FORMULATION AND EVALUATION OF NANO- NIOSOMAL TOPICAL GEL OF PACLITAXEL FOR SKIN CANCER

A THESIS SUBMITTED TO

Assam Science and Technology University (ASTU), Guwahati

In partial fulfillment for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

Under the guidance of

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CERTIFICATE FROM THE GUIDE

This to certify that the final dissertation project entitled **"**Formulation And Evaluation Of Nano-niosomal Topical Gel Of Paclitaxel For Skin Cancer**"** submitted to Assam Science and Technology University in the partial fulfillment of the requirement for the award of degree of Masters of Pharmacy in Pharmaceutics is a genuine and legitimate research work carried out by **NASRINA ABDIN** with Roll No **190520011011** and Regd. No.**388005219**, Dept. of Pharmaceutics at **Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS),** Azara, Guwahati-17, under my supervision and guidance.

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CERTIFICATE FROM THE PRINCIPAL

This to certify that the final dissertation project entitled **"Formulation and Evaluation of Nano- Niosomal of Nano-Niosomal Topical Gel Of Paclitaxl for Skin Cancer."** submitted to Assam Science and Technology University in the partial fulfillment of the requirement for the award of degree of Masters of Pharmacy in Pharmaceutics is a genuine and legitimate research work carried out by **NASRINA ABDIN** with Roll No. **190520011011** and Regd. No **388005219**, Dept. of Pharmaceutics at **Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS),** Azara, Guwahati-17.

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DECLARATION BY THE CANDIDATE

I hereby declare that the matter embodied in the dissertation entitled **"Formulation and Evaluation of Nano- Niosomal of Nano-Niosomal Topical Gel Of Paclitaxl for Skin Cancer"** is a bonafide and genuine research work carried out by me under the supervision of Dr. Bhanu Pratap Sahu M.Pharm, PhD HOD. Department of Pharmaceutics, Girijananda Chowdhury Institute of Pharmaceutical Science (GIPS), Hatkhowapara, Azara, Guwahati. The work embodied in this thesis is original and has not been submitted the basis for the award of any degree, diploma or fellowship in any other university or institution.

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List of Publication, Webinar, National and International Conferences and poster presentation

Publication

- Formulation and evaluation of nanoniosomal topical gel of paclitaxel for skin cancer (Research journal of pharmacy and technology)
- Recent nano technological approaches emphasis on liposomal based nano carriers and its future prospective in treating skin carcinoma 9 World journal of pharmaceutical research)
- A review on nano niosomes (World journal of pharmaceutical research)

Webinar

- Quantity by design in pharmaceutical industry, organized by pharma literati on 19th September 2020.
- International webinar on shaping peace together, organized by PRISAL pharmaceutical foundation, 20th September 2020
- World pharmaceutical day celebration, organized by MNR college of pharmacy on 25th September 2020
- Recent developments in Bio- pharmaceutical sciences, organized by MNR college of pharmacy, 8th February 2021

National Conferences

- Two days national seminar on current research in drug discovery and development, organized by department of pharmaceutical sciences, Dibrugarh university on 13th & 14th November
- Two days National conference on medicinal plants mega conference & exhibition on 13 & 14th January 2020

International Conferences

- International e- conference on "Nutrients Acquestion by plants and plant-based remedies against viral diseases on 7th & 8th august 2020, organized by internal quality assurance cell (IQAC), nougong college, nagoan, Assam in association with world researcher's association North eastern university of ayurvedic & homeopathy (CNF IAH), Govt of India shillong, Meghalaya.
- 3rd international conference on smart village & rural development (COSVARD 2020) by Dr Medalson Ronghang, department of civil engineering college Chandrapur, Kokrajhar.
- 3rd national conference on recent advances in science and technology

Poster Presentation

- Poster presented on two days national seminar on current research in drug delivery and development at Dibrugarh university, during 13th & 14th November, 2019
- Poster pre4sented on NCRAST on 16th, 17th, 18th & 19th of august 2020, organized by Assam science and technology university
- Poster presented on basic overview on covid – 19- pandemic on 16th may 2020 organized by Shirpur education society

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NASRINA ABDIN

LIST OF ABBREVIATIONS

S.No	Abbreviation used	Full form of Abbreviation
1	NDDS	Novel drug delivery system
2	%	Percentage
3	ml	Millilitre
4	USP	United States Pharmacopeia
5	^{0}C	Degree Celsius
6	FTIR	Fourier Transformer Infrared Spectrophotometer
7	g	Gram
8	mg	Milligram
9	µg	Microgram
10	µm	Micrometer
11	pH	Negative logarithm of hydrogen ion concentration
12	µg /ml	Microgram per millilitre
13	kg	Kilogram
14	L	Litre
15	min	Minute
16	ml/min	Millilitre per minute
17	hrs	Hours
18	nm	Nanometre
19	±	Plus-minus sign
20	g/mol	Grams to moles
21	λ max	Absorption maxima
22	Conc.	Concentration
23	abs	Absorbance
24	fig	Figure
25	RPM	Rotations per minute
26	KBr	Potassium bromide
27	DSC	Differential scanning calorimetry
28	UV	Ultra Violet
29	XRD	X- ray diffraction
30	HLB	Hydrophilic – lipophilic balance
31	SEM	Scanning Electron Microscopy
32	BCS	Biopharmaceutics Classification System
33	SD	Standard Deviation
34	RBF	Round bottom flask
36	PTX	Paclitaxel
37	No	Number

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CHAPTER-I

INTRODUCTION

1.1 Introduction

Skin cancer represents one of the most commonly occurring carcinoma in human and it is growing at a rate of one million new cases being reported annually [1]. Ultra-violet radiation is the prime contributing factor responsible for its occurrence [2]. It has been found that, the formation and histological alteration in skin cancer are orchestrated in the molecular level. The ultra-violet radiation generate and activates a wide range of molecules in the skin, where some of them act as receptors of ultraviolet radiation including DNA/RNA, reactive oxygen species (ROS) and several other as transcriptional targets [3]. Pro-inflammatory cytokines and environmental stress can induce the p38 MAPK cascade in the keratinocytes which in turn lead to the formation of skin cancer [4]. Ultra-violet ray activated p38 MAPK have profound effect on cell survival and apoptosis of the keratinocytes which leads to formation of skin cancer [5, 6, 7].

Research and study on skin cancer could bring down the suffering patient load in most of the countries throughout the world including America, Australia, and Africa as the novel drug delivery system will pave new routes towards its treatment throughout the globe. Rapid progress in the application of nanotechnology for therapy and diagnosis has made a new field called “nanomedicine” and related subfields such as “pharmaceutical nanocarriers”. Nanoscale aggregates called nanocarriers are available in various classes including: nanoparticles made of metals, polymers, hydrogel, ceramic; lipid based carriers such as liposomes and niosomes [8,9]; nanoburrs.[1]

A niosome is a non-ionic surfactant-based liposome. Niosomes are formed mostly by cholesterol incorporation as an excipient. Other excipients can also be used. Niosomes have more penetrating capability than the previous preparations of emulsions. They are structurally similar to liposomes in having a bilayer, however, the materials used to prepare niosomes make them more stable and thus niosomes offer many more advantages over liposomes. The sizes of niosomes are microscopic and lie

in nonometric scale. The particle size ranges from 10nm-100nm. [10,11] Nanomaterials having at least one dimension in the range between about 0.1 to 100 nm, exhibit novel physico-chemical proprieties that differ from the bulk material resulting in the novel characteristics. Niosomes contain two major components, including cholesterol and nonionic surfactants. Cholesterol provides rigidity and proper shape, while surfactants play a major role in the formation of niosomes. The non-ionic surfactants possess a hydrophilic head (non-polar) and a hydrophobic tail (Figure 2). The families of Spans (Span 20, 40, 60, 80, and 85), Tweens (Tween 20, 40, 60, and 80), and Brij (Brij 30, 35, 52, 58, 72, and 76) are commonly used as non-ionic surfactants in the preparation of niosomes.

Nanotechnological materials have been traditionally prepared by the top-down approach which involves the break-down of materials using techniques developed by solid state physicists. However, an alternative approach called “bottom-up” forms the foundations of nanochemistry and enables the synthesis of nanostructures and nanomaterials through the utilization of supramolecular and biomimetic materials. Both approaches have interfaces with biology and biomimetic chemistry, engendering the field of nanobiology [12]

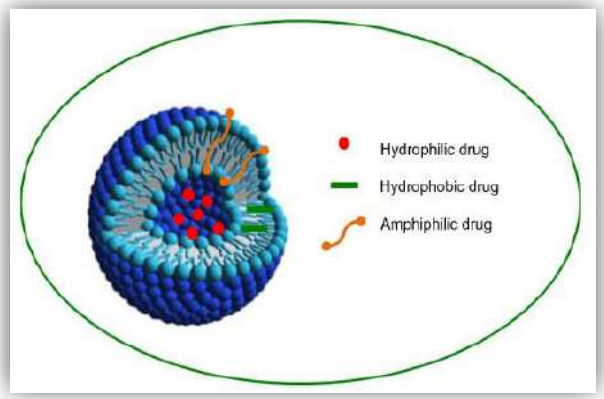


Figure 1.1. Structure of noisome and drug entrapment

1.2 ADVANTAGES

1. Niosome can accommodate a variety of drug moieties such as hydrophilic, lipophilic, as well as ampiphilic drugs.
2. They increase the stability of the entrapped drug.
3. Handling and storage of surfactants do not require any special conditions.
4. Can increase the oral bioavailability of drugs.
5. Can enhance the skin penetration of drugs.
6. They can be used for oral, parenteral as well topical.
7. The surfactants are biodegradable, biocompatible, and non-immunogenic.
8. Improve the therapeutic performance of the drug by protecting it from the biological environment and restricting effects to target cells, thereby reducing the clearance of the drug.
9. The niosomal dispersions in an aqueous phase can be emulsified in a non-aqueous phase to control the release rate of the drug and administer normal vesicles in external non-aqueous phase.

DISADVANTAGES

- Physical instability
- Aggregation
- Fusion
- Leaking of entrapped drug
- Hydrolysis of encapsulated drugs which limiting the shelf life of the dispersion.

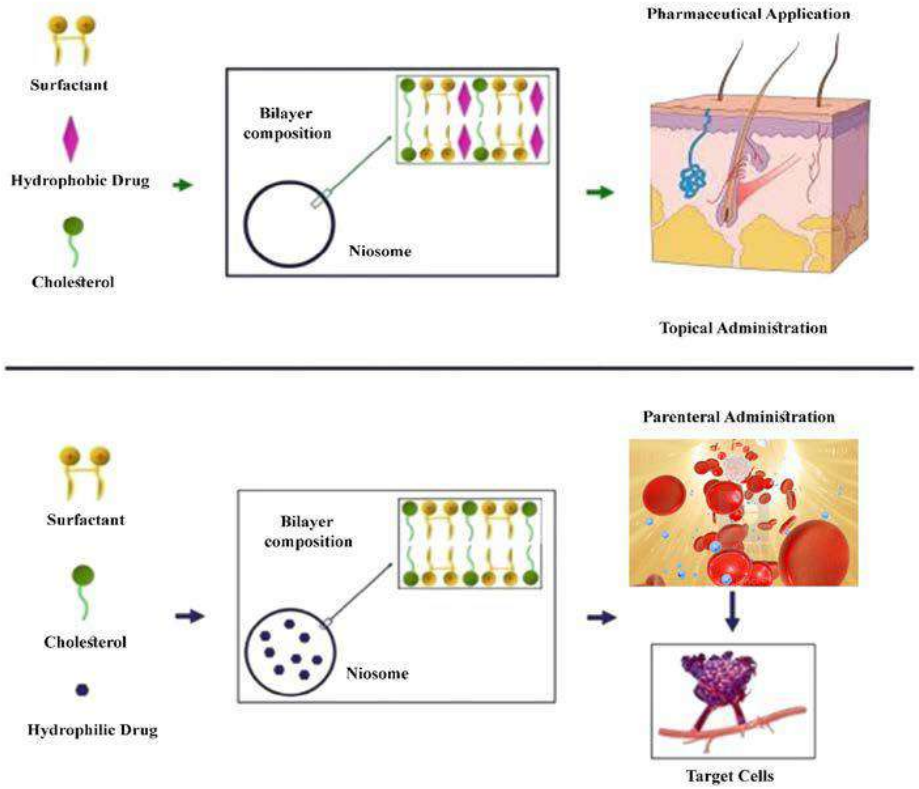


Figure 1.2. Formation of niosomes by non-ionic surfactant and cholesterol; entrapment of hydrophobic drug in vesicular membrane (up), entrapment of hydrophilic drug in aqueous part (down), topical administration to skin (up), and parenteral administration to target cells (down) (niosomes are similar to liposomes in having a bilayer.)

1.3 Preparation of Niosomes

a. **Thin-Film Hydration (TFH) Method:** The TFH method was first introduced by Bangham et al. for the preparation of liposomes [13]. TFH method is one of the most widely and simple methods utilized for the preparation of liposomes, which could be used for the preparation of the niosomes, as well. In TFH method, the surfactant and other additives are homogeneously dissolved in an organic solvent such as chloroform, or a mixture of organic solvents, in a round-bottom flask. Then, the solvent is evaporated completely using a rotary vacuum evaporator, and the thin film is obtained at the inner surface of the flask. The thin-film is then re-hydrated with an aqueous medium including water or phosphate buffer saline (PBS),

which commonly contain the drug for encapsulation. After the re-hydration is accomplished, MLV niosomes with various diameters are formed [14]. Recently, Ramadan et al. utilized TFH method for production of niosomes with various kind of surfactants and different ratios of surfactants to cholesterol. They encapsulated drug, timolol maleate which is used for treatment of glaucoma by lowering intraocular pressure (IOP), with an encapsulation efficiency (EE%) of 98.8%. [15]

b. **Solvent Injection (SI) Method:** In the SI method, the solvents such as diethyl ether, ethanol are used for dissolving the surfactants and other additives[16]. The homogenous solution is then put inside a syringe pump and is injected drop-wise through a needle to an aqueous solution (may contain drug) at a constant temperature, which is higher than the boiling temperature of the organic solvent. The remaining organic solvent is completely evaporated by a rotary vacuum evaporator. During this evaporation process, unilamellar vesicular niosomes are formed with different sizes, and the entrapped aqueous volume is relatively higher than other methods [17,18,19]. Kakkar and Kaur utilized this method for preparation of elastic niosomes composed of span 60 and tween 80 for encapsulation of lipophilic drug, ketoconazole. They used ethanol as solvent for span 60 and ketoconazole, the solution of which being injected into aqueous phase containing tween 80 [20] Reverse Phase Evaporation (REV) Method: The Rev. method was described by Szoka and Papahadjopoulos in 1978, for the preparation of LUV [21]. Two phases, namely organic and aqueous, are prepared beforehand. The organic phase is made from a mixture of ether and chloroform, containing a solution of surfactants and additives for membrane formation. The aqueous phase is usually water or PBS, in which the drug is dissolved inside. The organic phase is mixed with the aqueous phase, and then the mixture is vigorously shaken or sonicated to obtain an emulsion. Then, the organic phase is slowly evaporated by a rotary vacuum evaporator at a constant temperature, during which LUV niosomes are begun to form. The evaporation process is completed when the hydration of all of the niosomes are completed [22,23,24]. Villate-Beita et al. prepared niosomes with Rev. method. The cationic lipid DOTMA and squalene

were dissolved inside dichloromethane, and non-ionic surfactants were put into aqueous phase. Then, dichloromethane is added to the aqueous phase and emulsified. After the niosomes are prepared, plasmid DNA is added inside niosome solutions to obtain nioplexes [25].

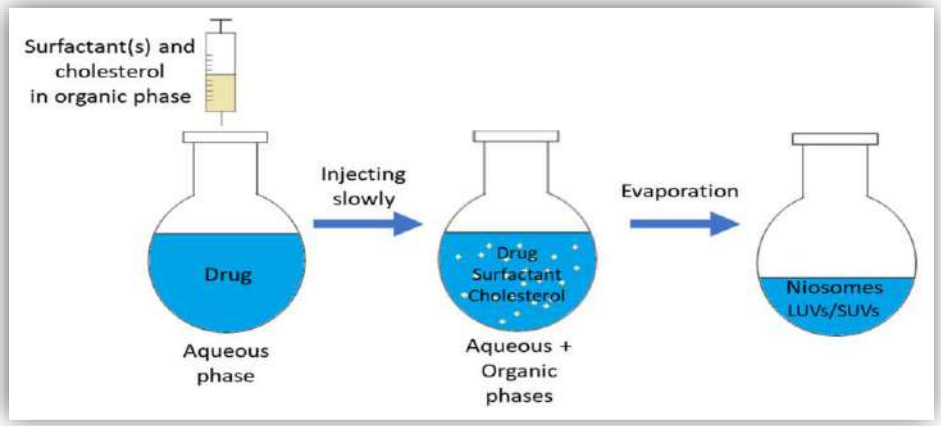


Figure 1.3. Schematic representation of Solvent Injection (SI) Method.

c. **The Bubble Method:** In the bubble method, niosomes are prepared without the help of organic solvents. The surfactants and additives are mixed in an aqueous phase such as PBS, and then the solution is transferred to a three-neck round-bottom flask. The three-neck flask is then positioned inside a water bath for controlling the temperature. The dispersion of surfactants and additives are occurred at 70 ◦C. At the start, by utilizing high shear homogenizer, the homogenous dispersion is obtained with stirring for 15–30 s, followed by the bubbling with nitrogen gas of solution at 70 ◦C [26,27,28].

d. **Freeze and Thaw Method:** The freeze and thaw method is an improved method for niosome preparation, which is derived from the TFH method. MLV niosomes suspension which was prepared by the TFH method is frozen in liquid nitrogen, and then thawed in a water bath for a number of cycles with short periods of time [29] Dehydration–Rehydration Vesicles (DRV) Method The DRV method was first explained by Kirby and Gregoriadis, in which they used SUVs

prepared by the TFH method, to form MLVs [30]. Briefly, SUVs, prepared by the TFH method, were separated by centrifugation. Afterward, SUVs were added to the aqueous phase with a drug, and this suspension was freeze-dried overnight. After rehydration of dried product, multilamellar DRVs were generated.

e. **Microfluidization Method:** The microfluidization method is developed recently for preparation of vesicular particles. In this method, two fluidized streams of organic and aqueous phases are moved forward through to a specific micro-scale channel and are interacted with very high speeds within the interaction chamber. The interface, where the two phases interact with each other and breach the thin liquid film, is arranged in a specific way that energy given to the system remains within the site of niosome production. [31,32]. Seleci et al. produced PEGylated niosomes that are encapsulated with topotecan for anti-glioma treatment, with utilizing microfluidic channel. Briefly, they dissolved span 60, cholesterol and PEG in chloroform (organic phase), and topotecan in aqueous phase. Then, organic phase and aqueous phase are given from different inlets and mixed inside the microfluidic channel, and channel is heated to 65 ◦C. The prepared niosomes collected from outlet. The drug EE% of niosomes were higher than 37.5% with sizes between 100 and 200 nm [33]

f. Supercritical Carbon Dioxide Fluid (scCO2) Method: In recent years, the scCO2 method was first demonstrated by Manosroi et al. as a novel niosome preparation method [34]. Briefly, they put surfactant, cholesterol, PBS with glucose and ethanol into a glass view cell, which had two windows and fixed volume. Then, CO2 was introduced to the system's view cell, while the pressure and the temperature are maintained at 200 bar and 60 ◦C, respectively. Niosomes are obtained after 30 min of magnetic stirring and the pressure is then released. LUV niosomes are obtained by this method with a size range from 100 to 440 nm. The main advantage of the scCO2 method is the one-step process that uses no toxic, inflammable, and volatile organic solvent [35,36].

g. **Heating Method (HM):** The HM is developed recently by Mozafari for the preparation of nano-carrier systems [37]. Briefly, the surfactants, cholesterol, and

drug are added to the aqueous phase such as PBS. The solution is prepared by stirring and heating the aqueous phase. Then, 3% v/v polyol such as glycerol is added to the solution. This method does not use any toxic, volatile organic solvent, and is introduced as an easy one-step process [38,39]

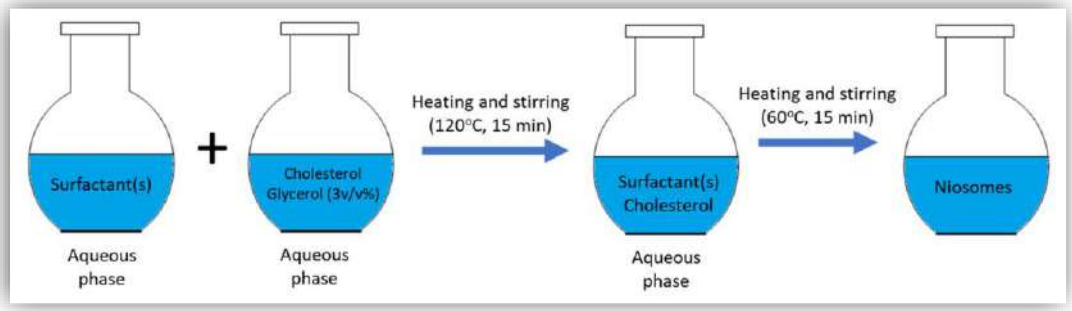


Figure 1.4. Schematic representation of Heating Method.

h. **Pronisome Method:** Proniosome has been utilized as a stable precursor for the production of niosomes for drug delivery applications. Proniosomes are made by coating some kind of water-soluble molecule (carrier) e.g., sorbitol, maltodextrin or mannitol, with non-ionic surfactants. The product of this method is dry, free-flowing formulations of a thin-film of surfactants coated onto the carriers. Proniosome-derived niosomes are then prepared by rehydrating the proniosome powder inside hot water with agitation, thus MLV niosomes are formed [40] The method has various advantages including good chemical and physical stability for longstanding storage, ease of transportation, and suitability to scale-up [41] Zeng et al. produced tacrolimus loaded niosomes by utilizing proniosome method. As an immunosurpressive agent, tacrolimus (FK506) is used for preventing the rejection reactions of corneal grafts. Briefly, surfactant, lipid, cholesterol and drug were mixed with ethanol. Then PBS added inside the mixture at 65 ◦C for 5 min. The gel of proniosomes was stored until reconstitution of niosomes.[42]

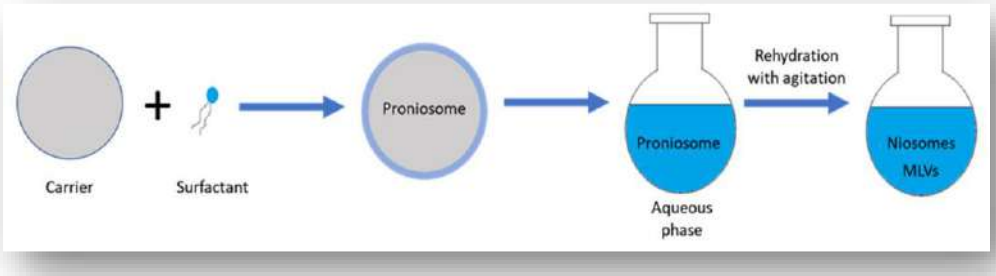


Figure 1.5. Schematic representation of noisome method.

1.4. Characterization of drug and mechanism

Paclitaxel is used as chemotherapeutic agent with the broad spectrum of activity for anticancers in humans. In 1992, paclitaxel was approved by the U.S. Food and Drug Administration (FDA) as Taxol for the use of advanced ovarian cancer. Two years later, it was also approved for treatment of metastatic breast cancer. This drug was originally isolated in 1971from the bark of the Pacific yew tree, *Taxus brevifolia*. Taxanes are the family of microtubule inhibitors. As a member of this family, paclitaxel suppresses spindle microtubule dynamics. This activity results in the blockage of the metaphase-anaphase transitions, and ultimately in the inhibition of mitosis, and induction of apoptosis in a wide spectrum of cancer cells.

Additional anticancer activities of paclitaxel have been defined that are independent of these effects on the microtubules and may include the suppression of cell proliferation as well as anti-angiogenic effects. Based on the drug targeting of a fundamental feature of the cancer phenotype, the mitotic complex, it is not surprising that paclitaxel has been found to be active in a wide variety of cancers in humans. The evidence in support of paclitaxel's broad anticancer activity and introduces the rationale for, and the progress in development of novel formulations of paclitaxel that may preferentially target cancers and that are not associated with the risks for hypersensitivity in dogs. On this note, a novel nanoparticle formulation of paclitaxel that substantially limits hypersensitivity was recently given conditional approval by the FDA Center

for Veterinary Medicine for use in dogs with resectable and nonresectable squamous cell carcinoma and nonresectable stage III, IV and V mammary carcinoma.

1.5. Evaluation methods

1.5.1. Preformulation Study: FTIR, zeta potential, Drug Entrapment, drug content, spreadibility, pH, viscosity, in vitro.

1.5.2. Zeta Potential (ζ): The zeta potential of the SLN were determined by performing the same dilution as done for particle size analysis and was measured by using a clear disposable Zeta cell for zeta potential analysis by electrophoretic mobility method using the Zetasizer NanoZS 90

1.5.3. Drug Entrapment Efficiency and Drug Loading Determination:

A volume of 2.0ml of each drug loaded sample was centrifuged at 12500rpm for 45min to separate the lipid and aqueous phase. The supernatant was then diluted with ethanol and analyzed by UV-VIS spectrophotometer (UV-1800 Shimadzu Spectrophotometer) at 228.20nm. The entrapment efficacy and drug loading of nanoparticle was calculated using:

$$EE = (W\alpha - Ws) / W\alpha \times 100 \quad \text{(i)}$$

$$DL = (W\alpha - Ws) / (W\alpha - Ws + Wl) \times 100 \quad \text{(ii)}$$

Where EE is entrapment efficiency, DL is Drug loading, Wα stands for the weight of PTX added to the formulation and Ws is the analyzed weight of drug in supernatant and Wl is weight of lipid added.[**43,44,45]**

1.5.4. FTIR Study

The compatibility study was carried out for the drug, excipients, physical mixture of the drug with excipients and lyophilized formulation SLN-F33 by scanning them over a wavenumber range of 4000-400cm-1 in FTIR spectrophotometer (Alpha E, Bruker Alpha, Ettlingen, Germany).

1.5.5. pH measurement

The pH of the gels was measured using a digital pH meter (Beckman Instruments, Fullerton, CA, USA).[46]

1.5.6. Viscosity measurement

Viscosity measurements of the gels were determined using a cone and plate rotary viscometer (Haake Inc., Vreden, Germany). The temperature was maintained at 37±0.5°C. One gram of each formulation was placed on the viscometer plate with a diameter of 2.9 cm. The torque value "S" was determined and the speed value "N" was maintained at 256 rpm. The following equation was applied for calculation of the viscosity:

$$\eta = GSN$$

where η is the viscosity in mPa s (mPa s=1 cP), G is the instrumental factor (14,200 mPa s/scale grade min), S is the torque (scale grade), and N is the speed (rpm)

1.5.7. pH of The Topical Gel

About 20gm of topical gel was subjected to pH measurement using a digital pH meter within 24h of manufacture [47]

1.5.8. Spreadability Study of SLN Gel

The spreadability of the gel formulation for SLN-F3 3G and PTXG was determined after 24h of preparation, by measuring the spreading diameter of 1gm gel between two horizontal plates (20 x 20cm2) after 1min. The standardized weight tied on the upper plate was 220g. The spreadability was calculated by using the following formula:

$$S = M \times L/t \quad \text{.....(iii)}$$

Where S is spreadability, M is weight tied on upper slide. L is the lengtof glass slide, t is time taken [**48].**

1.5.9. *In Vitro* Release Studies

In vitro release studies of niosomes suspension were carried out by dialysis bag method ("as discussed by Sathali and Rajalakshmi [11]"). A dialysis sac was washed and kept in distilled water for soaking. The vesicle suspension was pipetted into a bag made up of tubing and sealed followed by placing the dialysis bag into a beaker containing 500 mL of PBS pH 7.4. The beaker was placed over magnetic stirrer (50 rpm) and the temperature was maintained at 37°C ± 0.5°C. Samples were withdrawn at predetermined time intervals. Sink condition was maintained throughout the experiment by replacing the withdrawing sample with fresh medium. Samples were diluted and analysed for drug content by using UV/visible spectrophotometer at 278 nm[49]

DRUG PROFILE (Paclitaxel)

1.6. Description:

NAME – Paclitaxel

Brand name- Abraxane, Taxol

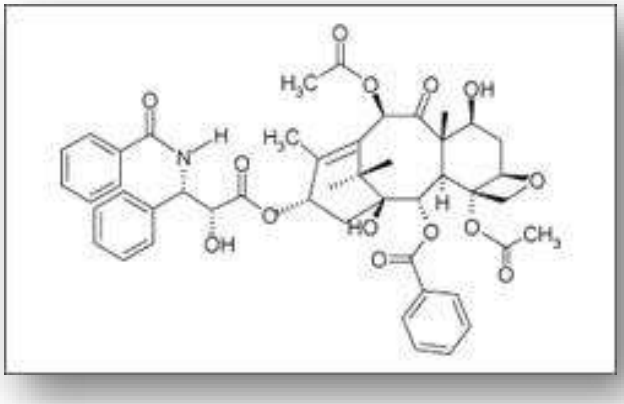
Generic Name- Paclitaxel

1.6.1. Identification, Chemical and physical Properties:

IUPAC NAME: 5 beta,20-Epoxy-1,2a,4,7 beta,10 beta,13 alpha-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2 R,3S)-N-benzoyl-3-phenylisoserine

CHEMICAL FORMULA: $C_{47}H_{51}NO_{14}$

CHEMICAL STRUCTURE:



Molecular weight- 853.906 g/mol

Solubility: Paclitaxel is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF), which should be purged with an inert gas. The solubility of paclitaxel in these solvents is approximately 1.5 mg/ml in ethanol and approximately 5 mg/ml in DMSO and DMF. Paclitaxel is sparingly soluble in aqueous buffers.

Bioavailability-6.5%

Protein Binding- 89 to 98%

Half-life- 5.8 hours

Melting point- 213°C

1.6.2. Therapeutic use:

- Paclitaxel for the treatment of ovarian cancer.
- Today the drug is used for a several types of cancer, including ovarian, breast, small cell and large cell lung cancers, and Kaposi's sarcoma.
- Paclitaxel is administered as a series of intravenous injections.

Administration: The usually effective dose Paclitaxel is injectable solution 6mg/mL for adults through Intravenous.

1.6.3. Mode of action: Paclitaxel-treated cells have defects in mitotic spindle assembly, chromosome segregation, and cell division. Unlike other tubulin-targeting drugs, such as colchicine, that inhibit microtubule assembly, paclitaxel stabilizes the microtubule polymer and protects it from disassembly.

1.6.4. Absorption: When a 24 hour infusion of 135 mg/m^2 is given to ovarian cancer patients, the maximum plasma concentration (Cmax) is 195 ng/mL, while the AUC is 6300 ng•h/mL.

1.6.5. Route of elimination: In 5 patients administered a 225 or 250 mg/m2 dose of radiolabeled paclitaxel as a 3-hour infusion, a mean of 71% of the radioactivity was excreted in the feces in 120 hours, and 14% was recovered in the urine.

1.6.6. Contraindication: The drug may cause birth defects. Patients should not take this drug if they are pregnant or plan to become pregnant. Breast feeding should also be avoided by patients taking paclitaxel. Immunosuppression may lead to increased risk of infection. Patients should tell their clinician if they are currently taking any other medications

1.6.7. Adverse effect: Common side effects include reduction in bone marrow function which may result in anemia, blood in stools or black stools, fast or irregular heartbeat, fever, chills, lower back pain, numbness or tingling of the hands or feet, mouth sores, painful, bloody, or difficult urination, swelling of the face, lips, or throat, nausea, vomiting, or diarrhea, unusual bleeding or bruising, wheezing or trouble breathing, hair loss.

1.6.8. Toxicity: Rat (ipr) LD_{50}=32530 µg/kg. Symptoms of overdose include bone marrow suppression, peripheral neurotoxicity, and mucositis. Overdoses in pediatric patients may be associated with acute ethanol toxicity

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CHAPTER-II

LITERATURE

SURVEY

2. LITERATURE SURVEY

2.1 Literature review

Brian E. Kilfoyle et al., (2012) TyroSpheres offer potential in the treatment of psoriasis, a disease resulting from over-proliferation of keratinocytes in the basal layer of the epidermis, by (a) enabling delivery of paclitaxel into the epidermis at concentrations >100 ng/cm^2 of skin surface area and (b) enhancing the cytotoxicity of loaded paclitaxel to human keratinocytes (IC_{50} of paclitaxel-TyroSpheres was approximately 45% lower than that of free paclitaxel). A potential topical psoriasis therapy has been developed consisting of tyrosine-derived nanospheres (TyroSpheres) with encapsulated anti-proliferative paclitaxel. TyroSpheres provide enhancement of paclitaxel solubility (almost 4,000 times greater than PBS) by effective encapsulation and enable sustained, dose-controlled release over 72 hours under conditions mimicking skin permeation. TyroSpheres were incorporated into a gel-like viscous formulation to improve their flow characteristics with no impact on homogeneity, release or skin distribution of the payload. The reports shows here, that the TyroSpheres provide a platform for paclitaxel topical administration allowing skin drug localization and minimal systemic escape.

Xiaoyan Yang et al., (2015) This study was conducted to development of formulations for hydrocortisone butyrate (HB)-loaded poly(D,L-lactic-*co*-glycolic acid) nanoparticles (PLGA NP) which will be suspended in thermosensitive gel for the improvement of ocular bioavailability of HB for the treatment of bacterial corneal keratitis. PLGA NP dispersed in thermosensitive gels can be considered as a promising drug delivery system for the treatment of anterior eye diseases. PLGA NP with different surfactants such as polyvinyl alcohol (PVA), pluronic F-108 and chitosan were prepared using oil-in-water (O/W) emulsion evaporation technique. NP was characterized with respect to particle size, entrapment efficiency, polydispersity, drug loading, surface morphology, zeta potential, and crystallinity. *In vitro* release of HB from NP showed a biphasic release pattern with an initial burst phase followed by a sustained phase. Such burst effect was completely eliminated when nanoparticles were suspended in thermosensitive gels and

zero-order release kinetics was observed. In HCEC cell line, chitosan-emulsified NP showed the highest cellular uptake efficiency over PVA- and pluronic-emulsified NP ($59.09 \pm 6.21\%$, $55.74 \pm 6.26\%$, and $62.54 \pm 3.30\%$, respectively) after 4 h. However, chitosan-emulsified NP indicated significant cytotoxicity of 200 and 500 μg/mL after 48 h, while PVA- and pluronic-emulsified NP exhibited no significant cytotoxicity.

Ashraf Alemi et al., (2018) This study investigated the augmentation of therapeutic effectiveness with the co-administration of paclitaxel (PTX; an effective chemotherapeutic drug for breast cancer) and curcumin (CUR; a chemosensitizer) in an MCF-7 cell line. The systemic administration of cytotoxic chemotherapeutic agents for cancer treatment often has toxic side effects, limiting the usage dose. To increase chemotherapeutic efficacy while reducing toxic effects, a rational design for synergy-based drug regimens is essential. So they optimized niosome formulations in terms of surfactant and cholesterol content. Afterward, the novel cationic PEGylated niosomal formulations containing Tween-60: cholesterol:DOTAP:DSPE-mPEG (at 59.5:25.5:10:5) were designed and developed to serve as a model for better transfection efficiency and improved stability. The optimum formulations represented potential advantages, including extremely high entrapment efficiency (~ 100% for both therapeutic drug), spherical shape, smooth-surface morphology, suitable positive charge (zeta potential $\sim +15$ mV for both CUR and PTX), sustained release, small diameter (~ 90 nm for both agents), desired stability, and augmented cellular uptake. Furthermore, the CUR and PTX kinetic release could be adequately fitted to the Higuchi model. A threefold and 3.6-fold reduction in CUR and PTX concentration was measured, respectively, when the CUR and PTX was administered in nano-niosome compared to free CUR and free PTX solutions in MCF-7 cells. When administered in nano-niosome formulations, the combination treatment of CUR and PTX was particularly effective in enhancing the cytotoxicity activity against MCF-7 cells. Most importantly, CUR and PTX, in both free form and niosomal forms, were determined to be less toxic on MCF-10A human normal cells in comparison to MCF-7 cells. The findings indicate that the combination therapy of

PTX with CUR using the novel cationic PEGylated niosome delivery is a promising strategy for more effective breast cancer treatment.

Rituraj Bharadwaj et al., (2016) In this study they attempted to prepare formulation of solid lipid nanoparticle (SLN) of paclitaxel for the effective treatment of various form of skin carcinoma. The SLN were prepared by high speed homogenization and ultrasonication method. The prepared SLN were characterised. The optimized paclitaxel SLN were loaded in carbopol gel. The prepared gels were evaluated for its gelling properties and finally studied for in-vivo anti-cancer efficacy and histopathological study. The particle size distribution was found to be in the range of 78.82 nm - 587.8 nm. The product yield (%) was found between 60-66% and showed a highest entrapment efficiency of 68.3%. The in-vitro release of the drug from SLN dispersion was found to be biphasic with the initial burst effect, followed by slow release. SLN loaded gel were subjected to permeability study and the results show steady-state flux (Jss), permeability coefficient (Kp), and enhancement ratio were significantly increased in SLN loaded gel formulation as compared with paclitaxel loaded gel. The histopathological study clearly reveals the efficacy of the SLN-F3 3G in the treatment of skin cancer. The experimental formulations show controlled release of paclitaxel and thus expected to show reduce dose related side effects.

Robin Foa et al., (1994) Taxol (paclitaxel), an anti-microtubule agent extracted from the needles and bark of the Pacific yew tre*eTaxus brevifolia*, has shown a remarkable anti-neoplastic effect in human cancer in phase I studies and early phase II and III trials thus far conducted. This has been reported primarily in advanced ovarian and breast cancer, although significant activity has also been documented in small-cell and non-small-cell lung cancer, head and neck cancers, and with lower activity in metastatic melanoma. The clinical utilization of Taxol had been previously somewhat restricted by its limited availability, a limitation that has recently been overcome by combined efforts of pharmaceutical, agricultural, and governmental agencies. In this review we shall address the pre-clinical data which have led to the use of Taxol in man, the main clinical results

thus far obtained, the toxicities associated with its use, current ongoing trials and future clinical directions of this promising agent.

Ibrahim A.Alsarra et al., (2005) Niosomes are nonionic surfactant vesicles that have potential applications in the delivery of hydrophobic and hydrophilic drugs. Permeation of a potent nonsteroidal anti-inflammatory, ketorolac, across excised rabbit skin from various proniosome gel formulations was investigated using Franz diffusion cells. Each of the prepared proniosomes significantly improved drug permeation and reduced the lag time (P<0.05). Proniosomes prepared with Span 60 provided a higher ketorolac flux across the skin than did those prepared with Tween 20 (7- and 4-fold the control, respectively). A change in the cholesterol content did not affect the efficiency of the proniosomes, and the reduction in the lecithin content did not significantly decrease the flux (P>0.05). The encapsulation efficiency and size of niosomal vesicles formed by proniosome hydration were also characterized by specific high performance liquid chromatography method and scanning electron microscopy. Each of the prepared niosomes achieved about 99% drug encapsulation. Vesicle size was markedly dependent on the composition of the proniosomal formulations. Proniosomes may be a promising carrier for ketorolac and other drugs, especially due to their simple production and facile up.

Ebtsam M Abdou et al., (2014) as treatment of vaginal fungal infection depends mainly on slow release of the drug and prolonged contact time of the delivery system with the vaginal mucosa, proniosomal gel was used as promising candidate to achieve this target. Terconazole, antifungal drug, proniosomal gels were developed based on span 60 and Brij 76 in different molar ratios (1:1, 1:1.5 and 1:2) relative to cholesterol. Proniosomal formulations were hydrated to form niosomes by incorporating into 1% carbopol gel. Proniosomal gel formulations were evaluated for their Entrapment Efficiency (EE %) and vesicle size. Increasing the molar ratio of cholesterol relative to surfactant has affected both EE and vesicle size of prepared niosomes. Drug release

profile from different prepared proniosomal gel formulations in simulated vaginal fluid (SVF) was studied in comparison to the commercial product of terconazole for 24 hours. Depending on the high EE% and in-vitro release profile of formulation SC1.5 (1:1.5 span60: cholesterol), it was selected for further evaluations of stability, mucoadhesion to the vaginal mucosa and inhibition of candidas growth. Results indicated that the selected formula, SC1.5, showed good stability and provided higher mucoadhesion and retention time then the commercial product which resulted in more efficient in-vitro inhibition of candida albicans.

Ketul K. Patel et al., (2012) the aim was to develop niosomal gel as a transdermal nanocarrier for improved systemic availability of lopinavir. Niosomes were prepared using thin-film hydration method and optimized for molar quantities of Span 40 and cholesterol to impart desirable characteristics. Comparative evaluation with ethosomes was performed using ex vivo skin permeation, fluorescence microscopy, and histopathology studies. Clinical utility via transdermal route was acknowledged using in vivo bioavailability study in male Wistar rats. The niosomal formulation containing lopinavir, Span 40, and cholesterol in a molar ratio of 1:0.9:0.6 possessed optimally high percentage of drug entrapment with minimum mean vesicular diameter. Ex vivo skin permeation studies of lopinavir as well as fluorescent probe coumarin revealed a better deposition of ethosomal carriers but a better release with niosomal carriers. Histopathological studies indicated the better safety profile of niosomes over ethosomes. In vivo bioavailability study in male Wistar rats showed a significantly higher extent of absorption (AUC0→∞, 72.87 h×μg/ml) of lopinavir via transdermally applied niosomal gel as compared with its oral suspension. Taken together, these findings suggested that niosomal gel holds a great potential of being utilized as novel, nanosized drug deliveryvehicle for transdermal lopinavir delivery.

R. Nazari-Vanania et al., (2019) Capecitabine (CAP) is an FDA-approved and frequently used chemotherapeutic agent for the treatment of various cancers. However, there are some side effects and chemoresistance limiting its use. Nanotechnological approaches can enhance the efficacy of anticancer drugs. In this study, CAP-loaded nanoniosomes were prepared. Nanoniosomes were prepared by the method of thin film hydration wherein CAP was loaded into the nanoniosomes. Nanoniosomes were then characterized by field emission scanning electron microscopy and (particle) vesicle size analysis. The cytotoxicity effect of the nanoniosomes were evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. CAP was loaded into the nanoniosomes and loading capacity and entrapment efficiency were determined. The vesicle size of the nanoniosomes was obtained in the nanometer scale, and CAP release profiles from the nanoniosomes were also obtained. Finally, the cytotoxicity effect of CAP and CAP-loaded nanoniosomes were evaluated toward MCF7 and PANC1 cell lines. The nanoniosomes with an amphipathic structure can penetrate into the cells with an enhanced release rate. These caused the toxicity of drug in the nanoniosomes to be higher than the free drug.

Priya Batheja et al., (2011) Tyrosine-derived nanospheres have demonstrated potential as effective carriers for the topical delivery of lipophilic molecules. In this investigation, a gel formulation containing nanospheres was developed for effective skin application and enhanced permeation. Carbopol and HPMC hydrophilic gels were evaluated for dispersion of these nanospheres. Sparingly water soluble diclofenac sodium (DS) and lipophilic Nile Red were used as model compounds. DS was used to determine the optimum polymer type, viscosity and release properties of the gel while fluorescent Nile Red was used in in vitro and in vivo skin distribution studies. In addition, the effect of a penetration enhancer, Azone, on the skin delivery was investigated. Dispersion of Nile Red-loaded nanospheres in 1% w/v HPMC gel produced a uniform and stable dispersion with suitable rheological properties for topical application, without any short-term cellular toxicity or tissue irritation. Invitro permeation studies using human cadaver skin

revealed that the deposition of Nile Red via the nanosphere gel in the upper and lower dermis was 1.4 and 1.8 fold higher, respectively, than the amount of Nile Red deposited via an aqueous nanosphere formulation. In vivo, the HPMC gel containing Nile Red-loaded nanospheres significantly enhanced (1.4 fold) the permeation of Nile Red to the porcine stratum corneum/ epidermis compared to the aqueous Nile Red-loaded nanospheres. An additional increase (1.4 fold) of Nile Red deposition in porcine stratum corneum/epidermis was achieved by incorporation of Azone (0.2 M) into the nanosphere gel formulation. Therefore, tyrosine-derived nanospheres dispersed in gels offer promise for the topical delivery of lipophilic drugs and personal care agents to skin for treatment of cancers, psoriasis, eczema, and microbial infections.

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CHAPTER 3

AIM AND OBJECTIVE

III. AIM & OBJECTIVE OF THE STUDY

3.1. Aim of the study:

The aim of the study is to Formulate and evaluate of nano- niosomal topical gel of paclitaxel for skin cancer.

3.2. Objectives of the study:

The objective of the study is

- For the development of paclitaxel loaded nanoniosomal topical gel by using thin film hydration methods
- Characterization, optimization and in-vitro, in-vivo evaluation of the optimised formulation for anticancer effect.

3.3. Plan of work:

1. **Literature review**.

2. Pre formulation study.

i. **Organoleptic properties**: colour, order, taste.

ii. **Solubility studies:** The purpose of the test is to determine how much of a solvent that can be dissolved in a solute, in other words, the highest concentration of a solute in a solvent. From a pharmaceutical perspective solubility tests can be used to determine: Maximal concentration that can be used in an in vitro activity assay.

iii. **Melting point determination:** The purpose of melting and boiling points in a lab experiment is to use them to help identify unknown substances. By taking a melting point of an unknown solid, we can compare it to a list of potential solids ad their meltig points and make a match to identify the solid.

iv. **Determination of pH:** This study is to calculate pH of formulation approximates the negative of the base 10 logarithm of the molar concentration of hydrogen ions in solution. More precisely, pH is the negative of the base 10 logarithm of the activity of the hydrogen ion.

v. **DSC:** the detection of transitions such as melts, glass transitions, phase changes and curing. DSC is used to determine the thermal phase transition in samples and interactions between the components of hydrogels, cyclodextrins, lipids and surfactants.

vi. **FTIR spectroscopy:** This study is performed to identify and characterize unknow materials i.e., films, solids, powers or liquids. Identify contamination on or in a material e.g., particles, fibers, powders or liquids. Identify additives after extraction from a polymer matrix.

3. Analytical method development:

i. Determination of Absorption Maxima (λ Max) in Phosphate Buffer
ii. Preparation of Standard Calibration in Phosphate Buffer

4. Formulation development:

i. Preparation of nano-niosomal topical gel of drug for skin cancer by thin film hydration method.

5. Evaluation and optimization:

i. Particle size analysis- This parameter gives information on the size distribution of particles. This can be used to calculate different properties of a particle and how they will act under certain conditions.

ii. Vesicle morphology- Shape and morphology of empty niosomal formulations and drug loaded niosomal formulations were determined by optical microscopy.

iii. Gel strength- Gel Strength is a measure of the ability of a colloidal dispersion to develop and retain a gel form. In the gelatine world, gel strength is traditionally referred to as Bloom. It is the force, expressed in grams, necessary to depress by 4mm the surface of a gelatine gel with a standard 0.5" diameter cylinder probe.

iv. Spreadability study- Spreadability of gels, creams, ointments and lotions is the net result of a combination of rheological contributions, of which viscosity is just one. In the study both the flow (i.e. viscosity) and structural (rigidity and strength) behaviors are compared.

v. Drug loading (loading capacity) and Drug entrapment efficiency – These two important parameters of nano-medicines, this reflects the mass ratio of drugs to nano-medicines and drug loading efficiency reflects the utilization of drugs in feed during the nano-medicine preparation process.

$$EE = \frac{weight\ of\ drug\ loaded}{weight\ of\ drug\ added}$$

$$LC = \frac{weight\ of\ drug\ loaded}{weight\ of\ nanoniosome}$$

vi. Zeta potential- To improve formulation stability and shelf life and reduce formulation time and cost.

vii. SEM- Scanning Electron Microscopy or SEM analysis provides high resolution imaging useful for evaluating various materials for surface fractures, contaminants or corrosion.

viii. TEM – This study is performed, to identify the growth of layers, composition and defects in semiconductors. To analysis the quality, shape, size and density of quantum wells, wires and dots high resolution is used. (Transmission electron microscopy (TEM) was used to determine the morphology of the niosomal vesicles. Few drops of optimized niosomal formulation (NC_2) were deposited on a carbon-coated copper grid and examined under transmission electron microscope.)

6. **In-vitro anticancer study-** In-vitro drug release testing, measures the release of active pharmaceutical ingredient (API) from the drug product matrix in controlled laboratory environment.
7. **In- vivo animal study-** This study is to stimulate the pathology of skin carcinoma of human in mice. Use of cell lines with high through screening is the primary screening method, due to the limitations like less relevance with clinical conditions further screening using suitable module.
8. **Histopathological study-** To check the skin cancer healing efficiency of the optimized gel by comparing with the standard marketed formulation and also by comparing with the non tested (healthy) animals after sacrificing of both the groups of animals in-vivo by taking mice as suitable animal.

•Animal checked by inducing chemical carcinogen DMBA (with acetone) per mice topically. After the induction period of 6weeks the formation of lesion on mice skin will be inspected. Therefore the treatments of gel (1.5 % paclitaxel) will be initiated according to the study design.
•The histological changes in the induced mice (in groups) skin cancer & normal mice skin needs to be studied after sacrificing the animal with cervical dislocation.
•Tumor inhibition, tumor reduction rates needs to be studied to evaluate the efficacy of the formulation.

CHAPTER-4

EXPERIMENTAL METHODOLOGY

IV . EXPERIMENTAL METHODOLOGY

4.1 List of equipments

Table 4.1: list of equipments used in the experiment

s.no	Name of equipments	Manufacturer
1	Fourier Transform Infrared	Spectroscopy (ATR), Bruker, ALPHA
2	Differential Scanning Calorimetric (DSC)	PerkinElmer , DSC 4000
3	High- Performance Liquid Chromatography (HPLC)	(Binary) Waters 515
4	Texture Analyser	Stable Micro System FD/1-077
5	Brook field Viscometer	Brook field , LBDV
6	Zeta Sizer	Malvern , (Nano-s90)
7	UV- Spectrophotometer	Shimadzu 1700 UV-Visible, spectrophotometer Japan
8	Dissolution Test Apparatus	Dissolution test apparatus-TDT-06T, Electrolab , Mumbai , India.
9	PH -Meter	Systronic, 361-micro pH meter
10	Balance	Sartorious electronic balance
11	Melting point apparatus	Macroscientific work 10A/UA, janwahar nagar, Delhi

12	Magnetic stirrer with hot plate	Rolex, India
13	Thermostatic water bath	Denver instrument, USA
14	Rotary flash evaporator	Labline stock centre, Mumai
15	Homogenizer	1KA T25 Digital Ultra Tunrak, Germany

4.2 List of materials

Table 4.2 List of materials used in the experiment

s.no	Ingredients	Source
1	Paclitaxel	Gips , chemical store
2	Cholesterol	Gips , chemical store
3	Span 40,60, 80	Gips , chemical store
4	Methanol	BS. Trading Rabinra, Sarani, Howrah
5	Chloroform	Gips , chemical store
6	Acetone , methanol	Krisshna Enterprise , Bhatta Bulding, PanbazarGuwahati
7	Disodium hydrogen phosphate	Gips , chemical store
8	Sodium hydrogen phosphate	Gips , chemical store
9	Whattman filter paper	S. D. Fine Chemicals Ltd., Mumbai, India

10	Sodium chloride, potassium chloride	Gips , chemical store

4.3. Pre formulation study

To detect the compatibility of drugs excipients used in the prepared formulations, the following preformulation studies were been carried out-

4.3.1. Organoleptic properties:

The organoleptic properties of the drug sample evaluated for its state, color, odor, and taste [1] Physical Appearance and homogenity of gel was observed visually, physical appearance includes inspection of color & texture of the gel and the result was obtained.

4.3.2. Solubility study:

The solvents are selected on a Polarity basis and the solubility is checked. A minute quantity of the drug taken in a test tube and solubility of the drug determined by dissolving the drug in 10ml of various solvents like methanol, ethanol, Dimethyl sulfoxide, Isopropanol, distilled water, chloroform , acetone, dimethyl formamide (DMF) and aqueous buffer.

4.3.3. Melting point determination:

A little amount of the drug sample in a dry capillary tube of 1mm internal diameter forming a column about 3mm high. Head the melting point apparatus to a temperature 5- 10°c below the expected temperature of melting and adjusting the heating so that the temperature in the chamber rises about 1°c per minute.

4.3.4. Fourier transforms infrared spectroscopy (FT-IR):

An FTIR spectrum reveals the characteristic peaks of all functional groups, present in a sample. In FTIR spectroscopic study, pure drug of PTX (Paclitaxol) performed individually and these physical mixture of an excipients were scanned over a wave

number range of 4000 - 400 cm^{-1} in FTIR Spectrophotometer (Alpha, Bruker model no: 100 59736, Germany)

4.3.5. Determination of pH

The pH of the individual gel (0.3&0.4) was delivered by using digital pH meter. Before measurements, pH meter was calibrated and readings were taken as by clipping the glass rod into the gel formulations.

4.3.6. Differential Scanning Calorimetric (DSC):

The physical state of drug inside the nano-niosome will be investigated by Differential scanning calorimetry (DSC). The thermo gram of the drug lead to nano-niosomes will be obtained by using DSC (Dever, instrument, USA). Accurately weighed samples were heated in aluminum pan using drug nitrogen as the efficient gas. The analysis was performed with a heating range of 20-300°c and at an increasing temperature rate of 10°c/minute.

4.4. ANALYTICAL DEVELOPMENT:

4.4.1. Determination of Absorption Maxima (λ Max) in Phosphate Buffer

Determination of absorption Maxima (λ max) in 7.4 phosphate buffer saline solution- 10ml of drug (PTX) was weight accurately and dissolved in 10ml of phosphate buffer saline solution in a volumetric flack (stock solution 1 mg/ml) The stock solution it diluted to make a concentration of (10mg/ml).The spectrum of this solution was run in the 400-800 mm range in a UV.

4.4.2. Preparation of standard calibration curve of drug quantitative by HPLC method

In this preparation method standard stock solution of PTx loaded nano-niosomes were prepared separately in the mobile phase and standard solution were prepared by dilution by using a pre-plotted calibration curve constructed to determine the

concentration of PTx in the samples was determined the concentration of PTx in the samples was determined.

Samples of PTx loaded nano-niosomes were analyzed using a water HPLC (MA, USA) equipped with a UV-Visible detector and Eurosphere C18 column (5µm, 4.6 mm x 250mm) from knauer (Berlin Germany). A mixture of methanol – acetonitrile water at a ratio of 75:75 v/v was used as mobile phase in the isocratic condition at a flow rate of 1ml min^{-1} for PTx analysis. The separation was carried out at temperature 20±1°c. The mobile phase was filtered through a 0.22 µm Millipore membrane filter and degassed by a wire ultrasonic cleaner for 5 mins before use.

The total run time was 4 min with a retention time of 2.7±0.1 min. The solvent flow rate was 1.6ml min^{-1}. A 60 µl volume of samples were injected into the system. Samples detection was carried out at 272nm. Standard solutions were prepared I the mobile phase as a serial concentration such as 100(10µ), 50 (5µ), 20(2.5 µg), 10(1.0 µg), 5(0.5 µg) in a range of 30-500 µg/ml of PTx loaded nano-niosomes. Standard calibration curves were plotted for PTx loaded nano-niosomes by taking absorbance vs. concentration.

4.5. FORMULATION DEVELOPMENT:

4.5.1. Drug loaded nano-niosome were performed by following the high speed homogenization and ultra-sonication method.

- Accurately weighed and measured excipient are taken in a clean and dry beaker and add organic solvents chloroform and methanol (ratio of 2:1) are added to dissolve all the excipients.

↓

- This mixture taken for magnetic stirring with the 500 rpm for 1 hour.

↓

- This solution is transferred in a round bottom flask attached to the rotatry flash evaporator at the temperature of 40-45°c for 1hour.

↓

- After the evaporation of the organic solvents it forms thin film, this film is suspended in the 7.4 phosphate buffer saline solution and it is heated for 1hour with the temperature at 45°c.

↓

- After 1 hour of heating the solution is transferred in the conical flask, it is attached to the rotary shaker for 1 hour with the rpm of 80.

↓

- After 1 hour, solution is transferred into the beaker and kept for homogenizer by maintaining the rpm 500 for 1 hour.

↓

- This solution was re-suspended with the distilled water of with the quantity of 1000µl and 800µl to prepare the final sample solution by using 20-22µl micropipettes.

↓

- Then, this solution was kept into the vortex shaker for 10 minutes (sample was taken into enclosed plastic tube).

↓

- To check the particle size solution was transferred to the zeta seizer (nano-s90) instrument, results were noted.
-

4.5.2. Preparation of nano-niosomal gel (blank gel)

- The nano-niosomal blank gel was prepared by taking 30 mg of carbopol 940 in 10ml of distilled water

↓

- and kept aside sometime for absorption then placed the mixture to the stirrer by maintained at the rpm at 120 and to the solution added NaOH (approximately 5ml) drop until a gel solution is formed.

↓

- Evaluation of nano-niosomal gel

FORMULATION TABLE

Table 4.3: Formulation of placebo using Span80

sno	Span80	soyalecithin	cholesterol	chloroform	methanol
F1	200 mg	100 mg	80 mg	8ml	4ml
F2	Span 60 145mg	200 mg	100 mg	10ml	5ml

Table 4.4: Formulation of placebo using Span 60

SLNO	Span 60	Cholesterol	soyalecithin	chloroform	methanol
F3	20mg	10mg	nil	12	6
F4	20mg	10mg	_	10ml	nil

Table 4.5: Formulation of placebo using Span 80

SLNO	Span 80	Cholesterol	soyalecithin	chloroform	methanol	Size	PDI
F5	50 mg	25mg	_	12	6	603.9	0.199
F6	50mg	25mg	25mg	12	6	762.3	0.136

Table 4.6: Formulation of placebo using Span 80

SLNO	Span 80 mg	Cholesterol	chloroform	methanol	Buffer	Size	PDI
F7	20 mg	10mg	12	6	10	762.3	0.136
F8	20mg	10mg	12	6	20	337.3	0.556
F9	20	10	20	0	20	477.6	0.349
F10	20mg	10mg	20ml	0	10	410.1	0.135

Table 4.7: Formulation with drug using Span 80

Formulation	Drug(PTX) (1:0.5:1)	Cholesterol	Span(80)	Chloroform (ml) (1:1)	Buffer (7.4)	Sonication	Size	PDI
F1	**20mg (2%)**	**10mg**	**20mg**	**12ml**	**12ml**	**30 min**	**237**	**0.2**
F2	**20mg (2%)**	**10mg**	**Span 60 (20mg)**	**12ml**	**12ml**	**30 min**	**295**	**0.5**
F3	**20mg**	**10mg**	**20mg span (80)**	**12ml**	**12ml**	**1 h**	**156**	**0.1**
F4	**20mg**	**10mg**	**Span 60 (20 mg)**	**12ml**	**12ml**	**_**	**249**	**0.3**

F5	**20**	**10mg**	**20(0.2%)**	**10**	**10**	**1 h**	**237**	**0.2**
F6	**20**	**20**	**20**	**10**	**10**	**1h**	**215**	**0.2**
F7	**40**	**10**	**20**	**10**	**10**	**1h**	**180**	**0.2**
F8	**20**	**20**	**20**	**10**	**10**	**1h**	**179**	**0.2**

4.6. EVALUATION AND OPTIMIZATION:

4.6.1. Particle size analysis:

The particle size and the poly dispersity index (PDI) of the SLN were determined by using dynamic light scattering after dispersing 1mg of lyophilized sample with 2ml of mili and water in zetasizer (Nano 75 -90) (Malve instruments, worestershire, UK). The measurement was at 25°c at a scattering angle of 90°.

4.6.2. Vesicle morphology:

It involves the measurement of size and shape of proniosomal vesicles. Size of niosomal vesicles can often measured by dynamic light scattering method in two conditions: without agitation and with agitation. Largest vesicle sizes are resulted by the hydration without agitation.

4.6.3. Gel strength:

Gel Strength is a measure of the ability of a colloidal dispersion to develop and retain a gel form. In the gelatine world, gel strength is traditionally referred to as Bloom. It is the force, expressed in grams, necessary to depress by 4mm the surface of a gelatine gel with a standard 0.5" diameter cylinder probe.

4.6.4. Spreadability study:

The spreadability of the gel formulation was determined after 24 hour of preparation by measuring the spreading diameter of 1gm of gel between two horizontal plates ($20\times20cm^2$) after 1min and the results was obtained.

4.6.5. Drug loading (loading capacity) and Drug entrapment efficiency:

Dialysis method and centrifugation method separation of un-entrapped drug from the niosomal suspension will be carried out. In dialysis tube the niosomal suspension to which one side of the tube is attached with the dialysis membrane. This tube is suspended in the 100 ml of 6.8 pH buffer, which was stirred on a magnetic stirrer. Through the dialysis membrane the un-entrapped drug and niosomal suspension were separated in the medium. After 6hrs of exhaustive dialysis the entrapped drug was estimated by UV spectrophotometric method. Optical density values were also noted.

4.6.5. Zeta potential:

The zeta potential of the nano-niosomes was determined by performing the same dilution as done for particle size analysis by electrophoretic mobility method using the zeta sizer nano-2s-90 (malvem instruments, worce tershire UK).

4.7. In-vitro anticancer study:

The release studies of noisomal formulations of drugs were performed using dialysis tube containing 2 ml of noisomal dispersion placed into appropriate containers (or small beakers) containing 25 ml of water/ethanol mixture of 70:30 ratios (v/v). Containers (or breakers) were placed in at ambient temperature and agitated with a rotation speed of 100 rpm. Collected samples in predetermined times. The release studies were performed in triplets and mean values were considered. Drug release mechanisms and kinetics are two characteristics of the dosage forms which play an important role in describing the drug dissolution profile.

In-vitro drug release studies were performed for all the niosomal gel formulations using Franz diffusion cells with dialysis membrane under the following conditions.

Diffusion test parameters:

- Medium : 15ml of pH7.4phosphate buffer

- Rotation speed : 100 RPM
- Temperature : 37±0.5°C
- Sampling volume : 3ml
- Time points : 0,0.25,0.5,1,2,4,6,8,12,24 hrs.

At pre-determined time intervals samples (3ml) were collected and replaced with same volume of fresh medium. The drug content in the samples was estimated using UV-visible spectrophotometer at 216 nm.

CHAPTER-V

RESULTS AND

DISCUSSION

5. RESULTS AND DISCUSSIONS

5.1 Pre-formulation Study of Drug

5.1.1 Organoleptic properties

The organoleptic properties like colour, odour, and taste were observed and the results were compared with the official requirement and found to be acceptable. The results are shown in Table 5.1.

Table 5.1: Organoleptic properties of drug

Sample	Taste	Colour	Odour
Paclitaxel	Bitter	Off white	Strong/ unpleasant

5.1.2 Solubility study

The solubility was checked in common solvents depending on their polarity such as water, span 20, span 60, span 80, tween 20, tween 80, pH-5.8, pH- 6.8, pH- 7.4. The results are shown in figure- 5.1, detailed explanation Table 5.2

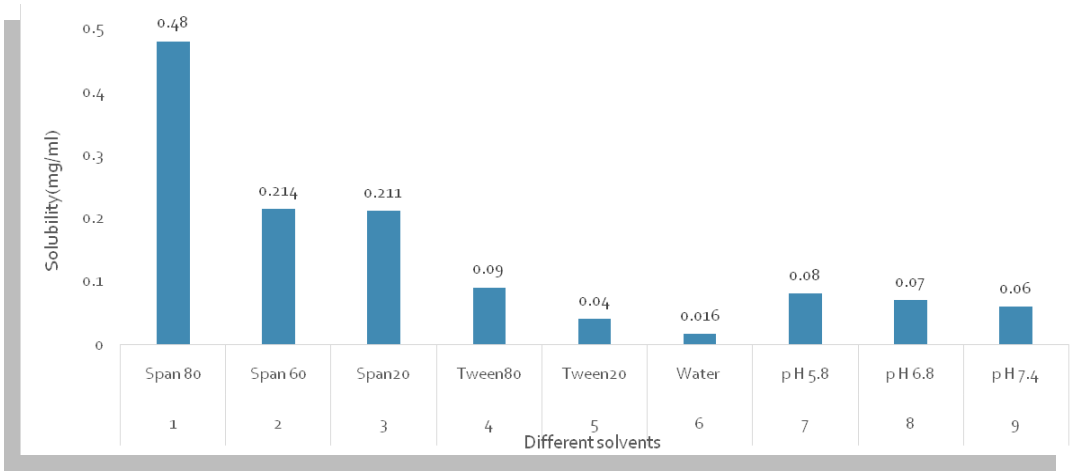


Figure 5.1: Solubility study of paclitaxel in different solvents

Table 5.2: Solubility study data

S.NO	VEHICLE	SOLUBILITY
1	Span 80	0.48
2	Span 60	0.214
3	Span 20	0.211
4	Tween 80	0.09
5	Tween 20	0.04
6	Water	0.016
7	pH 5.8	0.08
8	Ph 6.8	0.07
9	pH 7.4	0.06

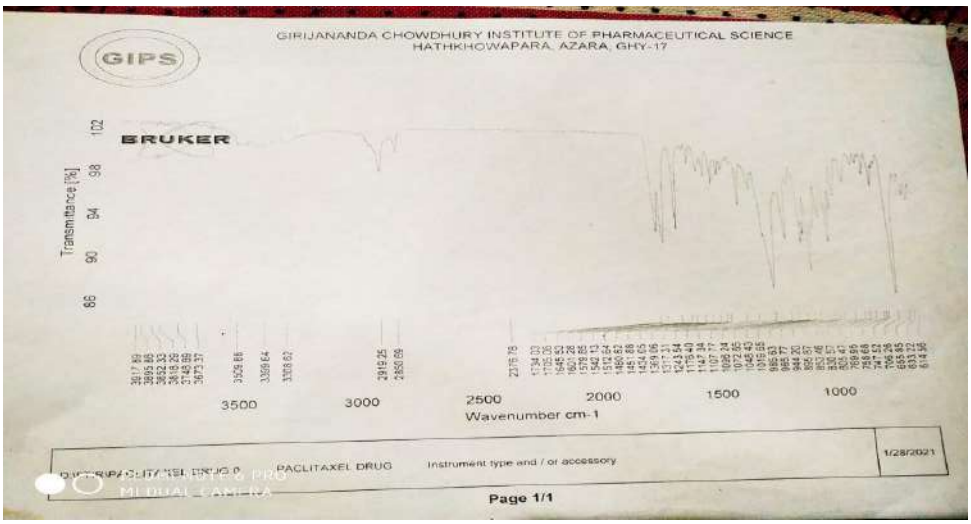
5.1.3 Melting point determination

The melting point was determined by using the melting point apparatus with the help of a capillary tube. The melting point of Paclitaxel was found to be 213°c. The melting range was compared with the official requirement and is found to be acceptable.

5.1.4. Compatibility study of drug and excipient: Fourier transforms infrared spectroscopy (FT-IR):

In order to evaluate the compatibility of pure drug with excipients FTIR study was done. Paclitaxel compatibility with excipients was studied by FTIR. The IR spectroscopy was obtained by a FTIR spectrophotometer using KBR pellets. The FTIR spectra both of pure drug and various excipients individually and in combination are shown in the **Figure 5.3 and the results are shown in Table no 5.4**

Figure: 5.2 (A) FTIR spectra of pure drug (Paclitaxel) (B) FTIR spectra of Cholesterol, Soyalecithin. Span 60, maltose (C) FTIR spectra of pure drug (Paclitaxel) and excipients



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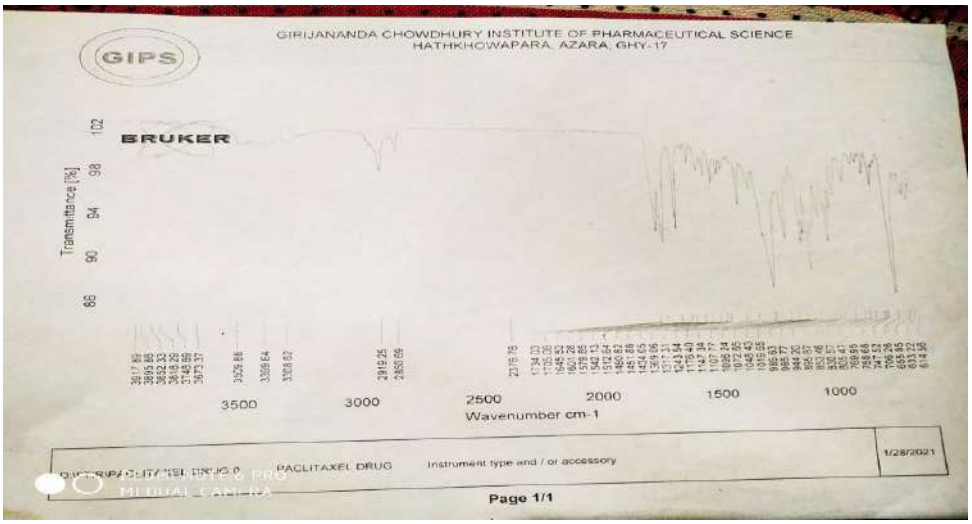
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Transmittance [%]
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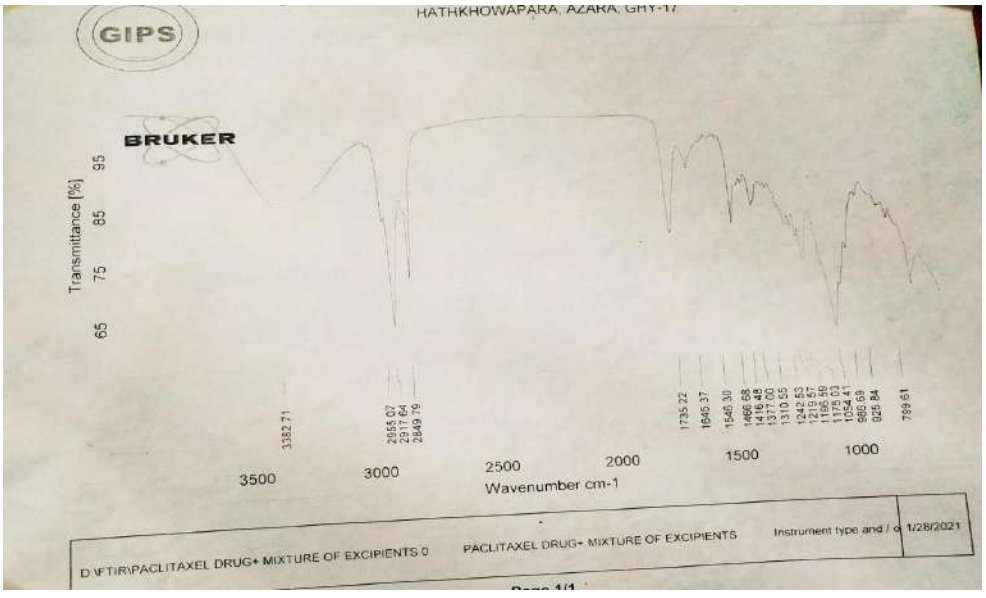
94
90
86
3500
3000
2500
2000
1500
1000
Wavenumber cm-1
PACLITAXEL DRUG
Instrument type and / or accessory
1/28/2021
Page 1/1

(B)

(C)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PACLITAXEL DRUG+ MIXTURE OF EXCIPIENTS
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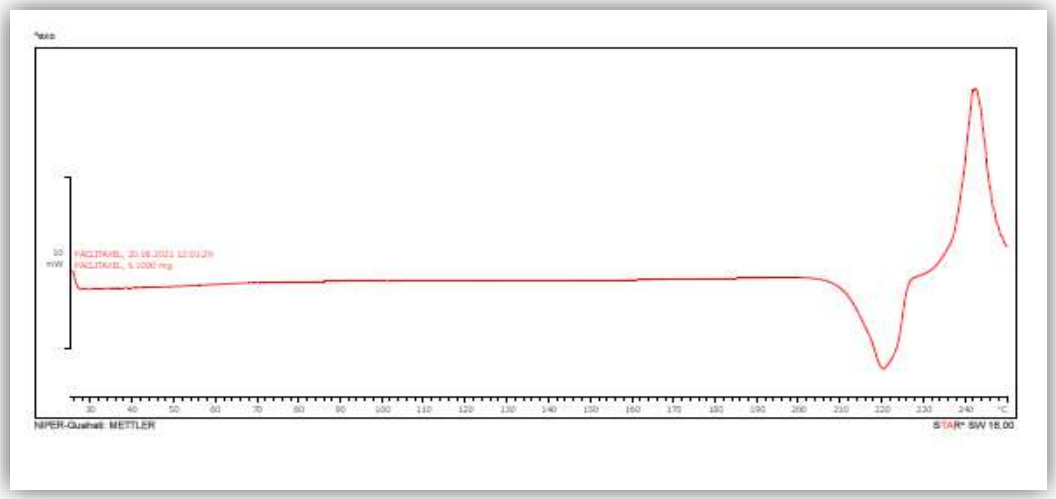
1/28/2021

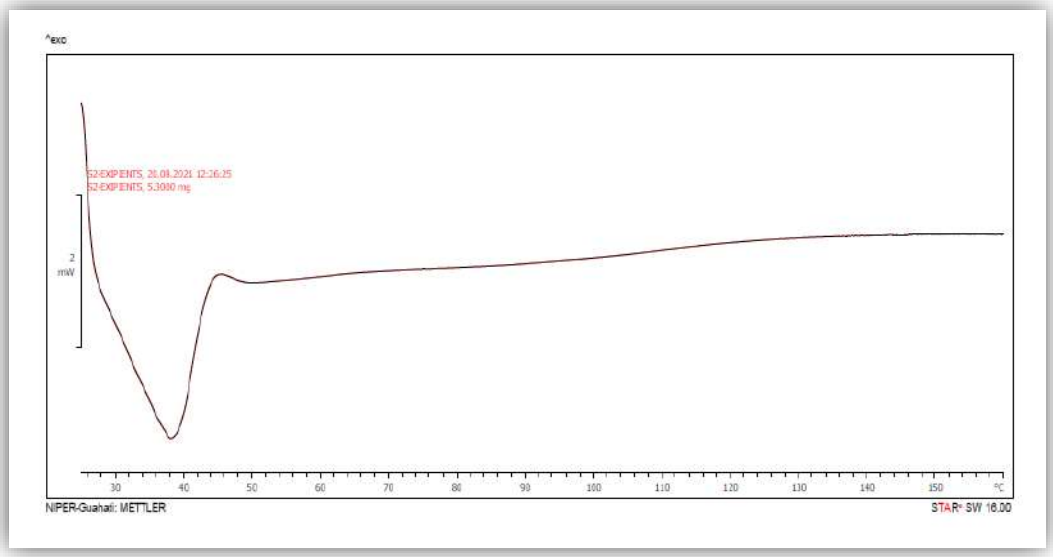
Table 5.3. FTIR interpretation of Paclitaxel

s.no	Frequency Region in (cm-1)		Bond
	FTIR	FT Raman	
1	3339	3319	N-H / O-H stretching
2	2973-2541	2971 - 2519	CH_3 / C-H stretching
3	1727- 1720	1727 - 1714	C=O stretching
4	1652 - 1579	1608 - 1581	C-C bending
5	1380 - 1330	1428 - 1336	CH_3 stretching
6	1274	1264	C-N stretching
7	1090 - 1049	1093 - 1050	C-O stretching
8	941 - 803	944 - 804	C- H in- plane deformation
9	689	566 - 250	C-H out of plane / C-C =r deformation

The FT-IR studies of pure (drug) Paclitaxel, with excipients were conducted. The FT-IR spectral analysis showed that the fundamental peaks and patterns of the spectra are similar in both pure drug and combination of drug and excipients indicating that there is no interaction between them and they are compatible with each other.

5.1.6. Differential Scanning Calorimetric (DSC):

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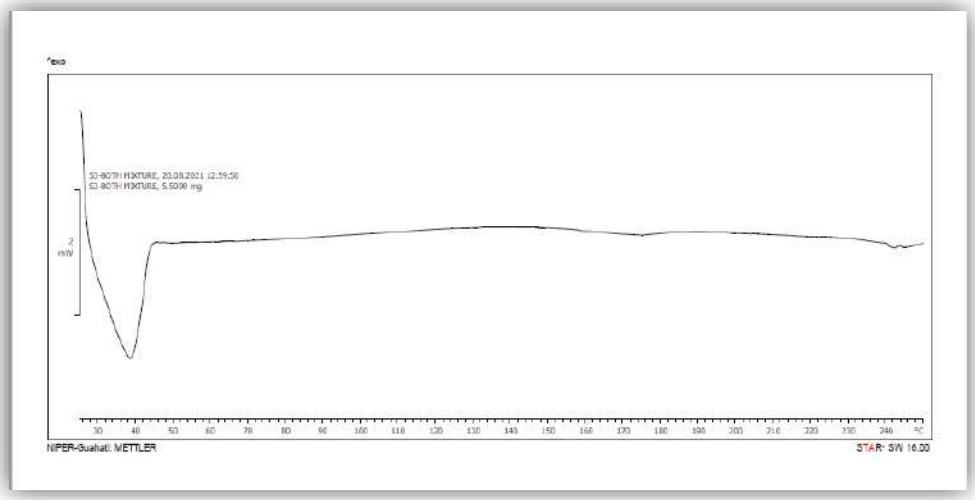
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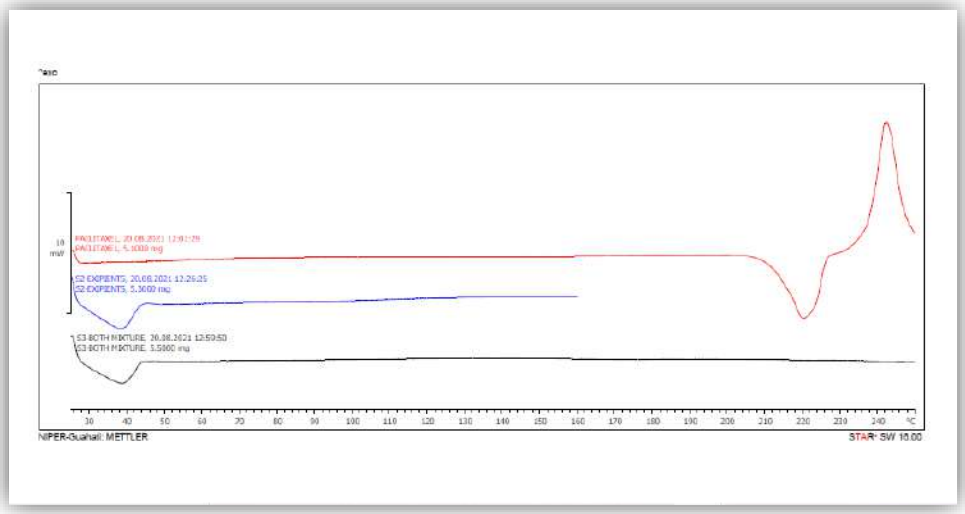


Figure 5.3: S1 view of Paclitaxel 5.1000 mg, S2 view of exicipents 5.3000 mg, S3 view of Both Mixture, 5.5000 mg, Overlay of all S1, S2, S3

5.2. ANALYTICAL DEVELOPMENT:

5.2.1 Determination of absorption maxima for the drug in phosphate buffer pH 7.4 by UV spectroscopy method

The spectrum of the solution was run in 200- 400 nm range in UV-visible spectrometer. The maximum paclitaxel maxima were found to be 227nm

Table 5.4: Data of calibration curve of paclitaxel in phosphate buffer 7.4 by UV spectroscopy

Concentration(µg/ml)n	Absorbance
2	0.276

4	**0.396**
6	**0.498**
8	**0.645**
10	**0.792**
12	**0.897**
14	**0.994**

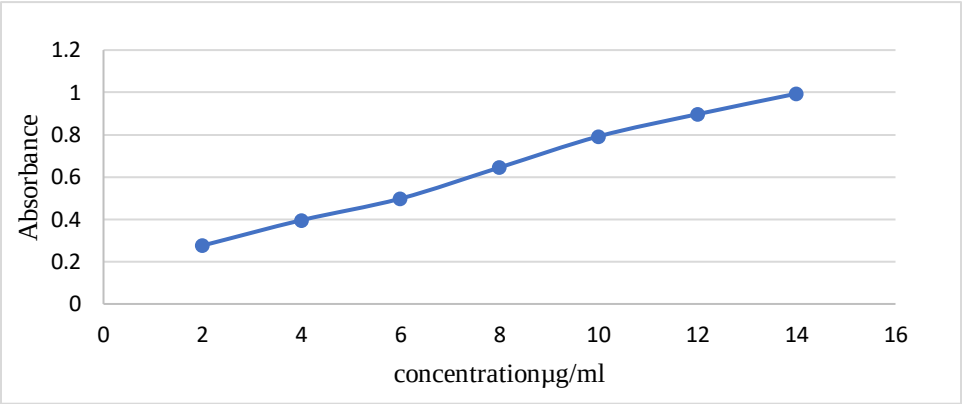


Figure 5.4: Standard graph of drug in phosphate buffer pH 7.4 by UV spectroscopy with y= 0.677x+0.0748; R^2=0.9822

5.2.2. Determination of absorption maxima for the drug in phosphate buffer pH 7.4 by HPLC method

In this preparation method standard stock solution of PTx loaded nano-niosomes were prepared separately in the mobile phase and standard solution were prepared by dilution by using a pre-plotted calibration curve constructed to determine the concentration of PTx in the samples was determined the concentration of PTx in the samples was determined.

Samples of PTx loaded nano-niosomes were analyzed using a water HPLC (MA, USA) equipped with a UV-Visible detector and Eurosphere C18 column (5µm, 4.6 mm x 250mm) from knauer (Berlin Germany). A mixture of methanol – acetonitrile water at a ratio of 75:75 v/v was used as mobile phase in the isocratic condition at a flow rate of 1ml min^{-1} for PTx analysis.

The separation was carried out at temperature 20±1°c. The mobile phase was filtered through a 0.22 µm Millipore membrane filter and degassed by a wire ultrasonic cleaner for 5 mins before use.

The total run time was 4 min with a retention time of 2.7±0.1 min. The solvent flow rate was 1.6ml min^{-1}. A 60 µl volume of samples were injected into the system. Samples detection was carried out at 272nm. Standard solutions were prepared I the mobile phase as a serial concentration such as 100(10µ), 50 (5µ), 20(2.5 µg), 10(1.0 µg), 5(0.5 µg) in a range of 30-500 µg/ml of PTx loaded nano-niosomes. Standard calibration curves were plotted for PTx loaded nano-niosomes by taking absorbance vs. concentration.

Table 5.5: Data of calibration curve of paclitaxel in phosphate buffer 7.4 by HPLC method

Concentration (ug/ml)	Absorbance (nm)
0.5	30623
1	35448

2	32747
5	89186
10	185654
25	568088
100	1457998

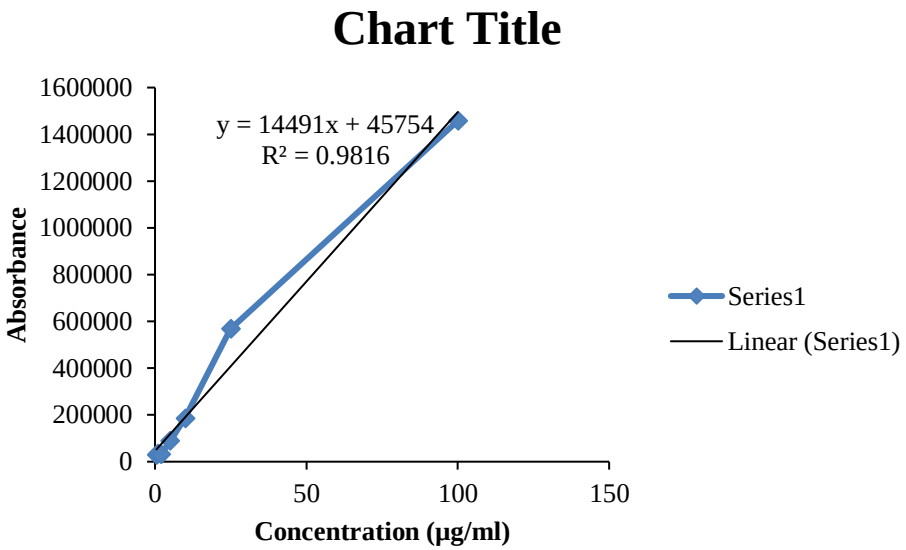
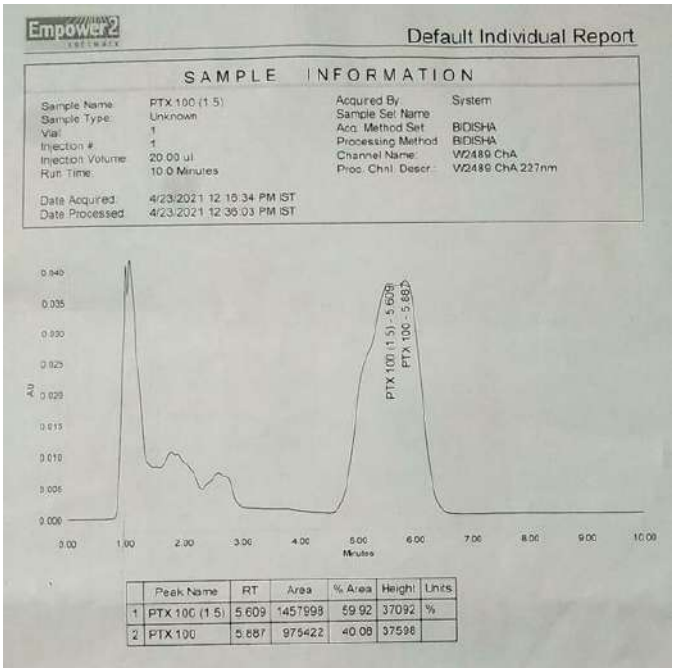


Figure 5.5: Standard graph of drug in phosphate buffer pH 7.4 by HPLC method



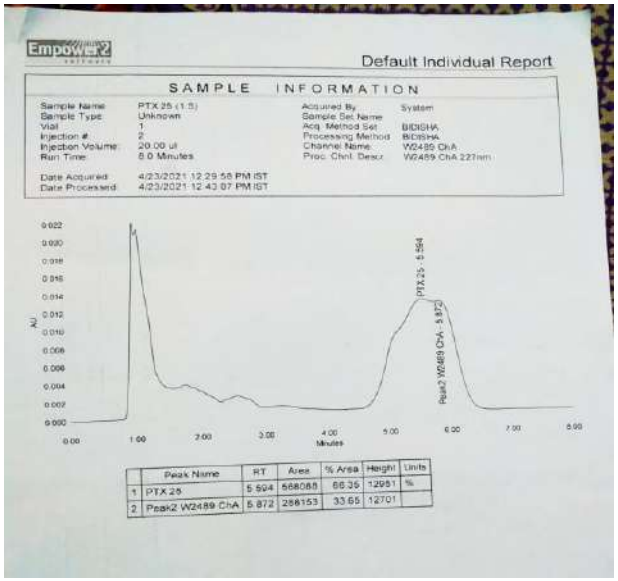
Empower2

Default Individual Report

SAMPLE INFORMATION

Sample Name:	PTX 100 (1.5)	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	
Vial:	1	Acq. Method Set:	BIDISHA
Injection #:	1	Processing Method:	BIDISHA
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	10.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 227nm
Date Acquired:	4/23/2021 12:16:34 PM IST		
Date Processed:	4/23/2021 12:36:03 PM IST		

	Peak Name	RT	Area	% Area	Height	Units
1	PTX 100 (1.5)	5.609	1457998	59.92	37092	%
2	PTX 100	5.887	975422	40.08	37598	



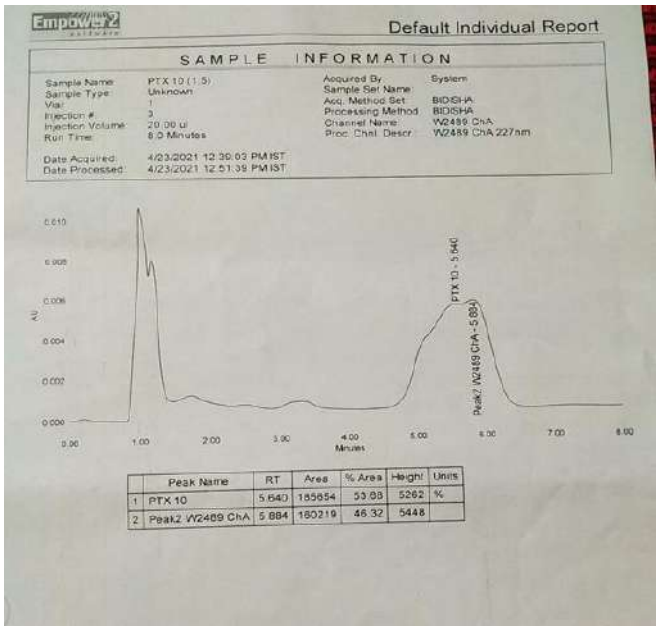
Empower2

Default Individual Report

SAMPLE INFORMATION

Sample Name:	PTX 25 (1.5)	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	
Vial:	1	Acq. Method Set:	BIDISHA
Injection #:	2	Processing Method:	BIDISHA
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 227nm
Date Acquired:	4/23/2021 12:29:58 PM IST		
Date Processed:	4/23/2021 12:43:07 PM IST		

	Peak Name	RT	Area	% Area	Height	Units
1	PTX 25	5.594	568088	66.35	12951	%
2	Peak2 W2489 ChA	5.872	288153	33.65	12701	



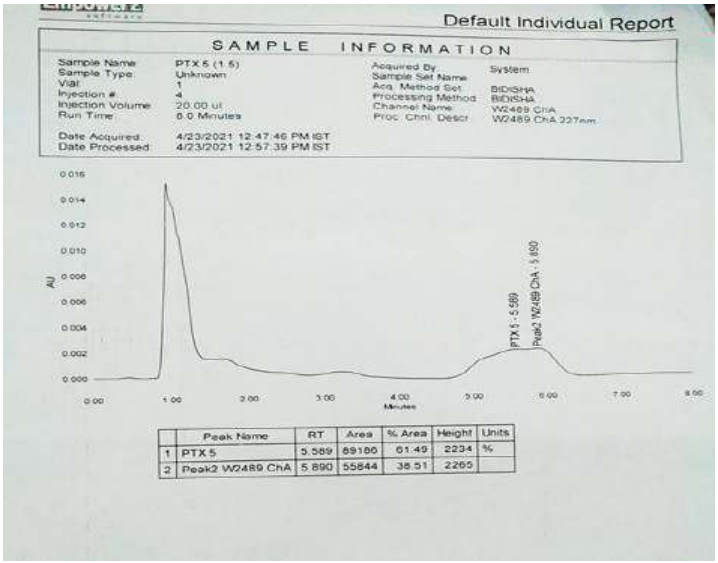
Empower 2 software

Default Individual Report

SAMPLE INFORMATION

Sample Name:	PTX 10 (1.5)	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	
Vial:	1	Acq. Method Set:	BIDISHA
Injection #:	3	Processing Method:	BIDISHA
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 227nm
Date Acquired:	4/23/2021 12:39:03 PM IST		
Date Processed:	4/23/2021 12:51:39 PM IST		

	Peak Name	RT	Area	% Area	Height	Units
1	PTX 10	5.640	185654	53.68	5262	%
2	Peak2 W2489 ChA	5.884	160219	46.32	5448	



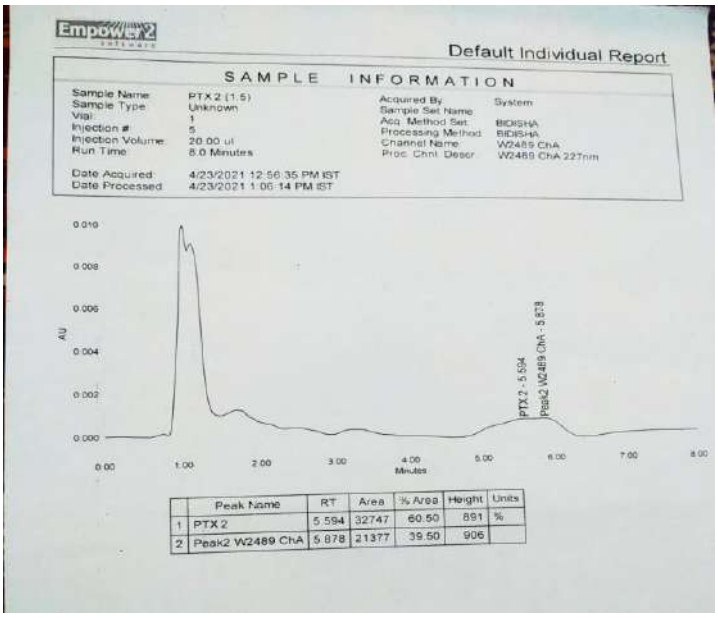
Empower 2 software

Default Individual Report

SAMPLE INFORMATION

Sample Name:	PTX 5 (1.5)	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	
Vial:	1	Acq. Method Set:	BIDISHA
Injection #:	4	Processing Method:	BIDISHA
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	8.0 Minutes	Proc. Chnl. Descr.:	W2489 ChA 227nm
Date Acquired:	4/23/2021 12:47:46 PM IST		
Date Processed:	4/23/2021 12:57:39 PM IST		

	Peak Name	RT	Area	% Area	Height	Units
1	PTX 5	5.589	89186	61.49	2234	%
2	Peak2 W2489 ChA	5.890	55844	38.51	2265	



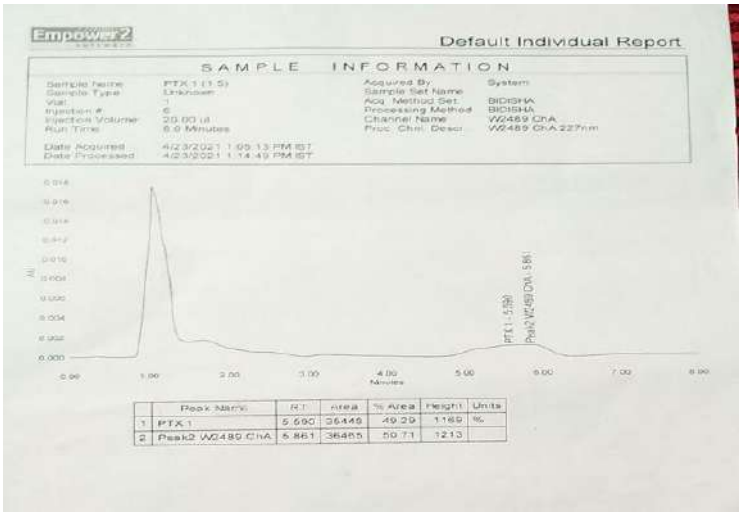
Empower 2

Default Individual Report

SAMPLE INFORMATION

Sample Name:	PTX 2 (1.5)	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	
Vial:	1	Acq. Method Set:	BIOSHA
Injection #:	5	Processing Method:	BIOSHA
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	8.0 Minutes	Proc. Chnl. Descr.	W2489 ChA 227nm
Date Acquired:	4/23/2021 12:56:35 PM IST		
Date Processed:	4/23/2021 1:06:14 PM IST		

	Peak Name	RT	Area	% Area	Height	Units
1	PTX 2	5.594	32747	60.50	891	%
2	Peak2 W2489 ChA	5.878	21377	39.50	906	



Empower 2

Default Individual Report

SAMPLE INFORMATION

Sample Name:	PTX 1 (1.5)	Acquired By:	System
Sample Type:	Unknown	Sample Set Name:	
Vial:	1	Acq. Method Set:	BIOSHA
Injection #:	5	Processing Method:	BIOSHA
Injection Volume:	20.00 ul	Channel Name:	W2489 ChA
Run Time:	8.0 Minutes	Proc. Chnl. Descr.	W2489 ChA 227nm
Date Acquired:	4/23/2021 1:05:13 PM IST		
Date Processed:	4/23/2021 1:14:49 PM IST		

	Peak Name	RT	Area	% Area	Height	Units
1	PTX 1	5.590	36446	49.29	1169	%
2	Peak2 W2489 ChA	5.861	36855	50.71	1213	

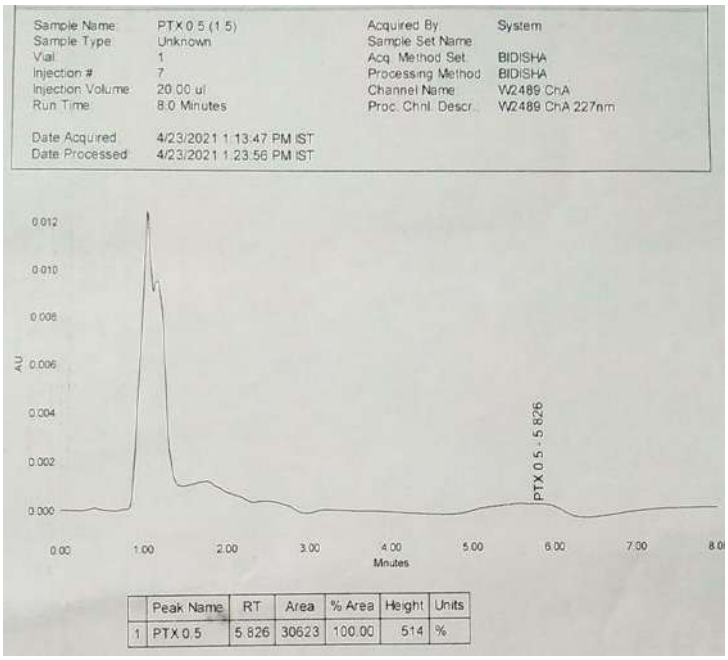


Fig5.6: Hplc Graph

5.3 EVALUATION AND OPTIMIZATION:

5.3.1 Particle size determination and polydispersity index:

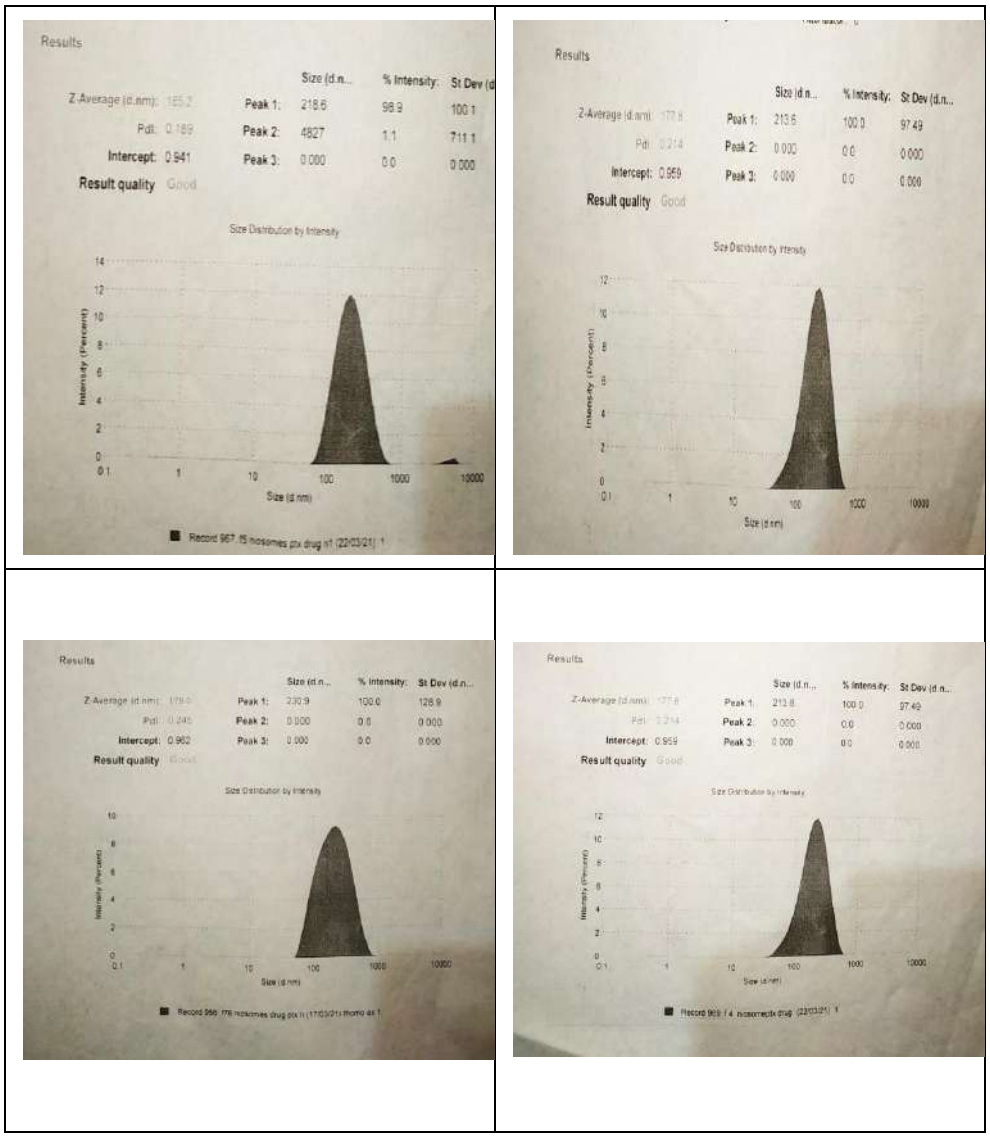
The particle size and the polydispersity Index (PI) of the former drug particles was measured immediately after precipitation by dynamic laser light scattering (Zetasizer Ver. 6.11Malvern).

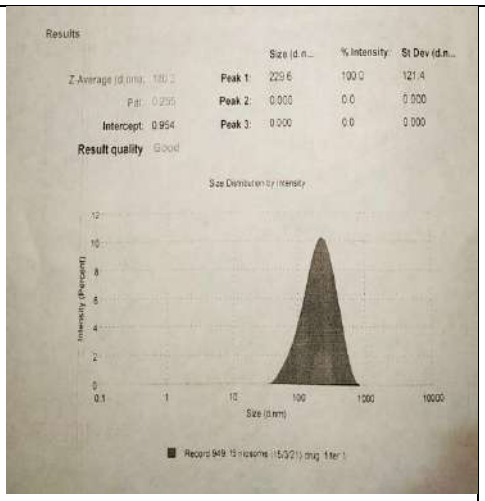
Table 5.6: Particle size determination and polydispersity index

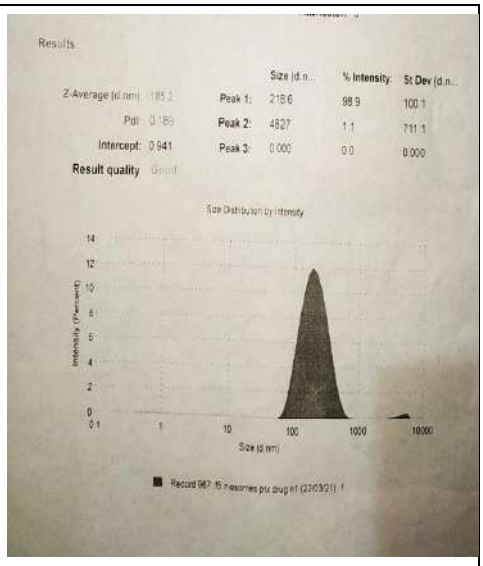
Formulation	Particle size (d.nm)	PDI±SD*
PF1	179.0	0.245
PF2	177.8	0214

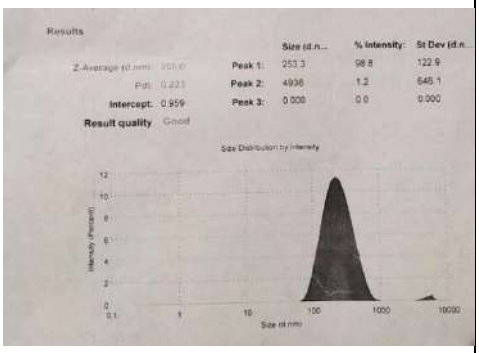
PF3	180.3	0.255
PF4	181.2	0.224
PF5	215.0	0.215
PF6	185.2	0.189
PF7	237.5	0.119
PF8	205.0	0.223

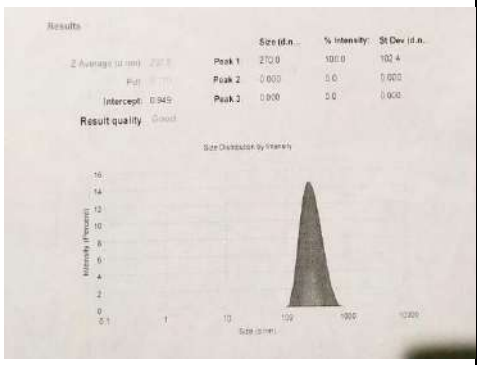
Table 5.7: Particle size and PDI of Formulations





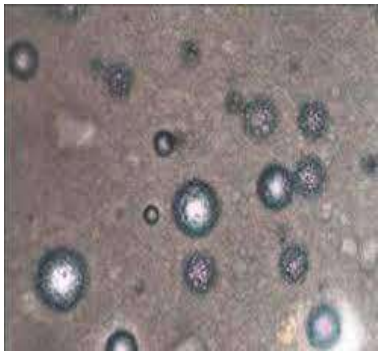


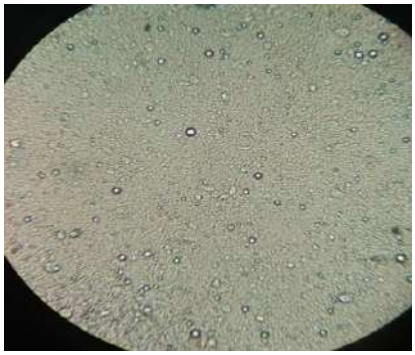


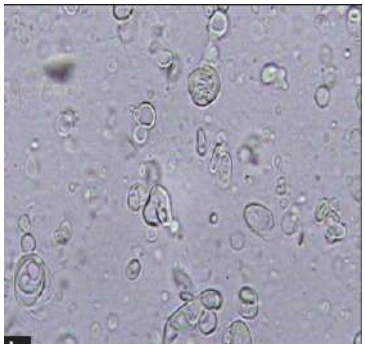


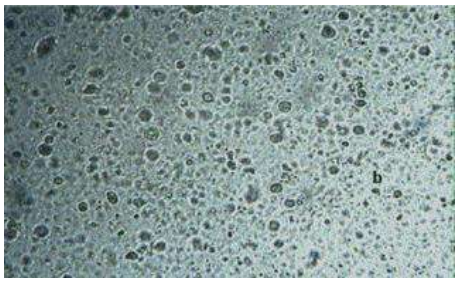
5.3.2. Vesicle morphology:

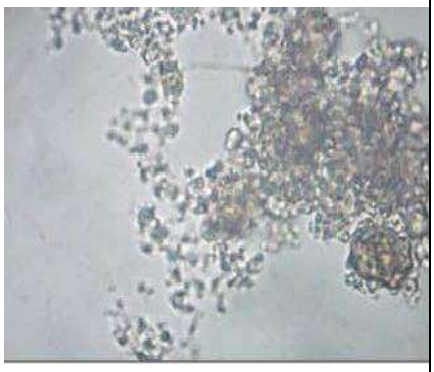
Table.5.8.Microscopic images of niosomal vesicles

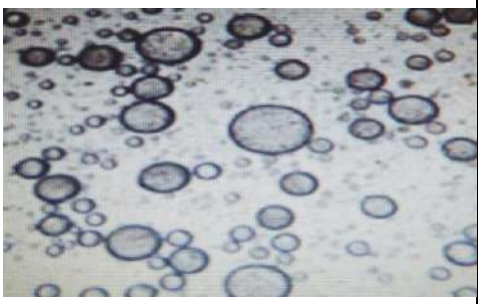


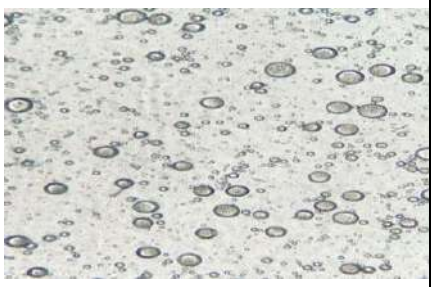






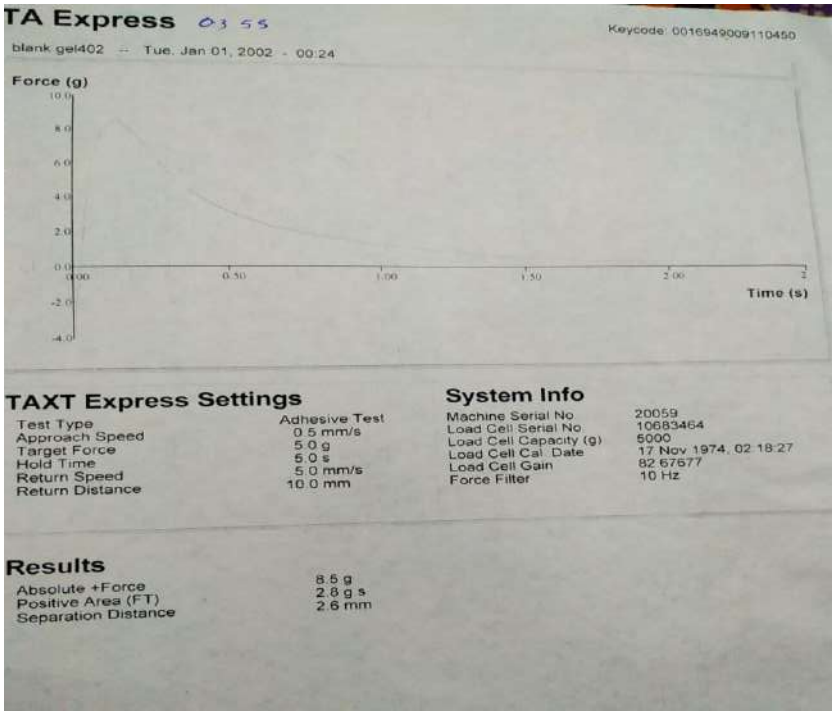


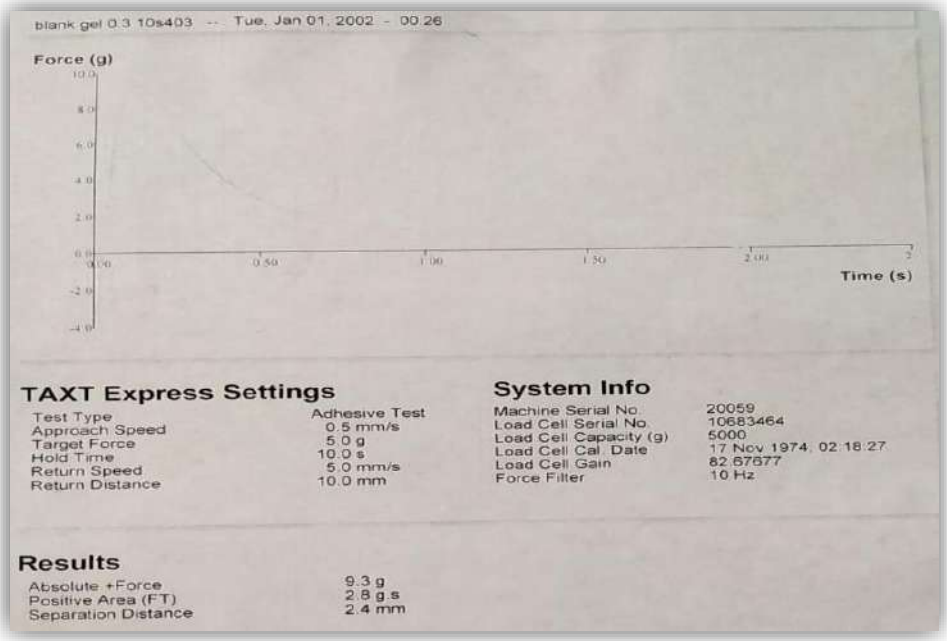


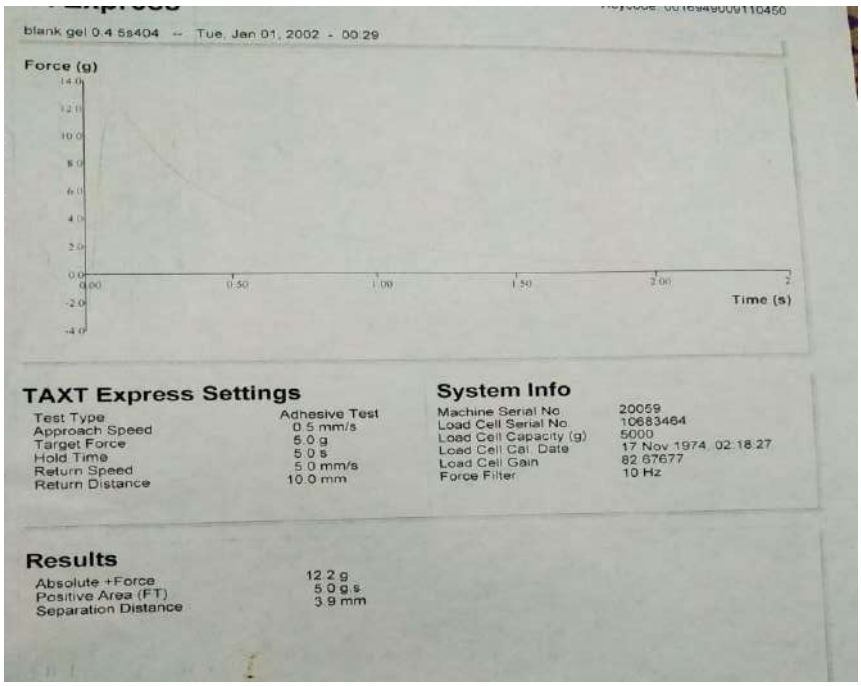


5.3.3. Gel strength:

Gel Strength is measured the ability of a colloidal dispersion to develop and retain a gel form.




blank gel 0.3 10s403 -- Tue, Jan 01, 2002 - 00:26
Force (g)
Time (s)
TAXT Express Settings
Test Type Adhesive Test
Approach Speed 0.5 mm/s
Target Force 5.0 g
Hold Time 10.0 s
Return Speed 5.0 mm/s
Return Distance 10.0 mm
System Info
Machine Serial No. 20059
Load Cell Serial No. 10683464
Load Cell Capacity (g) 5000
Load Cell Cal. Date 17 Nov 1974, 02:18:27
Load Cell Gain 82.67677
Force Filter 10 Hz
Results
Absolute +Force 9.3 g
Positive Area (FT) 2.8 g.s
Separation Distance 2.4 mm


blank gel 0.4 5s404 -- Tue, Jan 01, 2002 - 00:29
Force (g)
Time (s)
TAXT Express Settings
Test Type Adhesive Test
Approach Speed 0.5 mm/s
Target Force 5.0 g
Hold Time 5.0 s
Return Speed 5.0 mm/s
Return Distance 10.0 mm
System Info
Machine Serial No. 20059
Load Cell Serial No. 10683464
Load Cell Capacity (g) 5000
Load Cell Cal. Date 17 Nov 1974, 02:18:27
Load Cell Gain 82.67677
Force Filter 10 Hz
Results
Absolute +Force 12.2 g
Positive Area (FT) 5.0 g.s
Separation Distance 3.9 mm

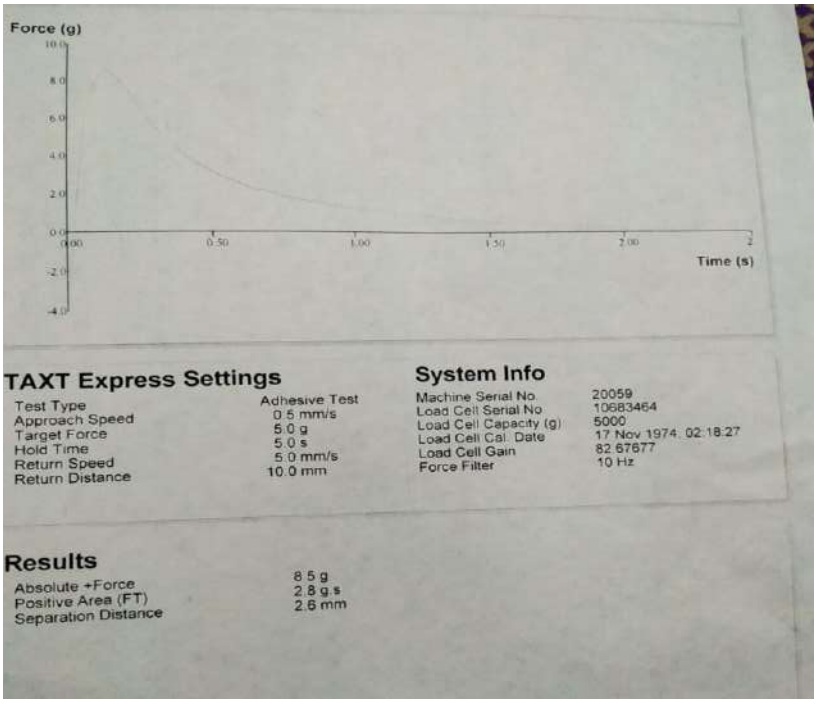


Figure 5.7: Gel strength of paclitaxel

5.3.4. Drug loading (loading capacity) and Drug entrapment efficiency:

Entrapment efficiency of nano-niosomal formulations ranged from 34.85 to 83.2. Span 60 bears a longest saturated alkyl chain and showed higher entrapment. The results are given in the **table no. 5.8.** Drug loading was performed according to the procedure given in experimental methodology. Drug content of all prepared formulations were found to be in range of 34.85 ± 2.61to79.6±4.11respectively. The maximum drug content was found to be 83.2±0.68 in FP6 formulation, indicates the uniform distribution of drug content in formulation. The results are given in **table. 5.8**

Table.5.9. Parameters of drug entrapment and drug content

Formulations	Drug entrapment	Drug content
FP1	79.6±4.11	76.5± 0.97
FP2	34.85 ± 2.61	37.5 ± 1.08
FP3	48.3±1.85	38.4±0.88
FP4	45.19 ± 3.12	82.4±0.88
FP5	73.1±5.15	85.2±0.77
FP6	83.2±0.68	39.2±4.3
FP7	50.94 ± 4.56	43.6±4.7
FP8	54.44 ± 5.15	33.42±0.45

CHAPTER-VI

SUMMARY AND CONCLUSION

VI. SUMMARY AND CONCLUSION

In this present study, we have tried to develop a topical gel containing PTX- loaded noisome. However earlier attempts has been made to deliver PTX cutaneously using ethosomes, nano-emulsions, lamellar liquid crystalline phases and with different penetration enhancers. But nano-niosomal topical gels have certain advantages over other nano-particulate delivery system.

The niosomes have efficient control over the release kinetic of the encapsulated drug with greater stability. Nano-niosomes have been shown to improve the efficiency and residence time of cytotoxic drugs with subsequent reduction of side effects associated with cytotoxic drugs. After the preparation of nano-niosomes by high speed homogenization and ultrasoication methods the FTIR spectra have been observed to check the integrity of the drug in the core of the nano-niosomes. The nano-niosomes (F1, F5, F6) found to be homogenous and with uniform distribution showing PDI of 0.1 ±0.3. The particle size of the nano-niosomes found to get decrease with increase in the concentration of the surfactants. This may be due to the higher concentration of the surfactants which allows better stabilization and preventing them from coalescing into large droplets. The particle size of nano-niosomes play a key role in permeation through the skin as well as bio-distribution gives smaller the particle size more will be the permeation. The increase in the entrapment efficiency is due to increase in the amount of emulsifier used for its preparation. The increased concentration of emulsifier may results in formation of layer at the surface of nano-niosomes and which account for higher entrapment efficiency. Followed by slower gradual release of the drug, it enchances the penetration of the drug for an effective therapy, whereas the slower gradual release helped in maintaining the requisite dose for a prolong period of time.