"FORMULATION AND EVALUATION OF PACLITAXEL LOADED LIPOSOMAL TOPICAL GEL FOR THE TREATMENT OF SKIN CARCINOMA"

Project Work

SUBMITTED TO

ASSAM SCIENCE AND TECHNOLOGY UNIVERSITY
IN THE PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE COMPLETION OF M. PHARM COURSE, DEPARTMENT OF PHARMACEUTICS, GIRIJANANDA CHOWDHURY INSTITUTE OF PHARMACEUTICAL SCIENCE, GUWAHATI

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CERTIFICATE FROM THE PRINCIPAL

This is to certify that the thesis entitled "***FORMULATION AND EVALUATION OF PACLITAXEL LOADED LIPOSOMAL TOPICAL GEL FOR THE TREATMENT OF SKIN CARCINOMA"*** submitted to Assam Science and Technology University in partial fulfillment of the requirements for the award of Degree of **Master of Pharmacy** in **Pharmaceutics** was carried out by **Bidisha Bordoloi** , Assam Science and Technology University ***Registration No***: ***387105219 of 2019-20, Roll no- 190520011002*** at ***Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Hatkhowapara, Ghy-17*** under my supervision.

I wish her all success in life.

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CERTIFICATE FROM THE GUIDE

This is to certify that the thesis entitled "***FORMULATION AND EVALUATION OF PACLITAXEL LOADED LIPOSOMAL TOPICAL GEL FOR THE TREATMENT OF SKIN CARCINOMA"*** submitted to Assam Science and Technology University in partial fulfillment of the requirements for the award of Degree of **Master of Pharmacy** in **Pharmaceutics** is a genuine and legitimate research work carried out by **Bidisha Bordoloi** , Assam Science and Technology University ***Registration No***: ***387105219 of 2019-20, Roll no-190520011002*** at ***Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Hatkhowapara, Ghy-17 ,***during the academic session 2020-2021 under my supervision and guidance.

I wish her all success in life.

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This is to certify that the thesis entitled **"*FORMULATION AND EVALUATION OF PACLITAXEL LOADED LIPOSOMAL TOPICAL GEL FOR THE TREATMENT OF SKIN CARCINOMA*"** submitted to Assam Science and Technology University in partial fulfillment of the requirements for the award of Degree of **Master of Pharmacy** in **Pharmaceutics** is a genuine and legitimate research work carried out by **Bidisha Bordoloi** , Assam Science and Technology University ***Registration No***: ***387105219 of 2019-20, Roll no-190520011002*** at ***Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Hatkhowapara, Ghy-17*** under my supervision**.,** during the academic session 2020-2021 under my co-guidance.

I wish her all success in life.

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DECLARATION BY THE CANDIDATE

I hereby declare that the matter embodied in the dissertation entitled "***FORMULATION AND EVALUATION OF PACLITAXEL LOADED LIPOSOMAL TOPICAL GEL FOR THE TREATMENT OF SKIN CARCINOMA*** is a bonafide and genuine research work carried out by me under the supervision of **Dr. Bhanu Pratap Sahu**, Associate Professor, Department of Pharmaceutics, ***Girijananda Chowdhury Institute of Pharmaceutical Science, Hatkhowapara, Azara, Guwahati-17.*** The work embodied in this thesis is original and has not been submitted for the award of degree, diploma, associateship or fellowship of any other university or institution.

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"FORMULATION AND EVALUATION OF PACLITAXEL LOADED LIPOSOMAL TOPICAL GEL FOR THE TREATMENT OF SKIN CARCINOMA"

ABSTRACT

CONTEXT: In the field of Nano technological research there have been many new studies representing the skin cancer treatment. The topical administration of anti-cancer drugs is considered to be a compelling alternative for increasing the drug therapy and drug targeting. The current therapy of skin cancer suffers from numerous side effects, so incorporating liposomes with anti-cancer drug paclitaxel can be considered to be a novel approach.

OBJECTIVE: In the present study we shall attempt to perform formulation of liposomal topical gel which will be loaded with anti- cancer drug paclitaxel for the effective treatment of various forms of skin cancer.

METHODS: The liposomes were prepared by thin film hydration method followed by high speed homogenization and ultra-sonication method. The prepared liposomes were characterized. The optimized paclitaxel liposomes were loaded in carbopol gel. The prepared gels were evaluated for its gelling properties.

RESULTS: The particle size distribution was found to be between 78.20 nm – 490.5 nm. The FTIR results shows stable peak. The DSC results show an endothermic peak of the drug, the mixture of the drug and excipients shows amorphous form during the preparation of liposomes. The HPLC analysis provides a precise result by making better separation and easier analysis of the sample. The topical gel was found to be homogenous as the ingredients were distributed evenly throughout the gel, moreover the pH of the topical gel was found to be compatible and stable. The topical gel was having a very good spreadability. The viscosity of the gel was found to be in the average ranges which give us a stable formulation. The strength of the gel was also calculated and

found to be a good and stable one which will facilitate the drug to penetrate into deeper skin.

CONCLUSION: The formulations shows stability and thus expected to show reduce dose related side effects. Liposome increases the drug concentration in the vehicle and therefore shows an increase the drug flux. The development of these new combined treatment will become the promising future treatments approaches which lie in the field of liposomes, which combines all the required ingredients that will meet all the requirements for an ideal treatment approach.

1.INTRODUCTION:

Skin cancer occurs when the body doesn't repair damage to the DNA inside skin cells, allowing the cells to divide and grow uncontrollably. Somatic cell damage could also be caused by a range of things, including genetics and skin type. Most cases of carcinoma result from overexposure to ultraviolet (UV) light produced by the sun. Carcinoma may appear as a dark spot, lesion, a wound that doesn't heal or a bump on the skin.

There are 2 main sorts of carcinoma: Non melanoma carcinoma (which includes basal cell skin cancer, squamous cell carcinoma and other rare types) and melanoma carcinoma. The first risk factor for melanoma and non-melanoma skin cancers is exposure to ultraviolet (UV) light, including sunlight and tanning beds, with the danger growing with the quantity of exposure.

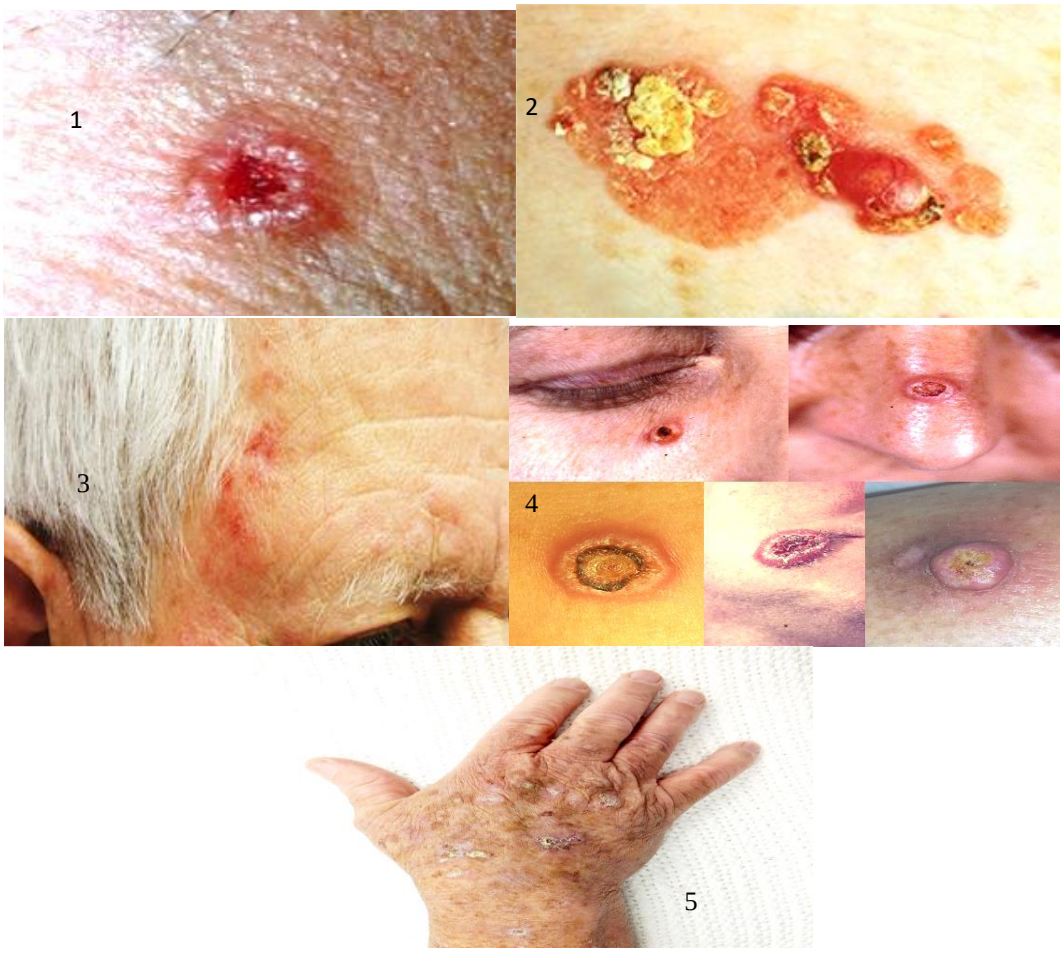


Figure: 1. Basal cell carcinoma, 2. Squamous cell carcinoma, 3. Bowen's disease, 4. Keratoacanthoma, 5. Actinic keratosis

Besides sun exposure, common carcinoma risk factors include:

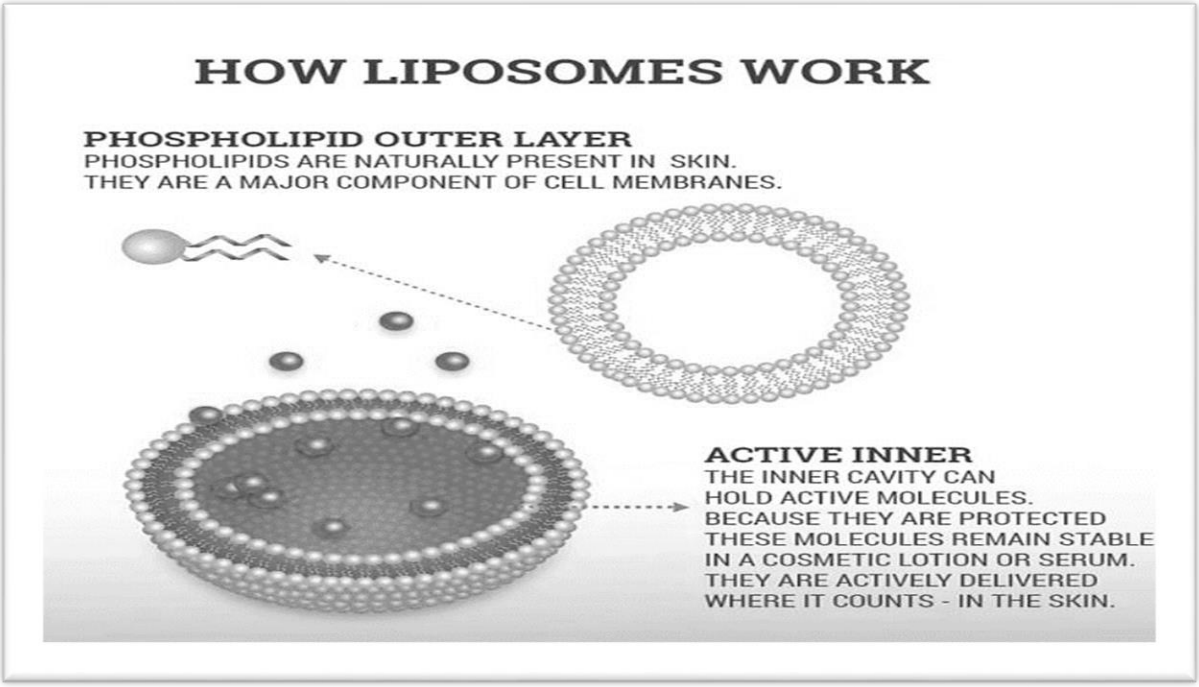
- Weakened system, from viruses, diseases or immune-suppression therapy related to organ transplantation
- Moles
- Chemical exposure to items like arsenic, industrial tar, coal, paraffin and certain varieties of oil
- Basal cell nervous syndrome, also called Gorlin syndrome
- Radiation therapy exposure

People who live in areas with bright, year-round sun exposure, or those that spend tons of time outdoors without sun protection or sunscreen, are at greater risk. Early exposure, particularly for people that had frequent sunburns during childhood, also increases carcinoma risks. Skin cancers can also be found in younger individuals who spend tons of time within the sun.

Pro-inflammatory cytokines and environmental stress can induce the p38 MAPK cascade within the keratinocytes which successively cause the formation of carcinoma. Ultra-violet ray activated p38 MAPK have profound effect on cell survival and apoptosis of the keratinocytes which results in formation of carcinoma. Research and study on carcinoma could bring down the suffering patient load in most of the countries throughout the planet including America, Australia, and Africa because the novel drug delivery system will pave new routes towards its treatment throughout the world.

Paclitaxel is understood together as the foremost effective anticancer drugs within the market today. Significant antitumor activity has been demonstrated in clinical trials against a good sort of tumors, including ovarian carcinoma, head and neck cancers and non-small cell carcinoma. The current clinical dosage form of paclitaxel, which is parenteral, is dissolved during a mixture of Cremophor® EL (poly-ox ethylated castor oil) and ethanol (50:50, v/v) and wishes to be diluted right before injection. However, after having a broad range of anti-neoplastic activity, PTX in intravenous formulations suffers from a variety of great side effects which incorporates hypersensitivity reactions, peripheral neuropathy, neutropenia and sometimes accompanied with nausea, thrombocytopenia, vomiting, microsites, decreased appetite and diarrhea which is especially because of the excipient utilized in the formulation to extend drug solubility. These untoward reactions are often minimized if the drug is directly applied at carcinoma site topically as just in case of carcinoma in order that least of medication

will reach the systemic circulation as compared to intravenous route of administration. Moreover, if the drug is applied locally to site of action, we will increase the quantity of accessible drug to the neoplastic cells. Taking under consideration all the pros and cons we've selected PTX for treatment of carcinoma and chosen the topical route to be the foremost efficient way for delivering it. PTX suffers from a really low solubility profile, thus to attenuate the side effects related to the solubilizes which was used previously and to extend its delivery to the location of action we've tried to form Nano liposomes encapsulating the drug. The liposomes are going to be one among the simplest ways to deliver the drug as because the lipids used for its preparation are generally well tolerated by the physiological system and moreover their residues are non-toxic to physical body. Liposomes is among one among the foremost stable sort of Nano carriers as they need a rigid core which is formed from phospholipids based artificial vesicles thus remain as solid both in room temperature and body temperature. As Nano carriers are often used for carcinoma targeting by improving the drug ability to succeed in and penetrate the tumor site evenly and moreover the drug will attain a greater stability and extended release within from it. On the opposite hand, liposomes are used to formulate a range of hydrophobic, poorly water soluble drugs.



In order to extend the therapeutic efficiency and reduce the side-effects caused by these vehicles, much effort has been dedicated to improving the aqueous solubility of paclitaxel without using Cremophor® EL. These alternatives include the utilization of solubilizing agents (e.g., Tween 80) (Singla et al., 2002) and the liposome-based formulations (Crosasso et al., 2000). A vehicle composed of ethanol and Tween 80 (50:50, v/v), to be diluted in glucose solution before use, was attempted to be developed for administration of paclitaxel (Singla et al., 2002). However, it had been reported that liposomal paclitaxel improved its solubility and showed similar in vitro cytotoxicity against a spread of tumor cell lines compared to that of the free paclitaxel in Cremophor® EL based vehicle (Taxol®) (Sharma et al., 1996). However, one among the main drawbacks of the liposomal formulation was its rapid clearance from blood due to the adsorption of plasma protein to the phospholipid membrane of the liposomes, thereby triggering the recognition and uptake of the liposomes by the mononuclear phagocytic system (MPS) (Schnyer and Huwyler, 2005).Fortunately, when the surface

of the liposomes was modified with a versatile hydrophilic polymer like polyethylene glycol (PEG), the uptake by MPS might be retarded (Torchilin and Trubetskoy, 1995).

The current topical treatment for carcinoma includes formulation containing 5-Fluorouracil and imiquimod. After approval by US FDA, 5-Fluorouracil has been considered a topical treatment of choice for actinic keratosis but it suffers from variety of drawbacks including intense inflammatory reactions, long period of treatment and ineffective in treatment of hyperkeratotic actinic keratosis. It's been tried to alleviate by researchers from time to time with different group of medicine and novel drug delivery system. Still studies were being administered throughout the world trying to seek out some newer chemical entity with new route of administration but till date no such appropriate formulation adequate to cure carcinoma is out there within the drug market.

In the present study, PTX loaded Liposomes are prepared and dispersed in topical gel for administration to treat carcinoma. The study involves preparation of a topical gel where the PTX loaded Liposomes are dispersed homogenously and may be applied on to site of carcinoma. Topical gel is chosen because the best way to deliver PTX as upon the appliance of the gel at skin, most of the nanoparticles will penetrate through the layers of skin releasing the drug entrapped within at the location of action. Moreover, a number of the particles will remain suspended just above the location of cancer having a surface contact with the skin; this arrangement will assure a slow and persistent release of PTX to the tumor. Thus, controlled release of drug are going to be achieved till the gel remains at the location of cancer moreover as only a little amount of drug will get into the systemic circulation via skin, thus the toxicity and other side effects of PTX formulation reported previously are often minimized to a substantial extent. Based on the effectiveness of PTX against different kind of tumor as gathered from wide spectrum of its anti-neoplastic activity, this aim of the study is to assess the

activity of PTX against carcinoma. The studies include formulation and evaluation of a topical gel containing PTX loaded liposomes, to quantify the in-vitro extent of PTX release from it and eventually to see its efficacy against chemically induced carcinoma in mice model.

1.1 DRUG PROFILE

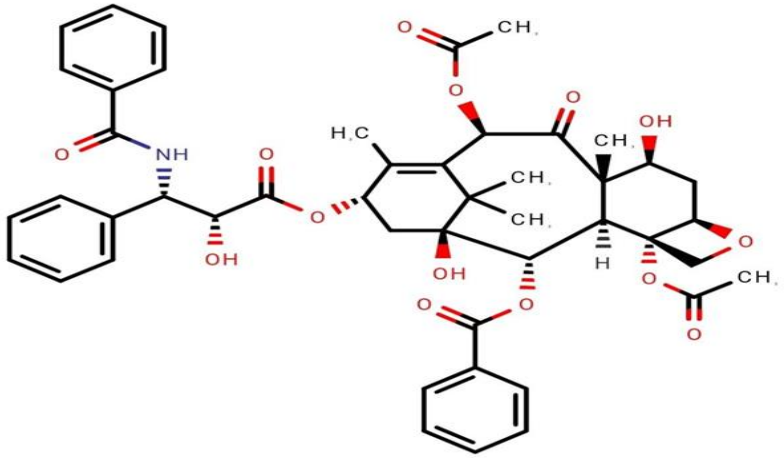
Paclitaxel (also known as Taxol) is a mitotic inhibitor used in cancer chemotherapy. It is an antitumor drug and it plays a major role in cancer chemotherapy. Paclitaxel is a toxoid chemotherapeutic agent used as first-line and subsequent therapy for the treatment of advanced carcinoma of the ovary, and other various cancers including breast and lung cancer.

- **Brand names: Abraxane, Taxol**
- **Generic name: Paclitaxel**

History:

Paclitaxel was first discovered in a US National Cancer Institute program in 1962. Monroe E. Wall and Mansukh C. Wani isolated it from the bark of a Pacific yew tree. Upon doing more research, they discovered that endophytic fungi in the bark of the tree synthesize paclitaxel. In 1977, scientists were able to confirm antitumor activity in mouse melanoma. Ever since 1992, paclitaxel has been used for the treatment of various cancers.

Structure:



Paclitaxel is a complex diterpene having a taxane ring with a four-membered oxetane ringand an ester side chain at position C-13.

- **Molecular Formula: C47H51NO14**
- **Molecular weight: 853.92 Da**
- **Melting point: 213°C**
- **Boiling point: 218-222°C**
- **ƛ max: 230 nm**

2. LITERATURE REVIEW:

- ***Tao Yang, et.al.(2007)*" Enhanced solubility and stability of PEGylated liposomal paclitaxel: *In vitro* and *in vivo* evaluation",** found in their research study that an improved PEGylated liposomal formulation of paclitaxel has been developed with the purpose of improving the solubility of paclitaxel as well as the physicochemical stability of liposome in comparison to the current Taxol® formulation. The use of 3% (v/v) Tween 80 in the hydration media was able to increase the solubility of drug by freeze-drying with sucrose as a lyoprotectant. Therefore, this PEGylated liposome formulation of paclitaxel could serve as a better alternative for treating human breast cancer.

- ***Rituraj Bharadwaj, et.al.(2016)" Topical* delivery of paclitaxel for treatment of skin cancer",** from the study it may be concluded that, PTX can be successfully encapsulated within the SLN following high speed homogenization and ultra-sonication method. The SLN can be homogenously dispersed in the topical gel and the gel showed a sustained release parameter. The in-vivo study confirmed that, the SLN entrapping PTX which was loaded in topical gel is quite efficient in the treatment of the skin carcinoma in mice as compared to normal PTZ loaded topical gel. Therefore, we conclude that topical administration of PTZ can be used for the treatment of skin cancer.

- ***SHIH-TING HUANG, et.al.(2018)"*** **Liposomal paclitaxel induces fewer hematopoietic and cardiovascular complications than bioequivalent doses of Taxol"** In the present study, a novel liposomal PTX (lipo-PTX) formulation was optimized with regards to encapsulation rate and long-term stability, arriving at a molar constituent ratio of soybean phosphatidylcholine: cholesterol. Comparable doses of lipo-PTX and Taxol were bioequivalent in terms of therapeutic efficacy in xenograft tumor models. However, the systemic side effects, including hematopoietic toxicity, acute hypersensitivity reactions and cardiac irregularities, were significantly reduced in lipo-PTX-treated mice compared with those infused with reference formulations of PTX. In conclusion, the present study reported that lipo-PTX exhibited a higher therapeutic index than clinical PTX formulations.

- ***Paola Crosasso, et.al.(2000)*** **"Preparation, characterization and properties of sterically stabilized paclitaxel-containing liposomes"** We prepared different conventional and PEGylated liposomes containing paclitaxel and determined encapsulation efficiency, physical stability and drug leakage in human plasma. In vitro cytotoxic activities were evaluated on HT-29 human colon adenocarcinoma and MeWo melanoma cell lines. Bio distribution 1 / 2 1 / 2 studies showed a considerable decrease in drug uptake in MPS-containing organs (liver and spleen) at 0.5 and 3 h after injection with PEGylated compared to conventional liposomes. They successfully obtained long-circulating liposomes containing paclitaxel, by including PEG – 5000. DPPE into a formulation composed of ePC/PG and cholesterol. This formulation also had the

advantage, of avoiding the use of Cremophor EL, a vehicle causes significant hypersensitivity problems.

- ***Stephan Koudelka, Jaroslav Turanek*** **(2012) "Liposomal paclitaxel formulations"** Over the past three decades, taxanes represent one of the most important new classes of drugs approved in oncology. Paclitaxel (PTX), the prototype of this class, is an anti-cancer drug approved for the treatment of breast and ovarian cancer. However, notwithstanding a suitable premedication, present-day chemotherapy employing a commercial preparation of PTX (Taxol®) is associated with serious side effects and hypersensitivity reactions. Liposomes represent advanced and versatile delivery systems for drugs. Generally, both in vivo mice tumor models and human clinical trials demonstrated that liposomal PTX formulations significantly increase a maximum tolerated dose (MTD) of PTX which outperform that for Taxol®. Liposomal PTX formulations are in various stages of clinical trials. LEP-ETU (NeoPharm) and EndoTAG®-1 (Medigene) have reached the phase II of the clinical trials; Lipusu® (Luye Pharma Group) has already been commercialized.

3. MATERIALS & METHODOLOGY:

1. Collection of excipients:

Materials used such as soya lethin, cholesterol, chloroform, methanol, poloxamer 188, disodium hydrogen phosphate, possaium dihydrogen phosphate, sodium chloride, sodium hydroxide, tween 80, acetonitrile, triethanolamine, carbopol 940 are collected from GIPS chemical store.

2 Apparatus used for preparation of Liposomes:

BUCHI R-200 rotary vacuum flash evaporator¨ by Flawil, Switzerland and water bath (BUCHI B-490).

3 Formulation of Liposomes:

Liposomes are prepared by using Modified thin film hydration method.

PREPARATION OF LIPOSOMES BY THIN FILM HYDRATION METHOD:

Procedure:

Accurately weight Soya lecithin/Cholesterol (90:10, molar ratio), drug paclitaxel (2% or 3%) and organic solvents chloroform/methanol (2:1, molar ratio) were dissolved & transferred into a suitable conical flask. Then mixture of the solution is kept under magnetic stirrer by maintaining the rpm 500 for 1 hour. Now transfer the solution into the rotatory flash evaporator and run the apparatus by maintaining the temperature 40 - 45 for 1 hour. After evaporation of organic solvents, remove the flask (formation of thin film occurs), so now the thin film was re suspended in phosphate buffer saline pH (7.4) with tween 80 (v/v), which was heated in water bath for 1 hour by maintaining the temperature at 45 degrees Celsius. Then the solution is transferred into the conical flask

and kept into the rotatory shaker for 1 hour at rpm 80.After that keep the solution in ultrasonicator for 15 mins or 30 mins. Then the solution is transferred into 50 ml beaker and kept it in the homogenizer by maintaining the rpm 500 for 1 hour. Then the solution was suspended into distilled water, here distilled water was taken (1000 µL and 800 µl) and sample solution was taken by using 20-200 µl. Then the solution was kept into magnetic shaker for 10 mins. Then the solution was taken into the glass cubate and checked for the size of the particle by using the instrument zeta sizer and required results was obtained.

The results are shown in table 1 & table 2

4 CHARACTERIZATION OF LIPOSOMES:

PREFORMULATION STUDIES:

To detect the compatibility of drug and excipient used in the prepared formulation, the following preformulation studies were being carried out.

A. Particle Size and Particle Size Distribution

The particle size and the polydispersity index (PDI) of the liposomes were determined by using dynamic laser light scattering after dispersing 1mg of lyophilized sample with 2ml of Milli-Q water in Zetasizer Nano ZS 90. (Malvern Instruments, Worcestershire, UK). The measurement was done at 25ºC at a scattering angle of 90º.

The results are shown in table 3 & table 4.

B. Solubility Studies:

Add one drop of liquid sample or about 25 mg of solid sample to 0.5 ml of water or the required solvent system in test tube. Tap the tube with finger to mix or stir with glass rod. Observe the sample.

The result is shown in table 5.

C. Fourier Transform Infra-red (FTIR) Spectroscopy:

An FTIR spectrum reveals the characteristic peaks of all functional groups present in a sample. In FTIR spectroscopic study, pure drug PTX, soya lethein, cholesterol, tween 80 and their physical mixture were scanned over a wavenumber range of 4000-400cm-1 in FTIR spectrophotometer (Alpha E, Bruker Alpha, Ettlingen, Germany).

The results are shown in figure 1, figure 2 & figure 3.

D. HPLC Analysis of Paclitaxel:

Paclitaxel concentration in all samples was directly analyzed by a Waters HPLC system (Milford, MA, USA) equipped with a dual pump (Waters 515), UV detector (Waters 2487), and automatic injector (Waters 717 plus). A reverse phase LiChrospher R100 RP-18 column (250×4 mm, 5 µm, Merck, Germany) was used at room temperature. The detector wavelength was set at 227 nm and injection volume was 20 µL. The mobile phase was a mixture of acetonitrile and water (75:75, v/v) at a flow rate of 1.0 mL/min.

The results are shown in table 6.

E. Differential Scanning Calorimetry (DSC):

DSC will be used to determine the nature and specification of the crystallinity of drugs and excipients through the measurement of the glass transition temperature and melting point temperature and their associated enthalpies. This technique will be used to study the physical and chemical interaction between drug and excipients. The required amount of drug, drug- excipients mixtures will be taken and then the samples will be sealed in aluminum pans analyzed in an atmosphere of airflow rate 25 ml/min. A temperature range of 30ºC to 400ºC will be used where the rate of heating is 10ºC/min.

The results are shown in figure 4, figure 5, figure 6 & figure 7

PREPARATION OF TOPICAL BLANK GEL:

Procedure:

Weigh carbopol 940 (0.1%,0.2%,0.3%,0.4% mg) & add water. Let it absorb for about 15 mins.Add the mixture to the magnetic stirrer at 600 rpm for 1 to 2 hours until the solution is mixed properly.Add NaOH or triethanolamine solution drop wise until a gel solution is formed.

5 Evaluation of Topical Gel:

A. Physical Appearance & Homogeneity

Physical appearance and homogeneity of gel was observed visually. Physical appearance includes inspection of color and texture of the gel.

B. pH of The Topical Gel

About 2 gm of topical gel was subjected to pH measurement using a digital pH meter within 24h of manufacture.

C. Spread Ability Study Of Gel

Spread ability was determined using an apparatus which was adapted in-house. It consists of a wooden block, which was provided by a pulley at one end. By this method, spread ability was measured on the basis of “slip” and “drag” characteristics of the gels. A ground glass slide was fixed on this block. An excess of gel (about 2 g) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with a hook. A weight of 100 g was placed on the top of the two slides for 5 min to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to a pull of 20 g weight

with the help of a string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm was noted. A shorter interval indicates better spreadability.

Spreadability (S) was calculated as in.

S = M.L/t ……

where M is the weight (g) tied to the upper glass slide L is the length (cm) moved on the glass slide, and t is time (sec).

D. Viscosity of The Gel:

The rheological properties of the gels were evaluated using a rotational Brookfield viscometer of cone and plate structure (model DV III, USA). About 0.5 g of the gel was applied to the plate and left until the temperature of the cone (25 ± 1 C) was reached. Readings were taken over a large range of shearing rates (from 0.75 to 1875 s-1) corresponding to 0.1 to 250 rpm.

The results are shown in table 7 & table 8.

E. Gel Strength:

The gel strengths are also measured with the rotating viscometer. The sample should be stirred at high speed for 10 seconds (or constant reading), then allowed to stand undisturbed for 10 seconds. With the gears in neutral, a slow (about 3 rpm), steady motion on the hand wheel is applied. The maximum reading is the initial gel in pounds per 100 ft2. The mud is re-stirred for 10 seconds and allowed to stand for 10 minutes. The measurement is repeated as before and the maximum reading is recorded as the 10-minute gel strength in pounds per 100 ft2. When an electrically driven instrument is used, the gel readings are the maximum value obtained at the low-speed 3 rpm setting.

The results are shown in table 9 , figure 8 ,figure 9, figure 10, figure 11.

4. RESULTS:

1. FORMULATION OF LIPOSOMES WITHOUT PTX DRUG:

Table 1:

Sl no	Poloxamer 188(Mg)	Soyalecthin	Cholesterol	Chloroform	Methanol	Buffer
F1	29 mg	60 mg	30 mg	8ml	4ml	-
F2	29mg	60 mg	30 mg	8ml	4ml	-
F3	5mg	30mg	52mg	8ml	4ml	10ml

Sl No	Surfactant Span 80	Cholesterol	Soyalecthin	Chloroform	Methanol	Buffer
F4	70mg	20mg	90mg	10ml	5ml	10ml
F5	60mg	30mg	60mg	10ml	5ml	10ml
F6	60mg	30mg	60mg	10ml	5ml	15ml

Sl No	Surfactant Tween 80	Cholesterol	Soyalechtin	Chloroform	Methanol	Buffer
F7	150mg(1%)	10mg	90mg	10ml	5ml	10ml
F8	300mg (3%)	10mg	90mg	10ml	5ml	10ml
F9	200mg (2%)	10mg	90mg	10ml	00	10ml

1.1 FORMULATIONS OF LIPOSOMES USING PTX DRUG:

Table 2:

Sl no	Drug (Ptx)	Surfactant Tween 80	Cholesterol	Soylechtin	Chloroform	Methanol	Buffer
F1	0.030mg (3%)	300mg(3%)	10mg	90mg	10ml	5ml	10ml
F2	0.030mg	300 mg	10 mg	90mg	10ml	5ml	10ml
F3	0.020mg	300mg	10mg	90mg	10ml	5ml	10ml
F4	0.020mg	200mg(2%)	10mg	90mg	10ml	5ml	10ml
F5	0.020mg	400mg	10mg	90mg	10ml	5ml	10ml

2. PARTICLE SIZE AND PARTICLE SIZE DISTRIBUTION

Table 3: Particle size distribution without drug

Sl no	PDI	Z- Average Size(d.nm)	PDI Width(dm)
F1	Nil	Nil	Nil
F2	Nil	Nil	Nil
F3	Nil	Nil	Nil
F4	0.585	396.1	259.5
F5	0.776	444.7	270.9
F6	0.769	501.7	320.8
F7	0.523	238.4	172.5
F8	0.431	206	135.2
F9	0.517	398.5	166.5

Table 4: Particle size distribution with PTX

Sl no	PDI	Z- Average Size (d.nm)	PDI Width (dm)
F1	0.813	490.5	230.5
F2	1.000	1198	123.5
F3	0.352	94.74	128.9
F4	0.287	120.8	230.5
F5	0.705	78.20	168.3

OBSERVATIONS:

After formulating different formulations of liposomes, the following results are observed, based on which further observations are made. In F1, F2 & F3 of liposome without drug Paclitaxel (PTX) the results were not obtained as expected. Therefore, a clear milky solution is observed due to some impurities so further formulations are prepared changing the amount of the sample. After formulating the particle size and particle size distribution are observed using zeta sizer. The least average particle size and PDI of the formulating samples with drug PTX are taken for further experiments, as they are considered to be good and stable.

3. CHARACTERISATION OF LIPOSOMES:

PERFORMULATION STUDIES:

A. SOLUBILITY STUDIES:

Table 5: Solubility of excipients with drug PTX

Excipients	Solvent System	Solubility
Cholesterol	Methanol & Chloroform	Highly Soluble
Soyalethin		
Paclitaxel Drug		
Tween 80	Phosphate Buffer Saline Solution	Partially Soluble

OBSERVATION:

After performing the test, it was observed that cholesterol, soyalethin and paclitaxel (PTX) are highly soluble in the solvent system. However, tween 80 is partially soluble in phosphate buffer solution.

B. FOURIER TRANSFORM INFRA-RED (FTIR) SPECTROSCOPY:

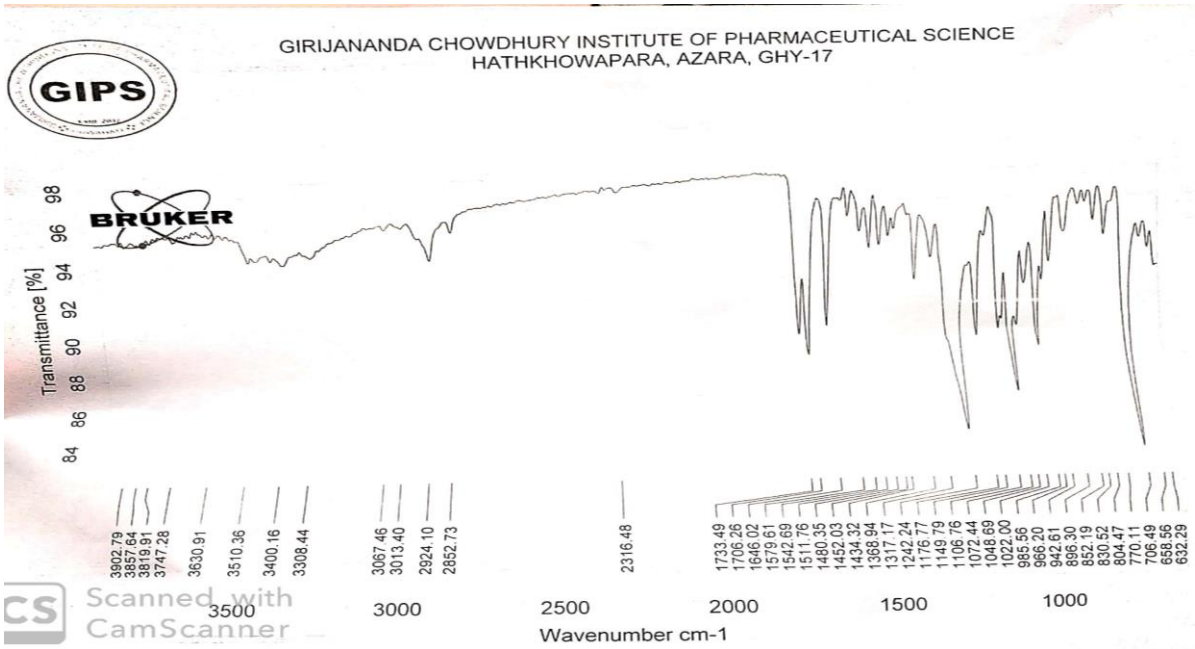


Figure 1: Paclitaxel drug

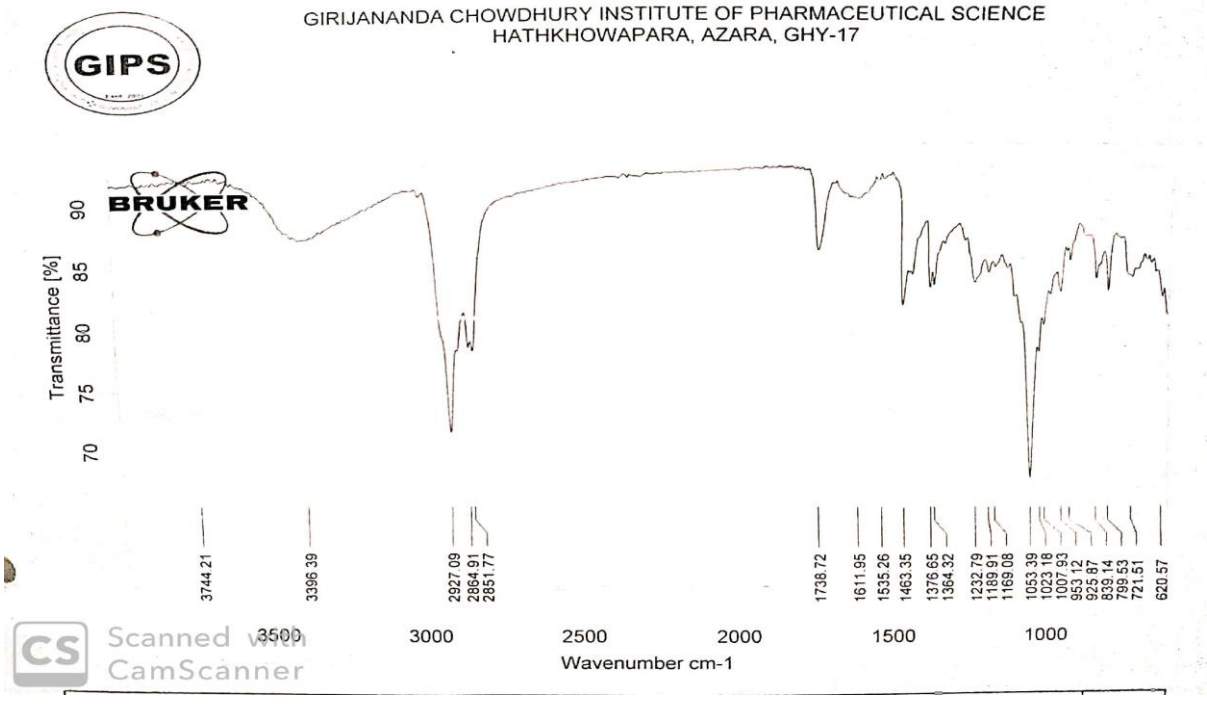


Figure 2: Liposomes excipients

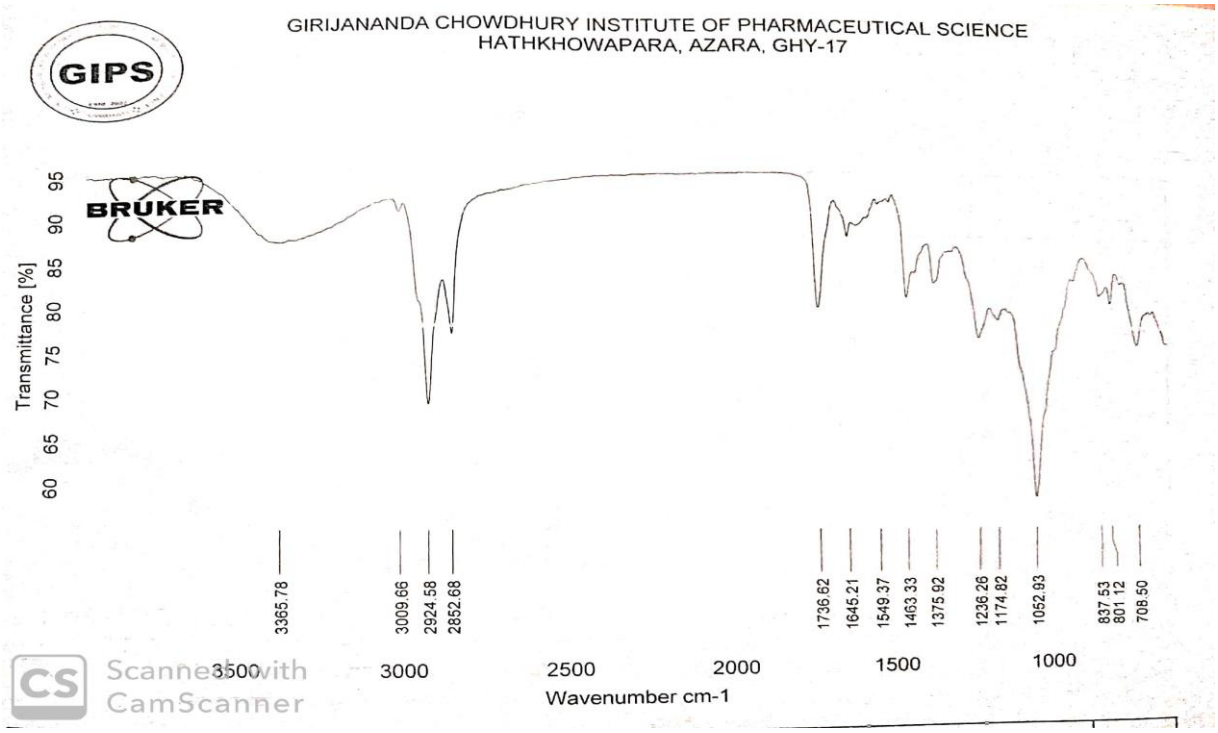


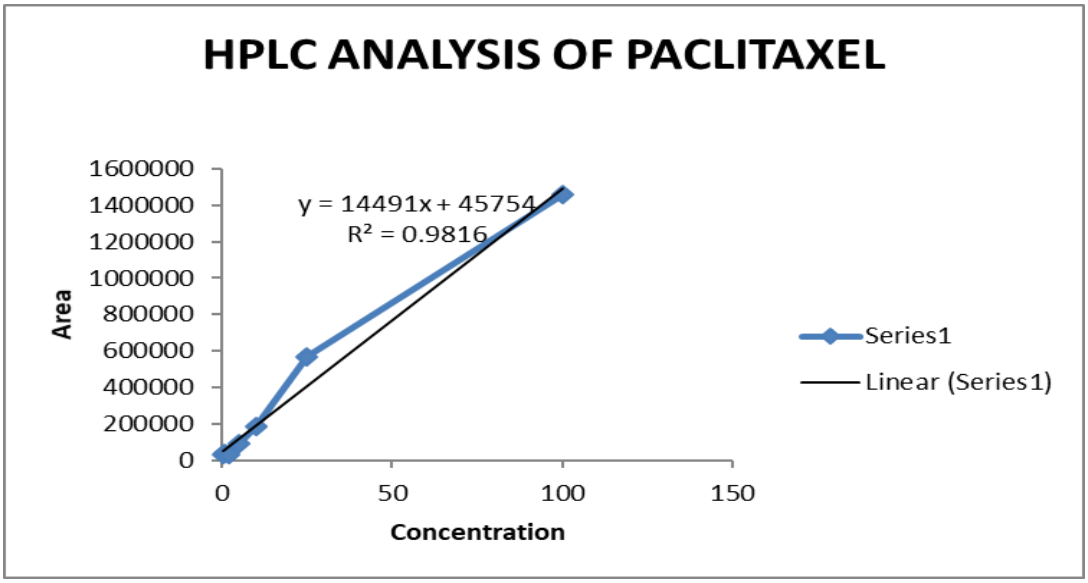
Figure 3: Physical mixture along with Paclitaxel

OBSERVATION:

The IR spectrum of pure drug PTX is shown in the above image (Figure 1). The excipients used for the preparation of liposomes are also checked like cholesterol, soyalethin, tween 80 (Figure 2) and physical mixture with the drug (Figure 3) was observed. The IR spectrum of physical mixture showed compatibility of the drug with the excipient.

C. HPLC Analysis of Paclitaxel:

Table 6:



OBSERVATION:

In the HPLC analysis the detector wavelength was set at 227 nm and injection volume was 20 µL. The mobile phase was a mixture of acetonitrile and water (75:75, v/v) at a flow rate of 1.0 mL/min. The following graph shows that the drug paclitaxel is efficient with the following excipients used for the preparation of liposomes.

D. Differential Scanning Calorimetry (DSC):

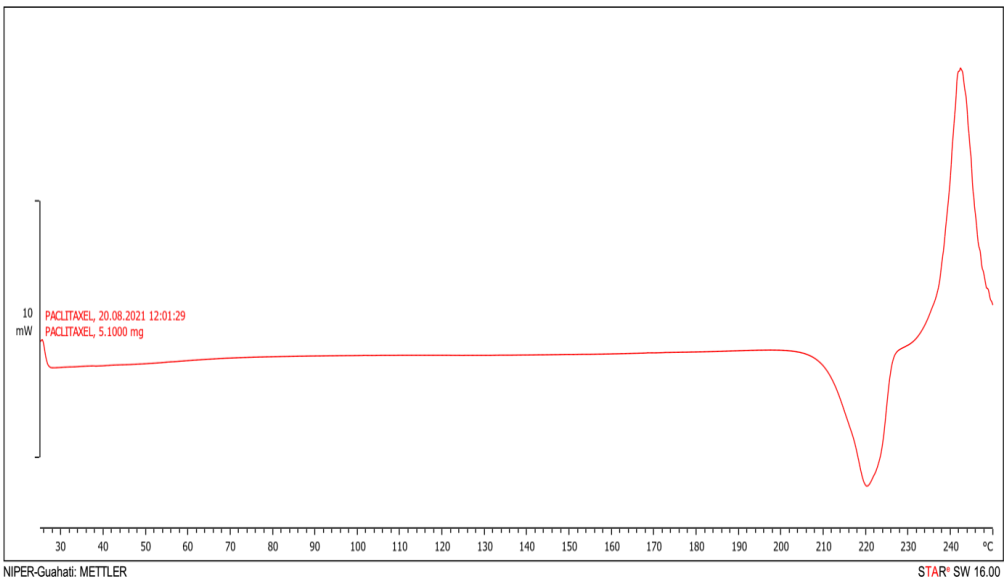


Figure 4: Paclitaxel Drug

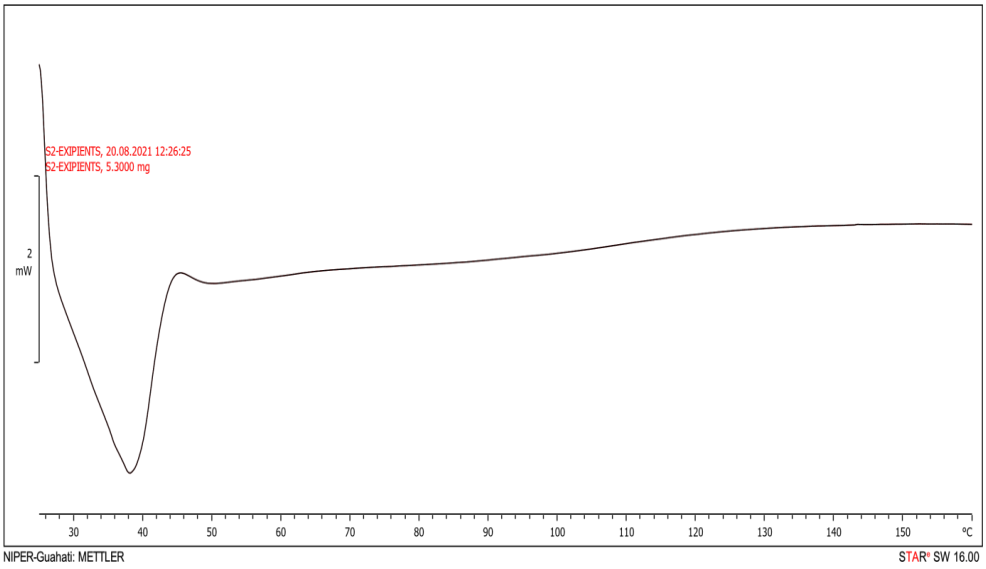


Figure 5: Excipients

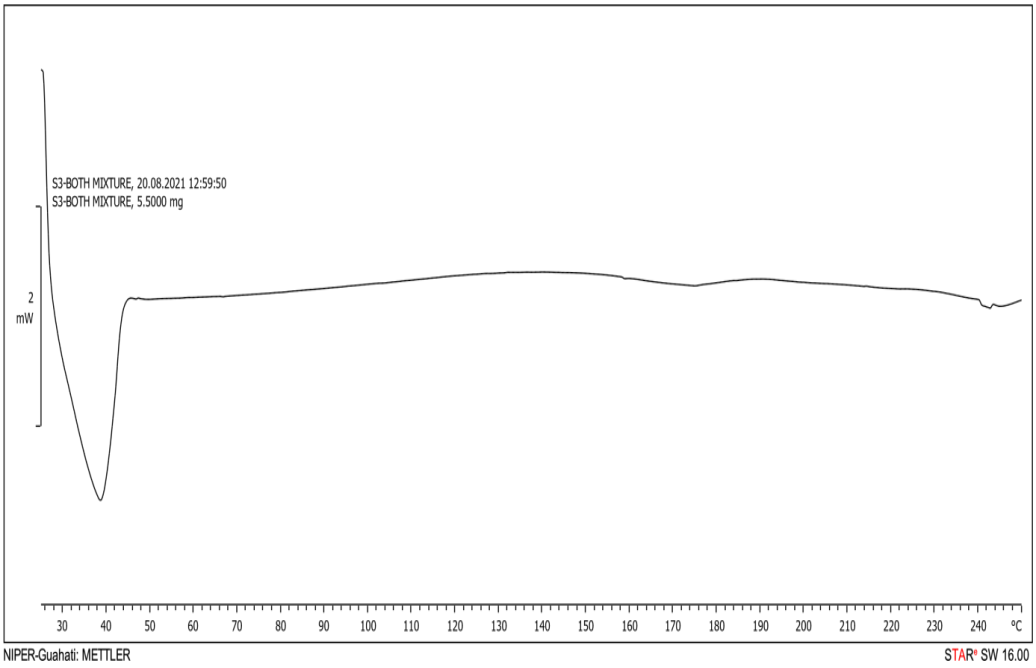


Figure 6: Excipients along with Paclitaxel drug

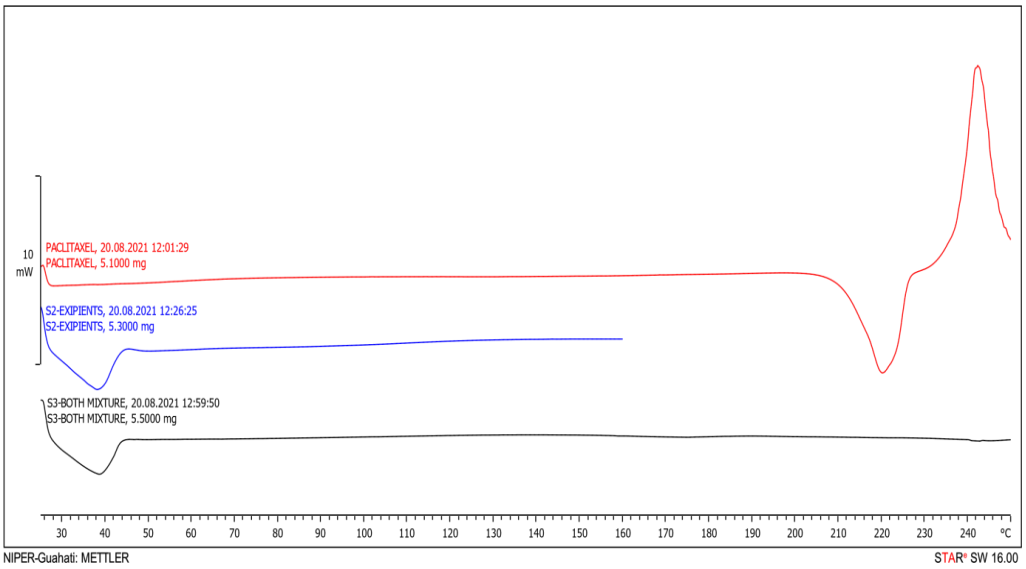


Figure 7: Overlay of paclitaxel, excipients along with both the mixture

OBSERVATION:

During the DSC analysis of liposomes, the endothermic peak of the drug at 240°C can be observed. However, the peak of the mixture of both the drug and excipients was absent which may be due to conversion of the drug to amorphous form during the preparation of liposomes and also it may have been homogenously dispersed in the liposomes.

TOPICAL BLANK GEL:

4. Evaluation of Blank Gel:

The following evaluations are done by preparing blank carbopol 940 gel with different concentration like 0.3% & 0.4%. The different observed results are obtained and shown below.

A. Physical Appearance & Homogeneity

Physical appearance and homogeneity of gel was observed visually. The physical appearance of the blank gel was found to be off white in color with smooth in texture and translucent. All the topical gels were found to be homogenous.

B. pH of The Topical Gel

The pH of different concentration of topical gel was found to be

- Topical gel of concentration 0.3%: pH is 9.
- Topical gel of concentration 0.4%: pH is 7.5.

The ph. value of concentration 0.4% was found to be compatible.

C. Spreadability Study of Gel

The topical gels of different concentration were evaluated with spreading diameter at 5 sec.

D. Viscosity of The Gel:

Table 7: Viscosity of Gel at 0.3%

Concentration 0.3%	
RPM	**RANGES**
3	**1000**
5	**3500**
10	**1680**
12	**1850**
20	**2460**
30	**2800**

Table 8: Viscosity of Gel at 0.4%

Concentration 0.4 %	
RPM	**RANGES**
2	**24300**
3	**9200**
5	**4200**
10	**2100**
12	**1350**
20	**1380**
30	**1280**

OBSERVATION:

The concentration of topical gel 0.3% & 0.4% is prepared and their viscosity is checked using different rpms. Different ranges of viscosities are found in different rpm like 2, 3, 5, 10, 12, 20 & 30. The following tables show the ranges of viscosities along with different rpms.

E. Gel Strength:

Table 9: Gel Strength for Conc 0.3% & 0.4%

Concentration	Hold Time	Absolute + Force	Positive Area	Separation Distance
0.3%	5 sec	8.5 g	2.8 g.s	2.6mm
	10 sec	9.3 g	2.8 g.s	2.4mm
0.4%	5 sec	12.2 g	5.0 g.s	3.9 mm
	10 sec	12.0 g	4.1 g.s	3.1 mm

For 0.3% Concentration:

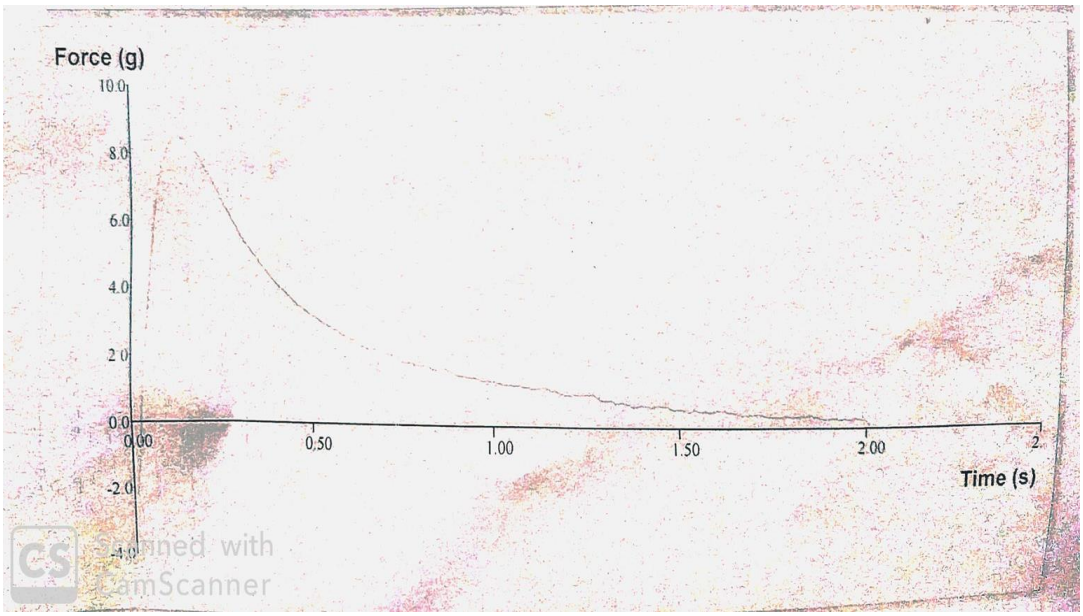


Figure 8: 0.3 % Conc for 5 Sec

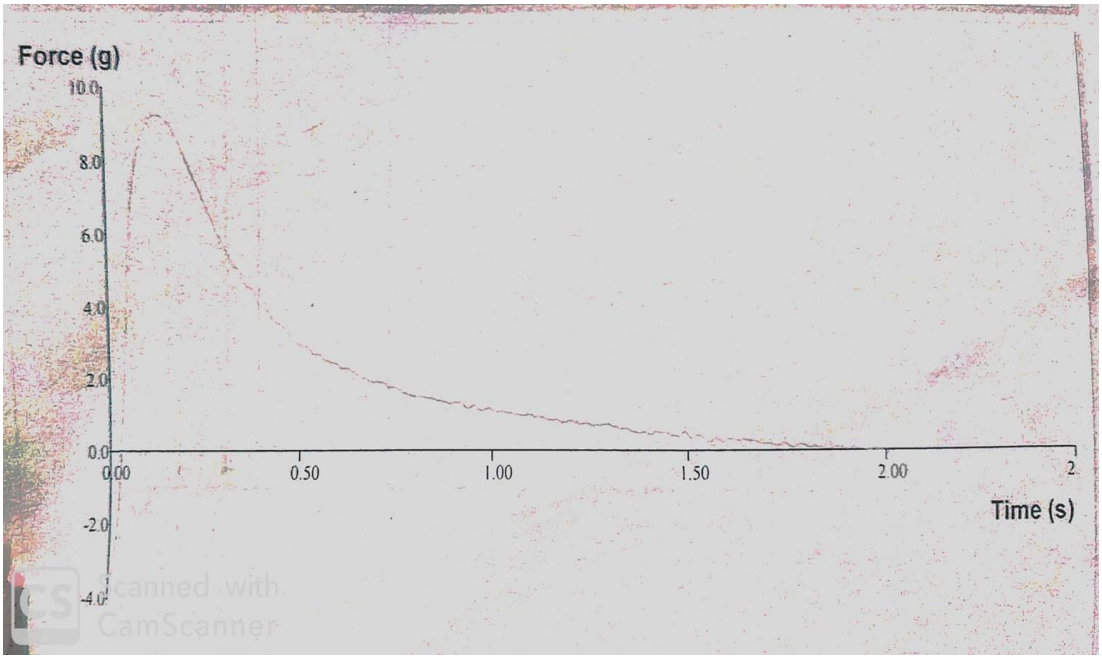


Figure 9: 0.3 % Conc For 10 Sec

For 0.4 % concentration:

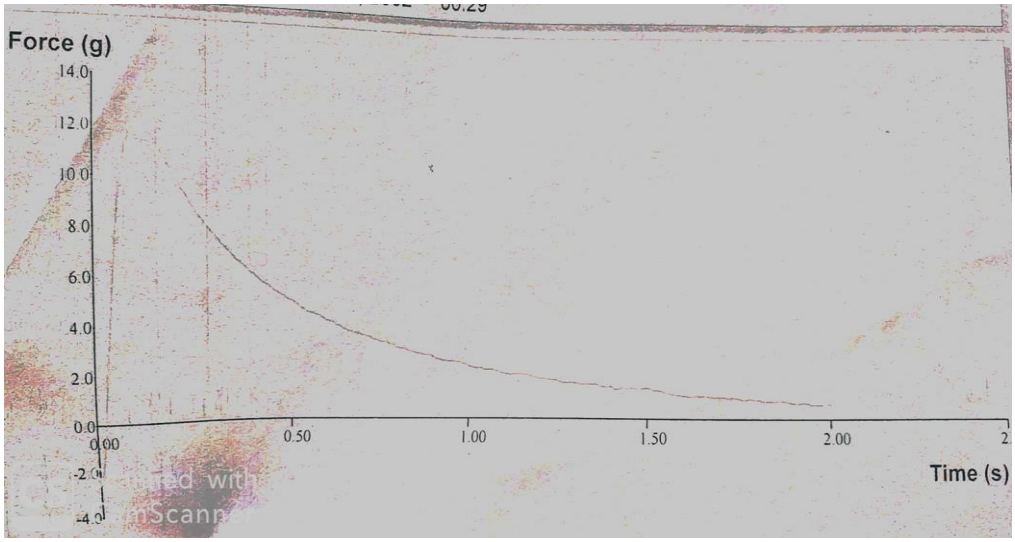


Figure 10 : 0.4 % Conc For 5 Sec

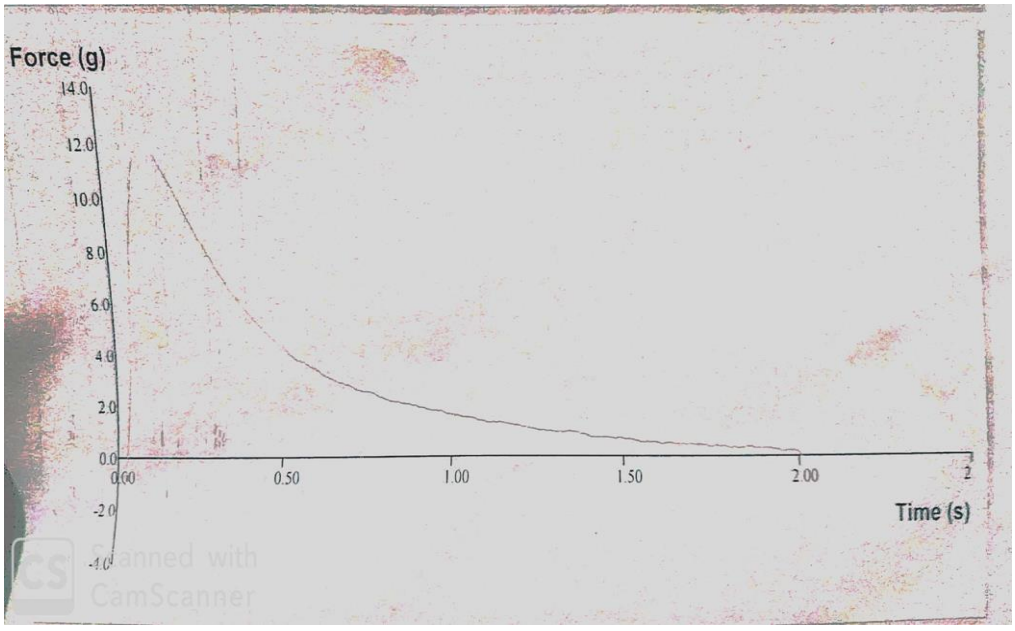


Figure 11: 0.4% Conc For 10 Sec

OBSERVATION:

Different concentration of gels is prepared and tested to check its strength. The following data are shown where different concentration of 0.3% & 0.4% of topical gel are taken to measure the strength withhold time of consisting 5 & 10 second. The sample which shows the best absolute strength will be taken for final preparation of gel and also for further experiments.

DISCUSSION:

In this present study, we have tried to develop a topical gel containing PTX loaded Liposome. From literature study we got to know that earlier attempts have been made to deliver PTX cutaneous and intravenously using ethosomes, Nano emulsions, lamellar liquid crystalline phases and tyrospheres with different penetration enhancers. But due to various side effects new development of formulations have been developed using Liposomes which have certain advantages over other Nano particles including biocompatibility, capacity for self-assembly, ability to carry large drug payloads, and a wide range of physicochemical and biophysical properties that can be modified to control their biological characteristics. Therefore, Liposomes prevent the drug from being metabolized prior to reaching target tissues, and simultaneously they minimize exposure of healthy tissue to the encapsulated drug during its circulation in the blood. It is also seen that liposomes have shown to improve the efficacy and residence time of cytotoxic drugs with subsequent reduction of side effects associated with cytotoxic drugs. After the preparation of Liposomes by thin film hydration method followed by high speed homogenization and ultra-sonication method, the particle size and particle size distribution have been observed by using Zeta-sizer, of different formulation of blank liposomes and PTX drug incorporated liposomes, the average size and its poly dispersity index (PDI) have been observed and the least average size among the formulations have been taken for further evaluation. The particle size of the liposomes is found to get increase with gradual increase in the concentration of the lipid used in the formulation, and also for a specific concentration of the lipid used the size of the liposomes are found to get decrease with increase in the concentration of the surfactant. The particle size of liposomes plays a key role in permeation through the skin as well as bio-distribution since smaller the particle size more will be the permeation (Table 3 &

4). The FTIR spectrums have been observed to check the integrity of the drug within the Liposomes. The presence of specific peak of PTX in the Liposome revealed the compatibility and integrity of the drug in the core of the Liposome.During the DSC analysis of liposomes, the endothermic peak of the drug at 240°C can be observed. However, the peak of the mixture of both the drug and excipients was absent which may be due to conversion of the drug to amorphous form during the preparation of liposomes and also it may have been homogenously dispersed in the liposomes. The HPLC analysis was done which provides a precise result by making better separation and easier analysis of the sample. The mobile phase was a mixture of acetonitrile and water (75:75, v/v) at a flow rate of 1.0 mL/min, the area and concentration of the detected sample was calculated (Table 6).

The topical gel was found to be homogenous as the ingredients were distributed evenly throughout the gel, moreover the pH of the topical gel was found to be compatible and stable. The topical gel was having a very good spreadability and because of this nature of the gel it will favors the drug to penetrate to the deeper site in skin tissue. The viscosity of the gel was found to be in the average ranges which give us a stable formulation. The strength of the gel was also calculated and found to be a good and stable one which will facilitate the drug to penetrate into deeper skin.

CONCLUSION

In the field of nanotechnology research there have been various new studies that have been carried out by combining new technological advances with conventional methods and treatment procedures for developing new potential drugs. The most studied Nano technological Nano carriers are the liposomes which appears to be affective against cytotoxic delivery systems, capable of tumor targeting and decreasing adverse effects and increasing the survival of skin cancer patients. With the new improved formulations of topically applied carcinoma gel combined with paclitaxel incorporated with liposomes as Nano carriers will present a developing field that will surely improve the skin cancer treatment for patients, by improving the quality of life or survival rate of affected patient. Finally we believe that the development of these new combined treatment will become the promising future treatments approaches which lie in the field of liposomes, which combines all the required ingredients that will meet all the requirements for an ideal treatment approach.

REFERENCES

1. Braun-Falco O, Plewig G, Wolff HH, Burgdorf WHC. Dermatology, 2nd edn. Springer-Verlag, New York, 2000, chapter 69: 1719–1721.
2. McGuire JF, Ge NN, Dyson S. Non melanoma skin cancer of the head and neck I: histopathology and clinical behavior. Am J Otolaryngology ,2009, 30,121-33
3. Warner KJ. All scripts EPSi. Skin cancer and its types. Mayo clinic, Rochester, Minn. July 19,2018.
4. Gary WC, William C. Skin cancer. Medicine Net, March 12,2020.
5. Stacey F. What is skin cancer. Health line journal, July 31, 2018.
6. Barrera, M. V., Herrera, E., Topical chemotherapy of actinic keratosis and no melanoma skin cancer: current options and future perspectives. Acts Dermo-Sifiliográficas, 2007,98 (8),556-562.
7. Hope MJ, Kittson CN. Liposomes: a perspective for dermatologists. Dermatology Clin 1993,11, 143–154.
8. Egbaria K, Weiner N. Liposomes as a drug delivery system. Adv Drug Del Rev 1990, 5, 287–300.
9. Lian, T., Ho, R.J., 2001. Trends and developments in liposome drug delivery systems. Journal of pharmaceutical sciences 90, 667-680.
10. Ulrich C. Topical treatment of field cancerization. Cancer Treat Res 2009,146,439-46.
11. Vatve M, Ortonne JP, Birch-Machin MA, Gupta G. Management of field change in actinic keratosis. Br J Dermatol 2007,157(Suppl 2),21-4
12. Maruyama, K., 2011. Intracellular targeting delivery of liposomal drugs to solid tumors based on EPR effects. Advanced drug delivery reviews 63, 161-169.

13. Mezei M, Gulasekharam V. Liposomes – a selective drug delivery system for the topical route of administration: lotion dosage form. Life Sci 1980,26,1473–1477.
14. Mezei M, Gulasekharam V. Liposomes – a selective drug delivery system for the topical route of administration: gel dosage form. J Pharm Pharmacol 1982,34,473–474.
15. Naseema A, Riaz AK. Liposome systems as viable drug delivery for skin cancer. Elsevier journal. 2016,10,1016.
16. McGillis ST, Fein H. Topical treatment strategies for non-melanoma skin cancer and precursor lesions. Semin Cutan Med Surg 2004,23,174-83
17. Askew DA, Mickan SM, Soyer HP, Wilkinson D. Effectiveness of 5-fluorouracil treatment for actinic keratosis -- a systematic review of randomized controlled trials. Int J Dermatol,2009,48,453-63
18. Dillaha CJ, Jansen GT, Honeycutt WM, Holt GA. Further studies with topical 5-fluorouracil. Arch Dermatol ,1965,92,410
19. Romagosa R, Saap L, Givens M, et al. A pilot study to evaluate the treatment of basal cell carcinoma with 5-fluorouracil using phosphatidyl choline as a transepidermal carrier. Dermatol Surg 2000, 26,338-400.
20. Gross K, Kircik L, Kricorian G. 5% 5-Fluorouracil cream for the treatment of small superficial Basal cell carcinoma: efficacy, tolerability, cosmetic outcome, and patient satisfaction. Dermatol Surg 2007,33,433-9; discussion 440.
21. Moreno G, Chia AL, Lim A, Shumack S. Therapeutic options for Bowen's disease. Australas J Dermatol 2007,48,1-8.
22. Neubert T, Lehmann P. Bowen's disease -- a review of newer treatment options. Ther Clin Risk Manag 2008,4,1085.

23. Gaspari A, Tyring SK, Rosen T. Beyond a decade of 5% imiquimod topical therapy. J Drugs Dermatol 2009,8,467.
24. Gebauer K, Shumack S, Cowen PS. Effect of dosing frequency on the safety and efficacy of imiquimod 5% cream for treatment of actinic keratosis on the forearms and hands: a phase II, randomized placebo-controlled trial. Br J Dermatol 2009,161,897-903.
25. Stockfleth E, Ulrich C, Lange-Asschenfeldt B, et al. Treatment of multiple, multiform actinic keratoses on the head with imiquimod 5% cream. Eur J Dermatol 2009, 19, 355.
26. Bath-Hextall FJ, Bong J, Perkins W, et al. Interventions for basal cell carcinoma of the skin (Cochrane review). Cochrane Database Syst Rev 2007,1, CD003412 42.
27. Schulze HJ, Cribier B, Requena L, et al. Imiquimod 5% cream for the treatment of superficial basal cell carcinoma: results from a randomized vehicle-controlled phase III study in Europe. Br J Dermatol 2005,152,939-47
28. Alessi SS, Sanches JA, de Oliveira WR, et al. Treatment of cutaneous tumors with topical 5% imiquimod cream. Clinics 2009, 64,961.
29. Micali G, Nasca MR, De Pasquale R. Erythroplasia of Queyrat treated with imiquimod 5% cream. J Am Acad Dermatol 2006,55,901.
30. Eberle J, Fecker LF, Forschner T, et al. Apoptosis pathways as promising targets for skin cancer therapy. Br J Dermatol 2007,156(Suppl 3),18-24.
31. Fecker LF, Stockfleth E, Nindl I, et al. The role of apoptosis in therapy and prophylaxis of epithelial tumours by nonsteroidal anti-inflammatory drugs (NSAIDs). Br J Dermatol 2007,156(Suppl 3),25-33.

32. Merk HF. Topical diclofenac in the treatment of actinic keratoses. Int J Dermatol 2007,46,12.
33. Dawe SA, Salisbury JR, Higgins E. Two cases of Bowen's disease successfully treated topically with 3% diclofenac in 2.5% hyaluronan gel. Clin Exp Dermatol 2005, 30,712.
34. Anjana AA, Babasaheb GD. Formulation and evaluation of transdermal path for targeted drug deliuvery of tamoxifen citrate for cancer. Indian journal of health science and research. 2016,9,40-48
35. Raquel Petrillia, Josimar O. Eloya, Fabiano P. Saggiorod, Deise L. Chescad, Marina Claro de Souzaa, Marcos V.S. Diase, Luis L.P. DaSilva, Robert J. Leeb, Renata F.V. Lopeza, Skin cancer treatment effectiveness is improved by iontophoresis of EGFR targeted liposomes containing 5-FU compared. Journal of Controlled Release,2018,283,151-162.
36. Barenholz, Y.C. Doxil®—The first FDA-approved nano-drug: Lessons learned. J. Control. Release 2012 ,160, 117–134.
37. Petre, C.E.; Dittmer, D.P. Liposomal daunorubicin as treatment for Kaposi's sarcoma. Int. J. Nanomed. 2007, 2, 277.
38. Batist, G.; Ramakrishnan, G.; Rao, C.S.; Chandrasekharan, A.; Gutheil, J.; Guthrie, T.; Shah, P.; Khojasteh, A.; Nair, M.K.; Hoelzer, K. Reduced cardiotoxicity and preserved antitumor efficacy of liposome-encapsulated doxorubicin and cyclophosphamide compared with conventional doxorubicin and cyclophosphamide in a randomized, multicenter trial of metastatic breast cancer. J. Clin. Oncol. 2001, 19, 1444–1454.
39. Hong, K.; Drummond, D.C.; Kirpotin, D. Liposomes Useful for Drug Delivery. U.S. Patent No. US20160030341 A1, 4 February 2009.

40. FDA. Center for Drug Evaluation and Research. MARQIBO New Drug ,2012.