and deposition.

WO2008140507 discloses a method of suppressing growth of cancer cells which are resistant to drug therapy, comprising administering to a subject in need thereof an amount of tetrac, triac, tetrac and triac nanoparticles or analogs thereof, effective for suppressing the growth. It does not disclose use of 5-FU nor does it disclose use of any lipid nano carrier based nanogel.

CN101485887A discloses 5-fluorouracil-sn2-phosphatidylcholine copolymers and a preparation method and application thereof, which have amphiphilic surface activity, can form liposome nano-capsules and a self-assembly delivery system, and can be taken as an intermediate preparation formulation and further prepared into a corresponding transdermal delivery preparation such as gel. In this invention, 5-FU gets incorporated in the structure of the liposomes, which can be incorporated in gels, and its release depends on the
enzymatic release by action of high-expression phospholipase A2. However, this invention involves use of a novel medicine i.e. 5-fluorouracil-sn2-phosphatidylcholine copolymers, which would not be considered as approved drug 5-FU and entire regulatory process will have to be gone through using the same for treatment.

WO2010125575 discloses novel compounds claim 1, claim 3 and method of treating cancer and related vascular diseases by administering at least one compound of General Formula I of claim 3 and specific compounds a) to p) of claim 44 which comprise the Selenophene compounds and Selenophene triazene compounds, their geometrical isomeric forms, stereoisomers, configurational isomers, polymorphs, hydrates, solvates and pharmaceutically acceptable salts. It does not disclose use of 5-FU nor does it disclose use of any lipid nano carrier based nanogel.

US20100166848 discloses liposomes which coencapsulate 5-fluorouracil (5-FU) and 2'-deoxyinosine (d-Ino). As per our understanding, the instant invention does not comprise liposomes which coencapsulate 5-fluorouracil (5-FU) and 2'-deoxyinosine (d-Ino) and a method for treating cancer in a patient, which method comprises administering an effective amount of a pharmaceutical composition comprising liposomes which coencapsulate 5-fluorouracil (5-FU) and 2'-deoxyinosine (d-Ino) in a patient in need thereof. There is no disclosure of any gel carrying 5-FU in general and lipid nano carriers in particular for topical administration of 5-FU.
US20080102127A1 discloses a nanoparticulate colloidal delivery vehicle where nanoparticles are composed of a) water insoluble biocompatible polymer and b) solid lipid material, uniformly distributed in nanoparticle polymeric matrix c) an outer layer, surrounding the particle and comprising of surfactant(s), said layer additionally may comprise phospholipid(s), pegylated phospholipid(s), water soluble or water swellable polymer(s) and targeting/recognizing compounds. The hybrid "lipid-water insoluble polymer nano-particles" are used as an ingredient of injectibles. There is no disclosure of any lipid nano carrier based nanogel formulation.

SUMMARY OF THE INVENTION

The invention comprises a lipid nano carrier based nanogel formulation of an active ingredient. The lipid nano carrier based nanogel formulation is useful for dermal delivery or topical application. The said active ingredient comprises a hydrophilic drug or a lipophilic drug. The said hydrophilic drug may be selected from a group 5-fluorouracil (5-FU), acyclovir, colchicine, diclofenac and glucosamine. The said lipophilic drug may be selected from a group isotretinoin, amphotericin B, dithranol, calcipotriol, coaltar and tacrolimus.

The invention also comprises a lipid nano carrier based nanogel formulation comprising 5-FU in a concentration of 0.001% to 10% w/w more particularly in a concentration of 5% w/w.

The invention also comprises a method of preparing lipid nano carrier based nanogel formulation of an active ingredient comprising the steps of: (a)
preparation of drug loaded vesicular lipid carrier dispersion using a drug solution in a hydrating medium, wherein the surface of vesicles of the vesicular lipid carrier contains a surfactant; (b) probe sonicating the drug loaded vesicular lipid carrier dispersion of step (a.) to prepare a lipid nano carrier dispersion in nanomeric size range, (c) concentrating the lipid nano carrier dispersion in nanomeric range by steps of subjecting the lipid nano carrier dispersion in nanomeric size range of step (b.) either to (i) rotary evaporation, or (ii) freeze drying with or without a cryoprotectant. (d) preparation of hydrophilic gel; and (e) mixing of lipid nano carrier dispersion of step (c.) with hydrophilic gel of step (b) to form a lipid nano carrier based nanogel formulation.

Method of preparation of drug loaded vesicular lipid carrier dispersion comprises steps of: (a) dissolving phospholipids, surfactant and others lipophilic additives in a small quantity of an organic solvent sufficient to dissolve the lipid, surfactant and additives, (b) removing the organic solvent by a means of evaporation or under reduced pressure, removing final traces under vacuum to get a lipid film deposited, (c) hydrating the deposited lipid film with drug solution in a hydration medium by rotation to form lipid carrier suspended in the said hydration medium.

When the drug used is 5-FU, the method of this invention comprises achieving loading of 5-FU to 1% w/w, the method comprising steps of: (a) hydration medium being water or Phosphate buffer saline (PBS 6.8) or Phosphate buffer saline (PBS 7.4). (b) dissolving 5-FU at a concentration of 10 mg/mL in the hydration medium, (c) hydrating the deposited lipid film
obtained by rotary evaporation with drug solution to form lipid carrier suspended in the said hydration medium, (d) probe sonicating the drug loaded vesicular lipid carrier dispersion of step (a.) to prepare a lipid nano carrier dispersion in nanometric size range, (e) concentrating the lipid nano carrier dispersion in nanometric range by steps of subjecting the lipid nano carrier dispersion in 100-200 nm or other nanometric size range of step (b.) either to (i) rotary evaporation, or (ii) freeze drying with or without a cryoprotectant. (f) preparation of hydrophilic gel; and (g) mixing of lipid nano carrier dispersion of step (e.) with hydrophilic gel of step (b) to form a lipid nano carrier based nanogel formulation.

Invention also comprises a method of making a lipid carrier based nanogel wherein the drug is 5-FU and hydration medium is an alkalizing medium. In this medium, the alkalizing medium may be 20% Tris buffer:25% Ammonia solution (1:1) wherein the loading achieved is 5% comprising steps of: (a) hydration medium being 20% Tris buffer:25% Ammonia solution (1:1). (b) dissolving 5-FU at a concentration of 100 mg/mL in the hydration medium, (c) hydrating the deposited lipid film claim 10 step (ii) with drug solution of step (b.) to form lipid carrier suspended in the said hydration medium, (d) probe sonicating the drug loaded vesicular lipid carrier dispersion of step (a.) to prepare a lipid nano carrier dispersion in nanometric size range, (e) preparing a lipid nano carrier dispersion in nanometric range by steps of subjecting the lipid nano carrier dispersion in nanometric size range of step (b.) either to rotary evaporation. (f) preparation of hydrophilic gel; and (g)
mixing of lipid nano carrier dispersion of step (e.) with hydrophilic gel of step (b) to form a lipid nano carrier based nanogel formulation.

Hydrophilic gel is prepared by a method comprising steps of: adding and mixing appropriate quantity of a polymer, preferably a cross-linked polyacrylate polymer, including, without limitations, polyacrylate polymer, methyl cellulose, Poloxamer, hydroxy ethylcellulose and hydroxy propylcellulose, to deionized water, allowing the dispersion to hydrate and swell for 60 min, neutralize the dispersion with an alkali accompanied by gentle stirring, agitating this mixture for 2 h with high speed stirrer until homogeneous clear hydrophilic gel is formed, adding a preservative, allowing to equilibrate the hydrophilic gel for at least 24 hours at room temperature prior to further use to make a lipid nano carrier based nanogel. The said alkali is 98% triethanolamine, Any other alkali may be used. Neutralization may be done to reach a pH 5.5 - 7.1. Preservative is selected from the group methyl paraben, propyl paraben or benzyl alcohol.

Method of this invention also comprises a method of preparation of lipid nano carrier based nanogel comprising step of mixing the lipid nano carrier dispersion with optimized hydrophilic gel under mechanical stirring for 1h to result in to lipid nano carrier based nanogel formulation.

The invention also comprises a method of treating a dermal disease capable of treatment by topical administration of a therapeutically effective amount the formulation of a lipid carrier based nanogel of 0.001% to 10% w/w containing therapeutically effective concentration of 5-FU.
The said dermal disease may be selected from acne, herpes lesions, inflammation, psoriasis cold sore, fungal and bacterial infection, wart, seborrheic eczema and cutaneous leishmaniasis by topical administration. Topical administration of a therapeutically effective amount the formulation of a lipid carrier based nanogel of 0.001% to 10% w/w containing therapeutically effective concentration of 5-FU.

An embodiment of this invention comprises enhanced Permeability and Penetration of 5 Fluorouracil reaching the Dermis resulting in reduced risk of occurrence of Bowen’s disease.

DETAILED DESCRIPTION OF THE INVENTION

In one embodiment the invention comprises a lipid nano carrier based nanogel formulation for topical application of an active agent, wherein the said Active agent is encapsulated within the said lipid nano carrier. In one embodiment, the said formulation is meant for skin targeting to increase local bioavailability. In a further embodiment, the invention comprises a method for making a lipid nano carrier based nanogel formulation for topical application of an active ingredient. In another embodiment of the invention, the components of lipid nano-carrier surface comprises at least one surfactant.

Drug selected for illustration is 5-FU (since it is an anti-cancer drug and considered as gold standard for treatment of skin cancer). Its topical application has also been proven to be valuable and safe treatment of actinic keratoses, solar keratoses and superficial basal cell carcinoma. From conventional topical cream formulation, 5-FU reaches cancer cells near to skin surface only, but it cannot reach cancer cells that may have invaded
deeply into the skin. For this reason, treatment with 5-FU generally is used only for pre-cancerous condition such as actinic keratoses and for some very superficial skin cancers. 5-FU can be used for the treatment of Squamous Cell carcinoma (SCC) and acne if topical formulation delivers the drug to deeper skin layers. In a further embodiment of this invention illustrated for 5-FU, the lipid nano carrier based nanogel has about 80% of 5-FU encapsulated in nano-carrier and rest about 20% in free form in the gel. Free form of drug provide the initial effective concentration followed by sustained release of encapsulated drug. In yet another embodiment this invention comprises a non-irritating topical application gel carrying 5-FU in a total concentration of at least 1% w/w. In one embodiment, the lipid nano carrier based nanogel of this invention shall have a minimum of at least about 0.001 % w/w 5-FU, more particularly at least about 0.5% w/w, more particularly at least about 1%, still more particularly at least about 5% w/w, still more particularly at least about 10%. In another embodiment, the lipid nano carrier based nanogel of this invention shall contain 5-FU at least more than about 0.001 % w/w, more particularly at least more than 0.5%, more particularly at least more than 1% w/w, still more particularly at least more than 2% w/w, still more particularly at least more than 5% w/w, still more particularly at least more than 10% w/w. The lipid nano carrier based nanogel of this invention shall have 5-FU in various range of concentrations, including but not limited to a range selected from a group of 0.001 - 0.5%, 0.001 - 1%, 0.001 - 2%, 0.001 - 5%, 0.001 - 10% w/w.
In yet another embodiment, this invention pertains to a method of preparing a lipid nano-carrier based nanogel composition useful for topical application of drugs on diseases or disorders of skin for topical application. The said drugs include hydrophilic as well as lipophilic drugs. The said skin diseases include, without limitation acne, herpes lesions, inflammation, psoriasis cold sore, fungal and bacterial infection, warts, seborrheic eczema and cutaneous leishmaniasis.

In yet another embodiment, this invention is a method of treatment by topical application of a lipid nano carrier based nanogel composition containing an active. In a further embodiment of this invention, the said active is an active Pharmaceutical Ingredient.

In yet another embodiment this invention comprises a formulation for topical administration of a hydrophilic drug entrapped in a lipid nano carrier based nanogel wherein the nano lipid carriers have ability to get absorbed below skin surface and create a depot in deeper layers of skin for better efficacy and better local bioavailability. Illustration has been provided for 5-FU.

In place of 5-FU, any other drug effective on topical delivery like isotretinoin, acyclovir, colchicine, diclofenac, amphotericin B, calcipotriol, dithranol, coaltar, tacrolimus, glucosamine, may be used in practicing this invention.

A lipid nano carrier based approach has dual advantages in topical delivery; first it reduces the direct contact of drug to skin surface so will minimize the skin irritation potential that is the major limitation of conventional 5-FU formulation and secondly it shall improve the skin deposition and local
bioavailability of the drug due to depot forming ability of nano carrier so that systemic absorption of the drug into blood circulation is minimized. A further advantage of a lipid nanocarrier over non-lipid nano-carriers is that a lipid nano-carrier will get absorbed into the skin far better. A further advantage of a lipid carrier having a surfactant on its surface is that efficiency of absorption into the skin increases still further.

5-FU is a hydrophilic anti-cancer drug used in the treatment of various forms of skin cancers. Moreover, therapy for basal cell carcinoma and squamous cell carcinoma does not end with treatment of initial lesion because almost 50% of patients with one non-melanoma skin cancer develop another one within next 5 years. Therefore, an improved percutaneous permeation of 5-FU is a fundamental requisite to achieve an effective topical therapeutic approach. Unfortunately, 5-FU shows a poor skin permeation thus reducing its anti-cancer effectiveness following topical administration.

In the present invention Vitamin E TPGS (TPGS, D-a-tocopheryl polyethylene glycol 1000 succinate) is selected as surfactant along with soya phosphatidyl choline for preparation of nano carrier formulation. Vitamin E TPGS reported to increase the intestinal permeability of number of poorly water soluble drugs like paclitaxel and Vancomycin. It is thought that this effect of TPGS could be applied to the topical delivery of 5-FU as one example for enhancing its skin permeation and local retention. In the present invention it is envies b prepare Vitamin E TPGS based nano carrier formulation. Any non-irritating and non-poisonous surfactant ,may be used in
place of Vitamin E TPGS, including, without limitation, sodium deoxycholate, sodium taurocholate, polyoxyethylene lauryl ether, polyoxyethylene-2-oleyl ether, and polyoxyethylene-2-stearyl ether.

**Brief description of Figures and legends:**

- **Figure 1:** Transmission electron microscopy photomicrograph of 5-FU lipid nano carrier formulation.
- **Figure 2:** Particle size measurement of 5-FU lipid nano carrier formulation.
- **Figure 3:** Comparative Rheogram of optimized 5-FU Nanogel and Marketed formulation.
- **Figure 4:** In vitro percutaneous absorption of 5-FU across the rat epidermal sheet from marketed and nanogel formulations of the instant invention.
- **Figure 5:** Percentage Hemolytic toxicity of different 5 FU formulations
- **Figure 6:** Confocal Laser Scanning Microscopy photomicrograph of rat skin treated with fluorescence marker loaded nanogel formulation (NG-8).

Throughout the specification, wherever the term "marketed formulations" or "Marketed 5-FU gel" or a term giving a similar meaning is used, it means a prior art formulation that is currently available in market that is not "lipid nano carrier based nanogel" in the meaning of this invention.

Throughout the specification, wherever the term "Active" or "Active agent" is used, it includes a drug.

The method of this invention for preparing the lipid nano carrier based nanogel formulation comprises following steps: (a) preparation of drug
loaded lipid nano carrier dispersion in nanometric size range, wherein the lipid nano carrier contains a surfactant; (b) preparation of hydrophilic gel; and (c) mixing of lipid nano carrier dispersion of step (a) with hydrophilic gel of step (b) to form a nanogel formulation.

Prior art method of preparing lipid nano carriers by Rotary evaporation sonication method comprises following steps: The phospholipids, surfactant and others lipophilic additives such as Butyl Hydroxy Toluene or other antioxidant are taken in a clean, dry round-bottom flask and this lipid mixture was dissolved in small quantity of an organic solvent. The quantity of solvent is determined so as to sufficiently dissolve the lipid, surfactant and additives. Any organic solvent considered suitable by a person skilled in the art can be used for the purpose. Examples of organic solvent suitable for this purpose are chloroform, methanol and diethyl ether. The amount of organic solvent in the mixture preferably ranges from 1-2% w/w of the lipid. The organic solvent was removed preferably by evaporation. In the present invention Rotary evaporator, Perfit, India has been used under reduced pressure at 40-42 °C to achieve the evaporation. Final traces of solvents were removed under vacuum. A lipid film gets deposited. The deposited lipid film was hydrated with drug solution by rotation at 60 rev/min for 1-2 hr to form lipid carrier suspended in water. The drug solution was prepared by dissolving 5-FU in distilled water or Phosphate buffer saline (PSB 6.8) or Phosphate Buffer saline (PSB 7.4) as specified in Table no. 3. To prepare dispersion in nanometric size (100-200 nm), these were probe sonicated (Probe sonicator,
VCX 505, Sonics, USA). However, this method was not useful for therapeutic purpose because 5-FU got precipitated in 5% w/w formulation and it also had lower encapsulation efficiency of 68.31±2.0%. (See Table 4). Best of the concentration / drug loading achievable in the formulation of the lipid nano carrier based nanogel was 1% of 5-FU.

Surprisingly, in one embodiment of this invention, a drug loading of about 5% was achieved in vesicular formation with encapsulation efficiency of 76.25±1.0% when freeze drying was used. In this embodiment, the lipid nano carrier was prepared using method as described above and resulting nanometric size dispersion was lyophilized. When lyophilization was done with a cryoprotectant such as Saccharose at 20%, the encapsulation-efficiency improved to 78.39±1.5%. The lyophilizaiton was done using Mini Lyodel freeze dryer (Delvac, India). Any other cryoprotectant could be used in appropriate concentration in the place of saccharose. In this method the formulation was first frozen at -40°C for 2 h followed by freeze drying until a free flowing powder was obtained. Formulation was reconstituted with distilled water to produce the formulation with 1 % and 5% w/w strength of 5-FU. However, use of this embodiment requires expensive equipment and time required to achieve the result is 24 hours.

In a still other embodiment of this invention, however, very surprisingly, 5-FU loading of 5% w/w in the formulation with Encapsulation efficiency of 80.24±3.2% was achieved with rotary evaporation sonication method when hydration medium no. 4 of Table 3 was used (20% Tris buffer:25% Ammonia solution (1:1) for hydration.
Table 8 shows that as compared to the Marketed gel, the 5-FU nanogel formulation (NG8) having no skin irritation since erythema did appear in the Marketed gel in 24 hours which doubled in 48 hours and persisted at that level upto 78 hours. However, in 5-FU nanogel formulation (NG-4) erythema did not develop at all.

Various polymers can be used as gel formers for nano-gel formation. This includes different types of Carbopols, like Carbopol 934 and Carbopol 980, which could be use as gel former. Carbopol 934 is a cross-linked polyacrylate polymer cross-linked with allyl sucrose and is polymerized in solvent benzene. Carbopol 980 is also crosslinked polyacrylate polymer widely used as gel former in topical formulation and polymerized in cosolvent system Ethyl Acetate and Cyclohexane. Cellulose derivative like methyl cellulose and Poloxomer can also be used as gel former. To form a gel, appropriate quantity of Carbopol 934 or 980 NF was added to deionized water and mixed. The dispersion was then allowed to hydrate and swell for 60 min. The pH of the un-neutralized sample was then measured to be 3.1. The Carbopol dispersion was then neutralized with 98% TEA (triethanolamine) until the desired pH value was approximately reached (5.5 - .7.1). During neutralization, the mixture was stirred gently with a spatula. This mixture wasagitated for 2 h with high speed stirrer until homogeneous clear gel was formed. Appropriate and pre weighed amounts of methyl paraben and propyl paraben were then added to the mixture as a preservative. Any other preservative could be added in the place of parabens, for example, without limitation, benzyl alcohol.. All the gel
preparations were allowed to equilibrate for at least 24 hours at room temperature prior to performing any further analysis.

Various phospholipids can be used for this invention, including, without limitations, Soya phosphatidylcholine, egg phosphatidylcholine, disteryl phosphatidyl choline, phosphatidylethanolamine and phosphatidylserine.

The lipid nano carrier dispersion was mixed with optimized Carbopol gel formulation under mechanical stirring for 1 h. This results in lipid nano carrier based Nanogel formulation.

Lipid nano-carriers made without incorporation of surfactant resulted in poor transdermal flux, lesser entrapment efficiency and poor skin deposition. Use of surfactant in lipid carrier nanogel formulation was found to increase the encapsulation efficiency, skin permeation and deposition that is required for increasing therapeutic performance of topical applied drugs. Hence, all further experiments were done incorporating a surfactant as the part of the lipid vesicle and formulations optimized by performing several experiments for the variables in the formulation. Hence optimization was made on several alternative parameters to get more efficacious formulation. The results are given in Table 1-8.

Tables 1 and 2 give data- on composition of lipid nano carrier formulated using, respectively, Sodium Deoxycholate and Vitamin TPGS as surfactants. Of various surfactants tried for incorporation into the lipid nano carrier, several of them had to be discarded on account of several limitations; and Sodium Deoxycholate and Vitamin E TPGS gave surprisingly improved results over Marketed formulation in all the aspects of Skin Permeation.
Parameters including Steady State transdermal flux, Drug deposited in the skin, "Enhancement Ratio" of transdermal flux from formulation to drug solution and the "Enhancement Ratio" of drug deposited from formulation to drug solution. (See Table 7). Between formulations containing Sodium Deoxy Cholate and the formulation containing Vitamin E TPGS, the formulation containing Vitamin E TPGS gave better results.

Table 3 gives data on optimization of hydration media. 20% Tris buffer:25% Ammonia solution (1:1) surprisingly gave solubility of drug to 100 mg/mL as against only 10 mg/mL for distilled water and Phosphate buffer saline (PSB 6.8) and Phosphate buffer saline (PSB 7.4) which resulted in achieving a maximum drug loading to 5% w/w (weight / weight) as against only 1% w/w for distilled water and Phosphate buffer saline (PSB 6.8) and Phosphate Buffer saline (PSB 7.4). It is this high solubility of 5-FU in 20% Tris buffer : 25% Ammonia Solution (1:1) that 5% drug loading of 5-FU in a lipid nano carrier is possible; otherwise, with solubility of only 10 mg/mL in other hydrating media, only a maximum of 0.5% drug loading becomes possible, which results in a formulation lower than the regulatory requirement of a formulation for topical treatment of skin cancer and related diseases. Thus, use of 20% Tris buffer : 25% Ammonia Solution (1:1) is an embodiment of invention in the method of making the lipid nano carrier based nanogel of this invention.

The lipid nano carrier based nanogel has improved penetration and drug concentration in the deeper layers of the skin over prior art formulations that are able to obviate the problems and disadvantages of therapy by prior art
formulations of 5-FU, the said disadvantages include the long length of treatment, significant inflammatory reactions, and concerns that the drug may not reach deep or hyperkeratotic lesions. It is also an embodiment of this invention that the lipid nano carrier based nanogel of this invention acts in the deeper layer of the skin would be able to target the atypia that these cells would be subjected to due to the original lesion, thus reducing the future malignant potential of these lesions following treatment that may lead to Bowen's disease, a neoplastic skin disease, which can be considered as an early stage or intraepidermal form of squamous cell carcinoma.

The invention is illustrated by examples given below. The examples provided in this specification are not to be considered limiting the scope of the invention but are to be considered as illustrative of how the invention is to be carried out with the specifically described examples. Any equivalents that can be used to get the same function or variations obvious to a person skilled in the art are to be considered included within the scope of this specification and claims.

EXAMPLES

EXAMPLE 1:

PREPARATION OF A LIPID CARRIER BASED NANOGEL WITHOUT A SURFACTANT

Lipid carrier based nanogel formulation without a surfactant was prepared for comparison purpose using the similar procedure as described for preparation of lipid carrier based nanogel formulation. This formulation was found to have lower drug loading and entrapment efficiency in comparison to surfactant
invented formulation. Steady state transdermal flux and skin deposition was also low for this formulation. When hydration media usually used in state of art i.e. water, PBS 6.8 or PBS 7.4 were used, the drug loading capacity was five times less i.e., 1 % w/w only.

<table>
<thead>
<tr>
<th>Compositions</th>
<th>Amount (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soya Phosphatidyl Choline</td>
<td>3.5%</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.5%</td>
</tr>
<tr>
<td>5-FU</td>
<td>5%</td>
</tr>
<tr>
<td>Butyl Hydroxy Toluene</td>
<td>0.05%</td>
</tr>
<tr>
<td>Hydrating media</td>
<td>q.s.</td>
</tr>
<tr>
<td>20% Tris buffer : 25% Ammonia solution (1:1)</td>
<td></td>
</tr>
<tr>
<td>Entrapment efficiency</td>
<td>32.23±0.9 %</td>
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<tr>
<td>Turbidity</td>
<td>0.2139</td>
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<td>Shape</td>
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EXAMPLE 2:
PREPARATION OF A LIPID CARRIER BASED NANOGEL INCORPORATING A SURFACANT

4.25 g of soya phosphatidyl choline, 0.75 g of Vitamin E TPGS or Sodium deoxy cholate were taken in a round bottom flask with butyl hydroxyl toluene in chloroform: methanol mixture (3:1) sufficient to dissolve the lipid, surfactant and butyl hydroxyl toluene. The organic solvent was removed by a means of evaporation under reduced pressure on a rotary evaporator (model Rotary evaporator, Perfit, India) to get a lipid film deposited on the
flask of the rotary evaporator. Final traces of the organic solvent were removed under vacuum by keeping in vacuum oven (model Perfit, India). The lipid film was hydrated with 5.0 mL 5-FU solution of 10 mg/mL in water or Phosphate buffer saline (PBS 6.8) or Phosphate buffer saline (PBS 7.4) or 20% Tris buffer: 25% Ammonia solution (1:1) by rotation to form lipid carrier suspended in the respective hydration medium, which was probe sonicated using probe sonicator (model VCX 505, Sonics, USA) for 10 minutes at 40% output frequency. From this, a lipid nano carrier dispersion was prepared by steps of subjecting the lipid nano carrier dispersion in 100-200 nm either to (i) rotary evaporation under reduced pressure of 15 psi in rotary evaporator, or (ii) freeze drying by first freezing to -40 degrees and then lyophilizing for 24 hours using Mini Lyodel freeze dryer (Delvac, India) with or without 20% sucrose as a cryoprotectant. The a lipid nano carrier dispersion obtained was 65 grams.

A hydrophilic gel was prepared by mixing 2.0 g of Carbopol 980 or 4.0 g carbopol 934 in 20 g of deionized water. The dispersion was allowed to hydrate and swell for 60 minutes, the dispersion was neutralized by 98% triethanolamine until the pH 5.5 was received, this mixture was agitated for 2 h with high speed stirrer until homogeneous clear hydrophilic gel is formed, Methyl and propyl paraben at 0.2 and 0.02% were dissolved in propylene glycol and added as a preservative. Remaining quantity of water was added with high speed stirring. The gel was allowed to equilibrate for at least 24 hours at room temperature prior to further use to make a lipid nano carrier based nanogel.
Lipid nano carrier dispersion prepared above was mixed with hydrophilic gel made above to get a lipid nano carrier based nanogel formulation. 100 g of final product of a lipid nano carrier based nanogel formulation was obtained.

EXAMPLE 3:

METHODS OF ANALYSIS

IN VITRO CHARACTERIZATION OF LIPID NANO CARRIER FORMULATION

Shape

For morphological characterization, Transmission Electron Microscopic (TEM) study using phosphotungastic acid as a negative stain was performed (Moragagni 268D FEI, Netherlands). A drop of the sample was placed on a carbon-coated copper grid to leave a thin film on the grid. Before the film dried on the grid, it was negatively stained with 1% phosphotungastic acid (PTA). A drop of the staining solution was added to the film and the excess of the solution was drained off with a filter paper. The grid was allowed to thoroughly dry in air and samples were viewed under a transmission electron microscope. A thin film of lipid nano carrier was spread on a slide and after placing cover slip it was observed under the optical microscope.

MAXIMUM AMOUNT OF DRUG LOADED

To determine the maximum amount of 5- FU that can be loaded in lipid nano carrier, formulations were prepared with 1% and 5% drug as using the
method described above as specified above. Drug loaded formulations were examined over a period of 14 days for the appearance of drug crystals under optical microscope.

5 ENTRAPMENT EFFICIENCY

The entrapment efficiency was determined after separating unentrapped drug by dialysis for 4 h in phosphate buffer saline (PBS 7.4). Drug loaded lipid nano carrier dispersion (2 mL) was taken into a cellulose acetate dialysis bag (Cellophane membrane, MW cut-off 12000-14000, HIMEDIA, India) immersed in 100 mL phosphate buffer saline (PBS 7.4) and magnetically stirred at 30 rpm. Samples, taken at time intervals from the receiver solution, were replaced with equal volumes of fresh solvent and drug content was analyzed by HPLC assay. The experiment was stopped when constant drug concentration values were obtained in subsequent withdrawals from the receiver phase (taking into account the progressive dilution of the medium). The dialyzed formulation was lysed using Triton-X 100 (0.1% v/v) and subsequently analyzed for drug content using HPLC assay. The percent of encapsulation efficiency EE %) was then calculated according to the following equation:

\[
EE\% = \frac{(Ct-Cr)}{Ct} \times 100
\]

Where Ct is the concentration of total drug and Cr is the concentration of free drug

EVALUATION OF NANOGEL
Visual appearance

The prepared nanogel was visually inspected for clarity, color and transparency. The prepared gels were also evaluated for the presence of any particles. Smears of gels were prepared on glass slide and observed under the microscope for the presence of any particle or grittiness.

Determination of pH

The pH of lipid nano carrier based Carbopol gels were determined by digital pH meter (Model E 181, kolkata, India). One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation for 30 min until constant reading obtained. The measurements of pH of each formulation were replicated three times and mean calculated.

Drug content

To 0.2 g of 5-FU nanogel formulation, weighed in a glass tube, was added in 20 ml of methanol and sonicated in a sonicator bath (TransonicT460/H, Elma, Germany). Volume of the solution was made up with water and drug content was assayed using HPLC method.

Extrudability study

This study was done for explaining whether the gel can be removed / or pressed out from the collapsible tube during application in proper manner or not. In conducting the test, a closed collapsible tube containing above 20 grams of gel was pressed firmly at the crimped end and a clamp was applied
to prevent any rollback. The cap was removed and the nanogel was thrust out until the pressure was dissipated.

5 Viscosity measurement

A Brookfield digital viscometer (Model RheolabQC SN80597032, US) with a suitable sample adaptor Accessories: TU1=C-LTD80/QC was used to measure the viscosity of the nanogel prepared in cp. Gels were poured into a removable sample chamber and the spindle was lowered perpendicularly. The spindle was rotated in the gel at increasing shear rates 10 to 100 rpm. At each speed, the corresponding shear stress and viscosity reading was noted.

Spreadability measurement

Spreadability of the formulation was determined by a locally fabricated apparatus. It consisted of a wooden block and provided with a pulley at one end. A rectangular ground glass plate was fixed on the wooden block. Excess of gel (about 2g) was placed on this ground glass plate, and then the gel was sandwiched between this plate and another glass plate having the dimensions of the ground glass plate attached with a hook. A 300 g weight was placed on the top of the two plates for 5 minutes to expel air and to provide a uniform film of the gel between the plates. Excess of the gel was scrapped off from the edges. The top plate was then subjected to a pull of 30 g with the help of a string attached to the hook and the time (in second)
required by the top plate to cover a distance of 12 cm was noted. The spreadability was calculated using the formula:

\[ S = \frac{mL}{t} \]

where, \( S \) = spreadability, \( m \) = weight tied to the upper glass slide, \( l \) = length of the glass slide and \( t \) = time taken in seconds.

**Skin Permeation and Deposition Study**

The *in vitro* skin permeation of 5 FU from different formulations was studied using Franz glass diffusion cell (ElectroLab, India) maintained at 37±1°C under non-occlusive conditions. The effective permeation area of the diffusion cell was 2.303 cm². The receptor compartment contained 6.0 ml phosphate buffer saline (PBS 6.8) and was constantly stirred at 100 rpm. Epidermal sheet of excised albino abdomen rat skin was mounted between the donor and the receptor compartment. 5-FU nanogel formulation (500 mg) was applied to the epidermal surface of skin. The samples (1 ml) were withdrawn through the sampling port of the diffusion cell at 1, 2, 3, 4, 5, 8, 18, and 24 hr time intervals and analyzed. An equal volume of fresh phosphate buffer maintained at 37±1°C was replaced into the receptor compartment after each sampling.

At the end of the permeation experiments the surface of the skin was washed five times with 50% ethanol to remove excess drug from the surface. The washing protocol was verified and was found to remove more than 95% of the applied dose at zero time. The skin was then cut into small pieces. The tissue was further homogenized with 50% ethanol (10 ml) and left for 24
hr at shaker incubator at 37+1 °C. After shaking for 5 min and centrifugation for 5 min at 3000 rpm, the 5 FU content in the upper phase was determined.

Skin localization Index

The marker 6-Carboxyflurescein (6-CF) loaded nanogel formulation was applied topically on the abdomen of the albino rat at a marked area of 1cm². The animals were caged individually after application of formulation and were sacrificed after 6 h of application. The skin was removed immediately, cut into pieces and washed with PBS. The skin was blotted and fixed in carny’s fluid The sections were viewed under Confocal Laser Scanning Microscope (CLSM, LSM 510, Carl Zeiss, Germany).

Skin Irritation Potential using Draize Test

The irritancy of different formulations was determined in male albino rabbits (1.9-2 kg) based on the method described by Draize et al. The animals were housed in an air-conditioned room (22±2.0 °C) and hair of the back was trimmed short, 24 h before the beginning of the assay. Three squares were drawn on each side of the back of each rabbit. The animals were divided into five groups each consisting of three animals. The first group did not receive any treatment and acted as a control, second group received 20% Sodium lauryl sulfate solution acting as a positive control. Third, fourth and fifth group received 5-FU solution, 5- FU nanogel (NG8) (see Table 5) and marketed formulation, respectively. At different time intervals of 0, 1, 24, 48 and 72 h after application the exposed area was scored for the erythema and oedema on grade of 0-4 as described by Draize score method.
**Hemolytic toxicity assay**

Cellular toxicity of carrier system using well known RBC lysis assay was conducted. Tested samples of 5-FU commercial/marketed formulation and lipid nano carrier formulations were diluted with 0.9% normal saline solution to concentration from 5000 mcg/ml to 78 mcg/ml. Human blood was freshly drawn from antecubital vein and centrifuged at 3000 rpm for 5-10 min and the supernatant was removed. The red blood cell (RBC) dispersion was diluted with saline solution to a concentration of 5% w/v. The RBC dispersion (0.5 ml) was mixed with distilled water, which was considered as producing 100% hemolysis, normal saline producing no hemolysis, hence acting as blank. The experiment was carried out on ELISA plate reader. After incubation at 37 °C for 4 hr in C02 incubator, the absorbance was taken at 540 nm against supernatant of normal saline diluted similarly as blank in ELISA plate reader. The percent hemolysis was thus determined for each sample by taking absorbance of distilled water as 100% hemolytic sample.

**HPLC Assay**

5-FU was estimated by the HPLC method as per official method. Degassed HPLC grade water was used as mobile phase and delivered at 1.0 mL min⁻¹. The injected fluid (20 µL) was eluted in C18 column at room temperature and 5-FU was monitored at 254 nm using a PDA detector (Agilent HPLC, 1200 Series, USA).

**STATISTICAL ANALYSIS**
Data are expressed as mean ± standard deviation (SD) of obtained results. Statistical analysis of the data was performed by analysis of variance (ANOVA) (Statpro, Version 2.01, and San Diego, CA). A p value <0.05 was considered statistically significant.

Table 1 and 2 summarizes the composition of 5-FU lipid nano carrier formulation for its topical administration.

Lipid nano carrier is vesicular carrier system morphologically similar to other vesicular carrier e.g. liposomes, niosomes but differ in composition and functionality. Lipid nano carrier consists of Phospholipid and surfactant as part of vesicle membrane in comparison to Phospholipid and cholesterol in conventional liposomal formulation. Penetration enhancer release in a sustained manner from vesicle membrane and facilitated the skin permeation and deposition of drug. Penetration enhancer used for preparation of lipid nano carrier formulation are biocompatible surfactant like Vitamin E TPGS, Sodium deoxy cholate. These surfactants also increase the fluidity of vesicle membrane results in increasing the encapsulation efficiency. In the present invention Vitamin E TPGS and sodium deoxycholate are selected as penetration enhancer for preparation of 5-FU lipid nano carrier formulation.
**Table 1: Composition and characterization of Lipid Nano Carrier formulation containing Sodium Deoxy cholate as a surfactant**

<table>
<thead>
<tr>
<th>Compositions</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soya Phosphatidyl Choline</td>
<td>5%</td>
<td>4.75%</td>
<td>4.5%</td>
<td>4.25%</td>
<td>4.0%</td>
<td>3.75%</td>
</tr>
<tr>
<td>Sodium Deoxy cholate</td>
<td>-</td>
<td>0.25%</td>
<td>0.5%</td>
<td>0.75%</td>
<td>1.0%</td>
<td>1.25%</td>
</tr>
<tr>
<td>5-FU</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
</tr>
<tr>
<td>Butyl Hydroxy Toluene</td>
<td>0.05%</td>
<td>0.05%</td>
<td>0.05%</td>
<td>0.05%</td>
<td>0.05%</td>
<td>0.05%</td>
</tr>
<tr>
<td>Hydrating media</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
</tr>
<tr>
<td>20% Tris buffer : 25% Ammonia solution (1:1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entrapment efficiency</td>
<td>71.19 ± 1.3</td>
<td>70.42± 1.3</td>
<td>69.47±2.2</td>
<td>79.39±1.5</td>
<td>54.38±2.8</td>
<td>44.23±1.8</td>
</tr>
<tr>
<td>Turbidity</td>
<td>0.2490</td>
<td>0.2487</td>
<td>0.3145</td>
<td>0.3356</td>
<td>0.2612</td>
<td>0.1347</td>
</tr>
<tr>
<td>Shape</td>
<td>Vesicular</td>
<td>Vesicular</td>
<td>Vesicular</td>
<td>Vesicular</td>
<td>Vesicular + Micelles</td>
<td>Micelles</td>
</tr>
</tbody>
</table>

Each value represents as mean ± standard deviation (n=3.)
Table 2: Composition and characterization of Lipid Nano Carrier formulation containing Vitamin E TPGS as surfactant.

<table>
<thead>
<tr>
<th>Compositions (%) w/w</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soya Phosphatidyl Choline</td>
<td>5%</td>
<td>4.75%</td>
<td>4.5%</td>
<td>4.25%</td>
<td>4.0%</td>
<td>3.75%</td>
</tr>
<tr>
<td>Vitamin E TPGS</td>
<td>-</td>
<td>0.25%</td>
<td>0.5%</td>
<td>0.75%</td>
<td>1.0%</td>
<td>1.25%</td>
</tr>
<tr>
<td>5-FU</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
</tr>
<tr>
<td>Butyl Hydroxy Toluene</td>
<td>0.1%</td>
<td>0.1%</td>
<td>0.1%</td>
<td>0.1%</td>
<td>0.1%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Hydrating media 20% Tris buffer : 25% Ammonia solution (1:1)</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
</tr>
<tr>
<td>Entrapment efficiency</td>
<td>72.27±2.2</td>
<td>74.67±2.5</td>
<td>75.27±2.7</td>
<td>80.24±3.2</td>
<td>56.21±2.4</td>
<td>49.17±1.6</td>
</tr>
<tr>
<td>Turbidity</td>
<td>0.2532</td>
<td>0.2765</td>
<td>0.3048</td>
<td>0.3423</td>
<td>0.2731</td>
<td>0.1412</td>
</tr>
<tr>
<td>Shape</td>
<td>Vesicular</td>
<td>Vesicular</td>
<td>Vesicular</td>
<td>Vesicular</td>
<td>Vesicular + Micelles</td>
<td>Micelles</td>
</tr>
</tbody>
</table>

Each value represents as mean ± standard deviation (n=3.)
Table 3: Optimization of hydration media

<table>
<thead>
<tr>
<th>Hydration media</th>
<th>Solubility of drug (mg/mL)</th>
<th>Maximum amount of drug loaded in formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1: Distilled water</td>
<td>10 mg/mL</td>
<td>1% w/w</td>
</tr>
<tr>
<td>No. 2: Phosphate buffer saline (PBS 6.8)</td>
<td>10 mg/mL</td>
<td>1% w/w</td>
</tr>
<tr>
<td>No. 3: Phosphate buffer saline (PBS 7.4)</td>
<td>10 mg/mL</td>
<td>1% w/w</td>
</tr>
<tr>
<td>No. 4: 20% Tris buffer : 25% Ammonia solution (1:1)</td>
<td>100 mg/mL</td>
<td>5% w/w</td>
</tr>
</tbody>
</table>
Table 4: Optimization of preparation method

<table>
<thead>
<tr>
<th>Preparation method</th>
<th>Amount of drug loaded in formulation</th>
<th>Appearance of drug crystal</th>
<th>Encapsulation efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotary evaporation sonication method using hydration medium no. 1, 2 and 3 of Table 3</td>
<td>1% and 5% w/w</td>
<td>No drug crystal was observed in 1% w/w but drug was precipitated out in 5% w/w formulation</td>
<td>68.31±2.0</td>
</tr>
<tr>
<td>Freeze drying method using hydration medium no. 1, 2 and 3 of Table 3</td>
<td>1% and 5% w/w</td>
<td>No drug crystal was observed in 1% and 5% w/w formulation</td>
<td>76.25±1.0</td>
</tr>
<tr>
<td>Freeze drying method using cryoprotectant using hydration medium no. 1, 2 and 3 of Table 3</td>
<td>1% and 5% w/w</td>
<td>No drug crystal was observed in 1% and 5% w/w formulation</td>
<td>78.39±1.5</td>
</tr>
<tr>
<td>Rotary evaporation sonication method using hydration medium no. 4 of Table 3</td>
<td>1% and 5% w/w</td>
<td>No drug crystal was observed in 1% and 5% w/w formulation</td>
<td>80.24±3.2</td>
</tr>
</tbody>
</table>

Each value represents as mean ± standard deviation, n=3.
Table 5: Composition and Characterization of lipid nano carrier based 5-FU nanogel formulation

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Carbopol 980 (%) w/w</th>
<th>Carbopol 1934 (%) w/w</th>
<th>5-FU Lipid nano carrier</th>
<th>Spreadability (g.cm/sec) (X ± S.D.)</th>
<th>Viscosity (cps)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG-1</td>
<td>0.25</td>
<td></td>
<td>LN-D</td>
<td>46.87±1.8</td>
<td>358</td>
<td>6.8</td>
</tr>
<tr>
<td>NG-2</td>
<td>0.35</td>
<td></td>
<td>LN-D</td>
<td>78.85±2.2</td>
<td>1250</td>
<td>6.9</td>
</tr>
<tr>
<td>NG-3</td>
<td>0.5</td>
<td></td>
<td>LN-D</td>
<td>105.02±4.1</td>
<td>2160</td>
<td>7.1</td>
</tr>
<tr>
<td>NG-4</td>
<td>0.75</td>
<td></td>
<td>LN-D</td>
<td>162.06±4.2</td>
<td>2990</td>
<td>6.9</td>
</tr>
<tr>
<td>NG-5</td>
<td>-</td>
<td>0.5</td>
<td>LN-D</td>
<td>76.4±1.9</td>
<td>1236</td>
<td>7.2</td>
</tr>
<tr>
<td>NG-6</td>
<td>-</td>
<td>0.75</td>
<td>LN-D</td>
<td>111.2±2.4</td>
<td>2050</td>
<td>6.0</td>
</tr>
<tr>
<td>NG-7</td>
<td>-</td>
<td>1.0</td>
<td>LN-D</td>
<td>125.0±3.1</td>
<td>2665</td>
<td>7.2</td>
</tr>
<tr>
<td>NG-8</td>
<td>-</td>
<td>1.5</td>
<td>LN-D</td>
<td>172.6±4.2</td>
<td>3072</td>
<td>6.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Carbopol 980 (%) w/w</th>
<th>Carbopol 1934 (%) w/w</th>
<th>5-FU Lipid nano carrier</th>
<th>Spreadability (g.cm/sec) (X ± S.D.)</th>
<th>Viscosity (cps)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG-9</td>
<td>0.25</td>
<td></td>
<td>*LN-J</td>
<td>52.34±1.2</td>
<td>374</td>
<td>6.7</td>
</tr>
<tr>
<td>NG-10</td>
<td>0.35</td>
<td></td>
<td>LN-J</td>
<td>80.49±3.4</td>
<td>1327</td>
<td>7.8</td>
</tr>
<tr>
<td>NG-11</td>
<td>0.5</td>
<td></td>
<td>LN-J</td>
<td>118.17±3.9</td>
<td>2238</td>
<td>7.0</td>
</tr>
<tr>
<td>NG-12</td>
<td>0.75</td>
<td></td>
<td>LN-J</td>
<td>167.12±4.5</td>
<td>3015</td>
<td>6.8</td>
</tr>
<tr>
<td>NG-13</td>
<td>-</td>
<td>0.5</td>
<td>LN-J</td>
<td>85.4±2.2</td>
<td>1328</td>
<td>6.4</td>
</tr>
<tr>
<td>NG-14</td>
<td>-</td>
<td>0.75</td>
<td>LN-J</td>
<td>120.4±3.1</td>
<td>2167</td>
<td>6.8</td>
</tr>
<tr>
<td>NG-15</td>
<td>-</td>
<td>1.0</td>
<td>LN-J</td>
<td>139.1±4.3</td>
<td>2706</td>
<td>7.0</td>
</tr>
<tr>
<td>NG-16</td>
<td>-</td>
<td>1.5</td>
<td>LN-J</td>
<td>181.4±6.7</td>
<td>3145</td>
<td>6.8</td>
</tr>
<tr>
<td>Mkt gel</td>
<td>-</td>
<td></td>
<td></td>
<td>156.01±7.3</td>
<td>2870</td>
<td>6.8</td>
</tr>
</tbody>
</table>

Each value represents as mean ± standard deviation (n=3.)

* Nanogel formulation of "optimized sodium deoxy cholate based lipid nano carrier formulation (LN-D) containing 5% 5-FU w/w

** Nanogel formulation of "optimized Vitamin E TPGS based lipid nano carrier formulation (LN-J) containing 5% 5-FU w/w

Mkt gel = Marketed 5-FU gel formulation
Table 6: Composition of Carbopoi gel (without drug)

<table>
<thead>
<tr>
<th>Compositions (% w/w)</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbopol 934</td>
<td>0.5-4.0%</td>
<td>-</td>
</tr>
<tr>
<td>Carbopol 980</td>
<td>-</td>
<td>0.25-2.0%</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>0.5%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Methyl Paraben</td>
<td>0.2%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Propyl Paraben</td>
<td>0.02%</td>
<td>0.02%</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>0.5%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Distilled water</td>
<td>q.s.</td>
<td>q.s.</td>
</tr>
</tbody>
</table>

Each value represents as mean ± standard deviation (n=3.)
Table 7: Skin Permeation Parameters for transport of 5 FU from rat skin

<table>
<thead>
<tr>
<th>S.No</th>
<th>Formulation Code</th>
<th>Jss* (µg/cm²/hr)</th>
<th>Drug deposited in the skin (µg)</th>
<th>Era</th>
<th>ERb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NG-8</td>
<td>126.717.2</td>
<td>5163188</td>
<td>3.2</td>
<td>18.1</td>
</tr>
<tr>
<td>2</td>
<td>NG-16</td>
<td>214.2±16.2</td>
<td>5697194</td>
<td>5.3</td>
<td>20.05</td>
</tr>
<tr>
<td>3</td>
<td>Marketed Formulation</td>
<td>80.69±4.2</td>
<td>1080122</td>
<td>1.4</td>
<td>3.8</td>
</tr>
<tr>
<td>4</td>
<td>5-FU solution</td>
<td>40.5511.3</td>
<td>28419.4</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Each value represents as mean ± standard deviation (n=3.)

*Jss = Steady state transdermal flux

ERa = Enhancement ratio, it is the ratio of transdermal flux from formulation to drug solution

ERb = Enhancement ratio, it is the ratio of drug deposited from formulation to drug solution

Marketed formulation containing 5% w/w of 5-FU in cream base

Table 8: Skin irritation score as per Draize method after application of different formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Erythema</th>
<th>Oedema</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 hr</td>
<td>24 hr</td>
</tr>
<tr>
<td>Sham control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Positive control 20% SLS</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>5-FU nanogel formulation (NG-8)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5-FU solution</td>
<td>0</td>
<td>0.66</td>
</tr>
<tr>
<td>Marketed gel</td>
<td>0</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Values represented as mean ± SD (n = 3).
Score are defined as 0 = no erythema, 1 = very slight erythema (light pink), 2 = well defined erythema (dark pink), 3 = moderate to severe erythema (light red), 4 = severe erythema (extreme redness).

Similarly defined for oedema.
CLAIMS

1. A lipid nano carrier based nanogel formulation of an active ingredient.

2. A lipid nano carrier based nanogel formulation of claim 1 for dermal delivery or topical application.

3. A lipid nano carrier based nanogel formulation of claim 2, wherein the said active ingredient comprises a hydrophilic drug.

4. A lipid nano carrier based nanogel formulation of claim 2, wherein the said active ingredient comprises a lipophilic drug.

5. A lipid nano carrier based nanogel formulation of claim 3, wherein the said active ingredient comprises a hydrophilic drug selected from a group 5-fluorouracil (5-FU), acyclovir, colchicine, diclofenac and glucosamine.

6. A lipid nano carrier based nanogel formulation of claim 3, wherein the said active ingredient comprises a lipophilic drug selected from a group isotretinoin, amphotericin B, dithranol, calcipotriol, coaltar and tacrolimus.

7. A lipid nano carrier based nanogel formulation of claim 5 comprising 5-FU.

8. A lipid nano carrier based nanogel formulation of claim 5 comprising 5-FU in a concentration of 0.001% to 10% w/w.

9. A lipid nano carrier based nanogel formulation of claim 2 comprising at least one active agent, a lipid, surfactant, hydrophilic...
polymer, alkalizing agent, cryoprotectant, buffer, preservative, antioxidant, hydrating fluid and pharmaceutically acceptable additives.

10. A lipid nano carrier based nanogel formulation of claim 9, wherein the lipid is a phospholipid selected from the group soya phosphatidylcholine, egg phosphatidylcholine, disteryl phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine.

11. A lipid nano carrier based nanogel formulation of claim 10 wherein the said phospholipid is in concentration range of 1-20% w/w.

12. A lipid nano carrier based nanogel formulation of claim 9, wherein the surfactant is selected from group of non-irritating surfactant.

13. A lipid nano carrier based nanogel formulation of claim 12 wherein the said non-irritating surfactant is selected from the group comprising Vitamin E TPGS, sodium deoxycholate, sodium taurocholate, polyoxyethylene lauryl ether, polyoxyethylene-2-oleyl ether, and polyoxyethylene-2-stearyl ether.

14. A lipid nano carrier based nanogel formulation of claim 13 wherein the said surfactant is used in concentration range of 0.25-5.0% w/w.

15. A lipid nano carrier based nanogel formulation of claim 9, wherein the hydrating fluid is selected from phosphate buffer saline (pH 6.8), phosphate buffer saline (pH 7.4) and 20% Tris buffer:25% Ammonia solution (1:1).
16. A lipid nano carrier based nanogel formulation of claim 9, wherein the hydrophillic polymers used as gel formers is selected from a cross-linked polymer,

17. A lipid nano carrier based nanogel formulation of claim 16 wherein the said cross-linked polymer is selected from the group comprising polyacrylate polymer, methyl cellulose, Poloxamer, hydroxy ethylcellulose and hydroxy propylcellulose.

18. A lipid nano carrier based nanogel formulation of claim 8 comprising 5-FU in a concentration of 5% w/w.

19. A lipid nano carrier based nanogel formulation of claim 8 having 60-80% of 5-FU in encapsulated form.

20. A lipid nano carrier based nanogel formulation of claim 8 wherein the said formulation has a good local bioavailability and sustaining the release of drug without showing the signs of skin irritation after the repeated administration.

21. A method of preparing lipid nano carrier based nanogel formulation of an active ingredient comprising the steps of:

a. preparation of drug loaded vesicular lipid carrier dispersion using a drug solution in a hydrating medium, wherein the vesicle membrane of the vesicular lipid carrier contains a surfactant;

b. probe sonicating the drug loaded vesicular lipid carrier dispersion of step (a.) to prepare a lipid nano carrier dispersion in nanomeric size range,
c. concentrating the lipid nano carrier dispersion in nanomeric range of step (b) by subjecting the same dispersion either to (i) rotary evaporation, or (ii) freeze drying with or without a cryoprotectant.

d. preparation of hydrophilic gel; and

e. mixing of lipid nano carrier dispersion of step (c.) with hydrophilic gel of step (b) to form a lipid nano carrier based nanogel formulation.

22. A method of claim 21 wherein:

a. preparation of drug loaded vesicular lipid carrier dispersion comprises steps of:

i. dissolving phospholipids, surfactant and others lipophilic additives including lipophilic drug to be used in a small quantity of an organic solvent sufficient to dissolve the lipid, surfactant and additives,

ii. removing the organic solvent by a means of evaporation or under reduced pressure, removing final traces under vacuum to get a lipid film deposited,

iii. hydrating - the deposited lipid film in a hydration medium by rotation to form lipid carrier suspended in the said hydration medium, wherein the said
hydration medium shall contain hydrophilic drug is intended for loaded

23. A method of claim 22 wherein the drug is 5-FU and loading achieved is 1% w/w comprising steps of:

a. hydration medium being water or Phosphate buffer saline (PBS 6.8) or Phosphate buffer saline (PBS 7.4).

b. dissolving 5-FU at a concentration of 10 mg/mL in the hydration medium,

c. hydrating the deposited lipid film claim 10 step (ii) with drug solution of step (b.) to form lipid carrier dispersed in the said hydration medium,

d. probe sonicating the drug loaded vesicular lipid carrier dispersion of step (a.) to prepare a lipid nano carrier dispersion in 100-200 nm or other nanometric size range,

e. concentrating the lipid nano carrier dispersion in nanometric range by subjecting the same dispersion either to (i) rotary evaporation, or (ii) freeze drying with or without a cryoprotectant.

f. preparation of hydrophilic gel; and

g. mixing of lipid nano carrier dispersion of step (e.) with hydrophilic gel of step (b) to form a lipid nano carrier based nanogel formulation.

24. A method of claim 22 wherein the drug is 5-FU and loading achieved is 5% w/w comprising steps of:
a. hydration medium being an alkalizing medium.

b. dissolving 5-FU at a concentration of 100 mg/mL in the hydration medium,

c. hydrating the deposited lipid film of claim 10 step (ii) with drug solution of step (b.) to form lipid carrier dispersed in the said hydration medium,

d. probe sonicating the drug loaded vesicular lipid carrier dispersion of step (a.) to prepare a lipid nano carrier dispersion in nanometric size range,

e. preparing a lipid nano carrier dispersion in nanometric range by steps of subjecting the lipid nano carrier dispersion in nanometric size range of step (b.) either to rotary evaporation.

f. preparation of hydrophilic gel; and

g. mixing of lipid nano carrier dispersion of step (e.) with hydrophilic gel of step (b) to form a lipid nano carrier based nanogel formulation.

25. A method of preparation of hydrophilic gel of claim 21 comprising steps of:

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a. adding and mixing appropriate quantity of a polymer to deionized water,

b. allowing the dispersion to hydrate and swell for 60 min.,

c. neutralize the dispersion with an alkali accompanied by gentle stirring,
d. agitating this mixture for 2 h with high speed stirrer until homogeneous clear hydrophilic gel is formed,
e. adding a preservative,
f. allowing to equilibrate the hydrophilic gel for at least 24 hours at room temperature prior to further use to make a lipid nano carrier based nanogel.

26. A method of preparation of lipid nano carrier based nanogel of claim 21 comprising step of mixing the lipid nano carrier dispersion with optimized hydrophilic gel under mechanical stirring for 1 h to result in to lipid nano carrier based Nanogel formulation.

27. A method of claim 24 wherein:
   a. the said solvent is selected from a group comprising chloroform, methanol and diethyl ether, and
   b. the amount of organic solvent in the mixture ranges from 1-2% w/w of the lipid, and
   c. said rotation for hydration are at 60 rev/min for 1-2 hr.

28. A method of claim 25 wherein:
   a. said polymer is a cross-linked polyacrylate polymer
   b. the said alkali is 98% triethanolamine,
   c. said neutralization is done to reach a pH of 5.5 - 7.1,
   d. said preservative is selected from the group methyl paraben, propyl paraben or benzyl alcohol.

29. A method of treating a dermal disease capable of treatment by topical administration of a therapeutically effective amount the
formulation of claim 7 containing therapeutically effective concentration of 5-FU.

30. A method of treating a dermal disease selected from acne, herpes lesions, inflammation, psoriasis cold sore, fungal and bacterial infection, wart, seborrheic eczema and cutaneous leishmaniasis by topical administration of a therapeutically effective amount the formulation of claim 7 containing therapeutically effective concentration of 5-FU.

31. A method of claim 21 wherein the nano-carrier dispersion is loaded with 5-FU.

32. A method of claim 31 wherein the said loading achieved is 0.5 to 5.0 % w/w similar to clinical recommended concentration 0.5%, 1.0%, 2.0% and 5.0% w/w for its topical administration

33. A method of claim 21 wherein the nano-carrier dispersion is loaded with acyclovir.

34. A method of claim 33 wherein the said loading achieved is 5.0 % w/w similar to clinical recommended concentration 5.0% w/w for its topical administration

35. A method of claim 24 wherein the said alkalizing medium is 20% Tris buffer:25% Ammonia solution (1:1).

36. A method of claim 30 of achieving enhanced Permeability and penetration of 5-FU reaching the dermis resulting in reduced risk of occurrence of Bowen's disease.

37. A lipid nano carrier based nanogel formulation of claim 7 comprising enhanced Permeability and penetration of 5-FU
reaching the dermis resulting in reduced risk of occurrence of Bowen's disease.
**Figure 2**

![Image of Figure 2](image-url)

<table>
<thead>
<tr>
<th>Peak</th>
<th>Diameter (nm)</th>
<th>Std. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.3</td>
<td>0.3</td>
</tr>
<tr>
<td>2</td>
<td>2.3</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>0.9</td>
<td>0.4</td>
</tr>
<tr>
<td>4</td>
<td>2.2</td>
<td>0.4</td>
</tr>
<tr>
<td>5</td>
<td>0.3</td>
<td>0.6</td>
</tr>
</tbody>
</table>

**Average**: 1.82 ± 0.2

**Cumulants Results**

- **Diameter** (d): 125.5 (nm)
- **Polydispersity Index (PDI)**: 0.284
- **Diffusion Coeff.** (D): 1 x 10^-5 cm^2/sec

**Measurement Condition**

- **Temperature**: 25°C
- **Pathic Name**: WATER
- **Refractive Index**: 1.337
- **Viscosity**: 1 x 10^-3 (cP)

- **Scattering Intensity**: 1 x 10^3 (cps)
Figure 3
Figure 4

Cumulative amount of drug release (μg)

Time (h)

- Marketed
- NG-8 (5%)
- NG-8 (7.5%)
- NG-8 (10%)
- drug sol
Figure 5

- Nanogel (NG-8)
- Marketed gel
- Drug solution
- Nanogel (NG-16)

Concentration (µg/mL) vs. % Hemolysis
# INTERNATIONAL SEARCH REPORT

## A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K9/127 A61K47/32

## B. MINIMUM DOCUMENTATION SEARCHED (CLASSIFICATION SYSTEM FOLLOWED BY CLASSIFICATION SYMBOLS)

A61K

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>GLAVAS-DODOV M ET AL: &quot;5-F1 uorouracil topical liposome gel s for anti cancer treatment—formulation and evaluation&quot;, ACTA PHARMACEUTICA, CROATIAN PHARMACEUTICAL SOCIETY, HR vol. 53, no. 4 1 December 2003 (2003-12-01), pages 241-250, XP009156709, ISSN: 1330-0075 Retrieved from the Internet: URL: <a href="http://pubic.carnet.hr/acphee/tapas">http://pubic.carnet.hr/acphee/tapas</a> pdf abstract page 242, paragraphs fourth, last page 243 -----</td>
<td></td>
</tr>
</tbody>
</table>

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

### Date of the actual completion of the international search

6 September 2012

### Date of mailing of the international search report

29/10/2012

### Name and mailing address of the ISA/Authorized officer

Vazquez Lantes, M
### INTERNATIONAL SEARCH REPORT

**Box No. II** Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. □ Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. □ Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. □ Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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**Box No. III** Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. □ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

   - 7, 8, 18-20, 23, 24, 27, 29-32, 35-37 (completely); 1-3, 5, 9-17, 21, 22, 25, 26, 28 (partially)

**Remark on Protest**

- [ ] The additional search fees were accompanied by the applicant’s protest and, where applicable, the payment of a protest fee.
- [ ] The additional search fees were accompanied by the applicant’s protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- [ ] No protest accompanied the payment of additional search fees.
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 7, 8, 18-20, 23, 24, 27, 29-32, 35-37 (completely); 1-3, 5, 9-17, 21, 22, 25, 26, 28 (partially)
   - Alipid nano carrier based nanogel formulation of 5-fluorouracil.

2. claims: 33, 34 (completely); 1-3, 5, 9-17, 21, 22, 25, 26, 28 (partially)
   - Alipid nano carrier based nanogel formulation of acyclovir.

3. claims: 1-3, 5, 9-17, 21, 22, 25, 26, 28 (all partially)
   - Alipid nano carrier based nanogel formulation of colchicine.

4. claims: 1-3, 5, 9-17, 21, 22, 25, 26, 28 (all partially)
   - Alipid nano carrier based nanogel formulation of diclofenac.

5. claims: 1-3, 5, 9-17, 21, 22, 25, 26, 28 (all partially)
   - Alipid nano carrier based nanogel formulation of glucosamine.

6. claims: 1, 2, 4, 6, 9-17, 21, 22, 25, 26, 28 (all partially)
   - Alipid nano carrier based nanogel formulation of isotretinoin.

7. claims: 1, 2, 4, 6, 9-17, 21, 22, 25, 26, 28 (all partially)
   - Alipid nano carrier based nanogel formulation of amphotericin B.

8. claims: 1, 2, 4, 6, 9-17, 21, 22, 25, 26, 28 (all partially)
   - Alipid nano carrier based nanogel formulation of diethanol.

9. claims: 1, 2, 4, 6, 9-17, 21, 22, 25, 26, 28 (all partially)
   - Alipid nano carrier based nanogel formulation of calcitriol.
10. claims: 1, 2, 4, 6, 9-17, 21, 22, 25, 26, 28 (all partially)
   A lipid nano carrier based nanogel formulation of coal tar.

11. claims: 1, 2, 4, 6, 9-17, 21, 22, 25, 26, 28 (all partially)
   A lipid nano carrier based nanogel formulation of tacrolimus.