" PREPARATION AND CHARACTERIZATION OF TAMOXIFEN LOADED TRANSDERMAL DRUG DELIVERY SYSTEM FOR ESTROGEN RECEPTOR POSITIVE BREAST CANCER"

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For the award of the degree of

Doctor of Philosophy
In the Faculty of Pharmacy

By

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(Registration No: KLEU/Ph.D./09-10/DOUN09014)

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Mrs. Anjana Adhyapak…

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LIST OF ABBREVIATIONS

1.	µg	microgram
2.	ACS	American Cancer Society
3.	cm^2	Square centimeter
4.	DBT	dibutyl phthalate
5.	DCM	dichloromethane
6.	DMEM	Dulbecco's modified eagle's medium
7.	DMSO	dimethyl sulfoxide
8.	DSC	Differential scanning calorimetry
9.	EC	Ethyl cellulose
10.	HPLC	High performance liquid chromatography
11.	HPMC K-50	hydroxypropyl methyl cellulose
12.	IPM	Isopropyl myristate
13.	LOD	Limit of detection
14.	LOQ	Limit of quantification
15.	mg	milligram
16.	mm	millimeter
17.	MRI	Magnetic resonance imaging
18.	ng	nanogram
19.	PET	Positron emission tomography
20.	Poly (SA: RA) 5:5	Poly (sebecic-co-recionolic acid anhydride) 7:3 w/w
21.	RH	Relative humidity
22.	RSD	Relative standard deviation
23.	SAXC	Small angle X-ray scattering
24.	SD	Standard deviation
25.	SEM	Scanning electron microscopy
26.	SLN	Solid lipid nanoparticles
27.	TC	Tamoxifen citrate
28.	v/v	volume by volume
29.	w/w	weight by weight
30.	XRD	X-ray diffraction

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ABSTRACT

Background and Objective: Conventional administration of drugs to target the cancer cell has the disadvantages of providing relatively low concentrations of the drug at the site of action. Hence, Tamoxifen citrate (TC) loaded matrix and solid lipid nanoparticle gel reservoir systems can deliver high therapeutic effective concentration of drug either in the site or site of tumor excision. In the present study, hydrophilic and lipophilic polymers are selected to develop transdermal patches. The objective of the present research work is to develop transdermal patches of tamoxifen citrate for estrogen receptor positive breast cancer using matrix and transdermal solid lipid nanoparticle reservoir systems.

Methods: The physical status and crystal structure of Tamoxifen citrate was confirmed by analyzing with DSC and XRD. To determine the TC content in formulations and pure API, the new UV-Visible analytical method was developed and standardized however, to determine the TC in blood samples a simple reproducible sensitive HPLC method was also developed. These developed UV-Visible and HPLC methods were resulted out excellent sensitive, accurate and provided good precision. The *In-vitro* studies of flux, permeation and diffusion coefficient variations of results observed in the TC loaded formulations were associated with factors such as hydrophilic nature of the polymer, water absorption towards the polymer surface, swelling properties. The estrogen positive MCF-7 breast cancer cell lines were used to determine the cytotoxic and effectiveness of the developed formulations. For the drug permeation of TC loaded matrix and reservoir systems, *in vivo* pharmacokinetic studies were investigated by using Female Albino Rats. Further, tamoxifen loaded transdermal patch was evaluated in rats to assess its efficacy on multiple biochemical parameters.

Results: The investagted *in vivo* studies reveled that drug permetion was very slow with penetration enhancer as compared to the *in vitro* drug permeation studies which was due to skin barriers. The TC loaded patch and solid lipid nanoparticle gel formualtions showed effective reduction rate of cell viability, depentent on amount of drug release from hydrophilic and hydrophobic combination based formualtions. As increasing the TC concentration in the formulations which results in a proportionate increase in dose and increased reduction in cell proliferation. The cytotoxicity against the estrogen positive MCF-7 cell line was affected significantly by the released amount of tamoxifen citrate. The pharmacokinetic studies revealed that the administered TC containing transdermal formulations, the drug was present in rat plasma for a much longer period compared to the oral administration, 18 h vs. 4 h. A relative bioavailability of formulation ranges in between 0.56-0.74 was estimated for transdermal vs. oral administration. Treatment with multiple formulations loaded with tamoxifen in transdermal patch showed improved anti-cancer activity by regulating hameatological parameters like haemoglobin content and various biochemical parameters like SGOT, SGPT and physical parameters like body weight.

Conclusion: The present study concluded that the transdermal patches of tamoxifen citrate for estrogen receptor positive breast cancer using matrix and transdermal solid lipid nanoparticle reservoir systems can be implicated in the treatment of breast cancer.

Key words: Tamoxifen citrate TDDS, SLN-Gel, PSRA, estrogen positive MCF-7 cell lines, Pharmacokinetic.

1. INTRODUCTION

Chemotherapy treatment is a complex treatment in which several factors are considered in investigating its success or failure. It transmits a high risk due to drug toxicity, and more effective drugs causes to be more toxic. There are many problems that still exist even for successful chemotherapy, and patients have to experience severe side effects and sometimes sacrifice their quality of life.[1] One of the major problems cladding cancer chemotherapy is the attainment of the required therapeutic concentration of the drug at the tumor site for a longer period of time without causing undesirable effects on the other organs while circulating in the body[2-6].

Breast cancer is one of the leading causes of cancer deaths among women. Nearly 1 million new cases are diagnosed each year. Oral administration of the non-steroidal antiestrogen like tamoxifen is the treatment of choice for the patients with all stages of estrogen receptor (ER) positive breast cancer[7].Antagonizing estrogen is a popular treatment strategy because estrogen receptor (ER) over expression is observed in about 70% of breast cancers, and about two thirds of breast cancers in postmenopausal women are ER-positive[8]. Oral tamoxifen undergoes extensive hepatic metabolism and the subsequent biliary excretion of metabolites[9]. Tamoxifen can have harmful long term side effects such as the development of endometrial cancer, or an acquired tamoxifen resistance leading to further tumor progression[7].Other side effects include liver cancer, increased blood clotting and ocular side effects such as retinopathy and corneal opacities. These effects were reported to be dose dependent. To overcome these undesirable side effects, transdermal drug delivery (TDDS) is necessary in order to achieve optimum therapeutic outcomes for breast cancer for a prolonged period of time. Transdermal drug delivery system (TDDS) allows delivery of contained drug into the systemic circulation via permeation through skin layers at a

controlled rate. All patch-type transdermal delivery systems developed to date can be described by three basic design principles: drug in adhesive, drug in matrix (usually polymeric), and drug in reservoir.

Preparation of TDDS consists of three basic designs: membrane control or RPs (reservoir patches), matrix or monolithic patches (MPs), and DIAPs (The target was to design) drug-in-adhesive patches. Several factors should be considered before choosing an appropriate design for a particular compound: drug solubility, stability and release rate. As a rule of thumb, if a drug permeates or crosses the skin faster than desired, RPs can slow down or control the permeation. Alternatively, if a drug passes through skin at a slower rate than the patch releases it, MPs probably containing a suitable chemical penetration enhancer may suffice. These systems consist of a backing layer, a polymeric matrix, an adhesive and a protective liner[10-15].

Treatment for breast cancer [16-19]

Surgery

Surgery is the oldest form of treatment for breast cancer. It also has an important role in diagnosing and staging (finding the extent) of cancer. It offers a great hope to cure different types of cancer, especially those that have not yet spread to other parts of the body.

Lumpectomy

A surgical procedure to remove a tumor (lump) and a small amount of normal tissue around it.

Partial mastectomy

A surgical procedure to remove the part of the breast that contains cancer cells and some normal tissue around it. This procedure is also called a segmental mastectomy.

Total mastectomy

A surgical procedure to remove the whole breast that contains cancer cells.

Radical mastectomy

A surgical procedure to remove the breast along with chest wall muscles under the breast and all of the lymph nodes under the arm. This procedure is sometimes called a Halsted radical mastectomy.

Radiation therapy

Radiation therapy is a cancer treatment that uses high-energy x-rays or other types of radiation to kill cancer cells. There are two types of radiation therapy. External radiation therapy uses a machine outside the body to send radiation towards the cancer. Internal radiation therapy uses a radioactive substance sealed in needles, seeds, wires, or catheters that are implanted directly into or near the cancer. The way the radiation therapy is given depends on the type and stage of the cancer being treated.

Chemotherapy[20]

Chemotherapy of cancer involves use of chemotherapeutical agents that are directed to kill or control the growth and proliferation of cancer cells. These agents are often toxic or even life threatening. Cancer chemotherapy was first successfully practiced in the 1950s when nitrogen mustard was found to be effective in inhibiting tumor growth. Due to its extreme toxicity, however, effective chemotherapy with anticancer drugs was not widely applied until the 1960s. In the beginning of 1970s

cancer chemotherapy grew rapidly as single and combination with other treatments, a possible way to cure some types of cancers or, at least, to lengthen the life expectancy of patients. So far there are number of anticancer agents available for clinical use, some are synthetic chemicals and some are natural extracts. Paclitaxel is a naturally occurring microtubule-binding agent, which has been shown to have tumoricidal activity against several human neoplasms, including non-small lung cancer, breast cancer and ovarian cancer.

Hormonal therapy[21]

Hormones, especially estrogens, have been linked to breast cancer because of their ability to stimulate cell proliferation, which in turn leads to accumulation of random genetic factors that may result in cancer. On the basis of this concept, chemoprevention of breast cancer is mostly aimed at reducing the rate of cell division through administration of ant hormones. Tamoxifen citrate and its derivatives are widely used in the hormonal therapy of breast cancer.

Limitation of surgery with treatment of Breast cancer[20]

Anatomical location of many tumors does not allow for total resection.

Possible side effects of mastectomy and lumpectomy include infection and blood or fluid collecting at the place where the incision is made. Axillary dissection causes swelling of the arm called lymphedema. It happens to between 1 to 3 out of 10 patients. High incidence of recurrence of tumor from the residual cells remaining after the resection/ surgery.

Limitation of radiation therapy with treatment of Breast cancer[2,3,5]

Normal tissues vary in their response to radiation. As with tumors, normal tissues that are dividing more rapidly may be affected during radiation treatment.

Since radiation is a local treatment, side effects are usually confined to the area being treated. Swelling and heaviness in the breast sunburn-like changes in the treated area. Fatigue is a general effect of radiation but the exact cause is unknown. The skin over the treatment area may become darker because of its effect on pigment producing cells. When radiation treatments include the chest area, the lungs can be affected due to decrease in the levels of surfactant, the substance that helps keep the air passages open. Radiation therapy is not given during pregnancy because it can harm the fetus.

Limitation of systemic chemotherapy with treatment of Breast cancer [2,3,5]

Chemotherapy is a complicated procedure in which many factors are involved in determining its success or failure. It carries a high risk due to drug toxicity, and the more effective drugs tend to be more toxic. All chemotherapeutic agents have significant dose limiting toxicities, such as bone marrow suppression, nephrotoxicity, ototoxicity, stomattis, nausea, neuropathy, myalgias, fatigue, alopecia, diarrhoea, mucosal toxicity, skin and nail changes. In case of hormonal therapy, hyperplasia, polyps, carcinoma and sarcoma, endometrial cancer, liver cancer, blood clotting and ocular side effects such as retinopathy and corneal opacities, hot flashes are reported. Multi drug resistance is the state, where the cell treated with a drug becomes resistant not only to the drug but also to other drugs, often structurally and functionally different. The tumor cells with multidrug resistance are characterized by lowered intracellular accumulation of the drugs.

Newer breast cancer therapy modalities being investigated[17-19]

Combination therapy involves administration of two or more drugs which belong to different classes or which are cytotoxic at different stages of the cell cycle.

Chemotherapy combined with radiation therapy involves administration of cytotoxic drugs, which sensitize the tumor cells for the radiation therapy.

Intratumoral or interstitial brachytherapy involves delivery of radiation to tumor cells by implanting the radiation needles/seeds into the tumor.

Intratumoral chemotherapy involves the delivery of cytotoxic and hormonal related drugs directly to the tumor site thereby increasing local drug concentration and minimizing systemic side effects[5].

Polymeric implantable and intratumoral injectable local drug delivery systems[22]

The production of drug loaded polymeric implant, wafer, microspheres and hydrogel introduced a new concept in drug administration. Drugs can be delivered to tumor in a sustained, continuous and predictable release fashion using polymers as delivery vehicles. Few implantable and intratumoral injectable microsphere drug delivery systems commercially available for the treatment of cancer are, Decapeptyl® (d-Trp)LH-RH, Decapeptyl® (d-Trp) LH-RH, Lupron Depot® (Leuprolide), Zoladex® (D-ser(Bu), AzGly-GnRH for prostate cancer and local delivery Gliadel® (Carmustine) for brain tumor. Intratumoral injectable micro particles for the treatment of cancer is Lupron Depot® (Leuprolide) for prostate cancer.

Non-biodegradable polymeric reservoirs and matrices[22, 23]

The first polymeric controlled release devices were based on non biodegradable polymers, principally silicone elsatomers. In 1964, researchers recognized that certain dye molecules could penetrate through the walls of silicone tubing. On observations, it leads to the development of reservoir drug delivery systems, which are hollow polymer tubes filled with a drug suspension. The drug is released by dissolution into the polymer from suspension followed by diffusion

through the polymer wall, a mechanism that works with silicone or poly (ethylene-co-vinyl acetate) (EVAc), which are most commonly, used non degradable polymers.

Biodegradable polymeric devices[23]

Polymeric devices like implants microspheres and hydrogels formed from biodegradable polymers dissolve after implantation and thereby release the drug. Most commonly used biodegradable polymer polyesters include poly (caprolactone) (PCL), poly lactic-co-glycolic acid) (PLGA), poly (lactic acid) (PLA) and polyanhydrides. Dissolution/degradation/diffusion of drug from these devices occurs by complex sequencual steps, including water penetration, hydrolytic and enzymatic degradation of the polymer molecules and degradation of polymer monomers and oligomers.

Oral vs transdermal: Pharmacokinetic differences

The oral and transdermal hormonal therapy (HT) have been proven and reported that the effectiveness for the relief of menopausal symptoms, several differences exist between these routes that may influence safety and patient acceptance of the regimen. Oral administration of estrogen is associated with extensive gut and first-pass liver metabolism as well as significant hepatic stimulation.[24] To overcome these metabolism processes, the investigation results revealed that the oral estrogens must be administered in relatively high doses to provide blood levels adequate to reduce menopausal symptoms. The extensive metabolism of oral estrogens results in conversion of a large portion of the dose to estrone and its conjugates, which have less estrogenic activity than estradiol has been reported. Significant metabolic conversion of oral estrogens to estrone results in a higher ratio of estrone to estradiol in the blood stream, which is opposite of the

physiological levels in pre-menopausal women.[25] In addition, some metabolites of conjugated estrogens formed during first-pass metabolism appear to have antiestrogenic or unrecognized pharmacologic activity in the human body.[26]

However, the transdermal dosage forms deliver estradiol directly to the systemic circulation through the skin, bypassing gut and first-pass hepatic metabolism.,[24] this prevents the gut and liver metabolism via transdermal administration helps maintain an estradiol to estrone ratio similar to that found in premenopausal women.[27] However, the clinical relevance of the estradiol-to-estrone ratio is currently unknown.

Significant variations in the metabolism of oral estrogens results in wide fluctuations in estrogen blood levels throughout the day, potentially resulting in inconsistent control of vasomotor symptoms (VMS).[28,29] Transdermal administration provides more consistent blood levels by avoiding the peaks and troughs inherent to oral estrogens.

Need for the study

There are three main approaches to deal with established breast cancer: surgical excision, irradiation and chemotherapy. The drugs that are commonly used for breast cancer are given below.

Natural and semisynthetic

Paclitaxel and docetaxel

Synthetic

Cyclophosphamide, methotrexate, fluorouracil, cyclophosphamide, doxorubicin and gemcitabine, mitoxantrone.

Hormonal therapy

Luteinizing hormone-releasing hormone (LHRH) analogs

Anstrazole and its derivatives

Tamoxifen and its derivatives.

Tamoxifen citrate[30,31]

Tamoxifen, a selective estrogen receptor modulator (SERM) is widely used in the treatment of breast cancer, and is effective in up to 80% of tumors that express both estrogen and progesterone receptors. It exhibits good bioavailability upon oral administration and is mainly employed for long term prophylactic therapy. Tamoxifen has a half life of 5 to 7 days and is administered in a dose of 10 to 20 mg, orally twice a day over a period of 5 years. Following long-term therapy, tamoxifen has some major side effects such as hot flushes, nausea, menstrual irregularities and endometrial cancer and development of drug resistance, which may lead to further progression of the tumor.

Hence a novel method of drug administration is needed to overcome the various disadvantages associated with above conventional therapy. This can be achieved by local extended drug delivery systems such as transdermal drug delivery. The said delivery systems have advantages like attaining the required therapeutic concentration of antineoplastic agents at the tumor site and target the delivery of drug to tumor cells and killing the malignant cells that survived the surgery and also preventing the systemic side effects of the conventional chemotherapy.

Advantage of local transdermal Patches (TDP)[27,28]

The transdermal polymeric based anticancer drug loaded patches offer sustained/controlled release of drug.

The transdermal polymer based anticancer drug loaded patches provide an opportunity to deliver high, localized doses of drug for a prolonged period directly into tumor.

Systemic side effects can be minimized by dose reduction with local drug Transdermal delivery. Frequent administrations of drug can also be eliminated. Microspheres provide prolonged continuous drug release into local tumor mass and drug which is not taken up by the tumor cells will diffuse into the local lymphatic and venous drainage and thus prevent the spread of metastases in its initial stage. Relatively non-invasive suitable for tumors that are not resectable/operateble.

Finally the proposed novel transdermal local drug delivery system may increase the tumor responsiveness to hormonal therapy and reduce relapse of the disease. In view of the above, it was considered worthwhile to formulate tamoxifen citrate local applicable patches with following:

Objectives

- Physicochemical characterization of tamoxifen citrate
- To formulate a new transdermal drug delivery patch to target estrogen positive breast cancer.
- To formulate a new solid lipid nanoparticles-reservior transdermal drug delivery patch to target estrogen positive breast cancer.
- Formulation of tamoxifen loaded transdermal patches by: using solvent evaporation or casting technique
- Formulation of tamoxifen loaded solid lipid nanoparticles by solvent deposition/Rotary flash evaporation method or any other suitable reported method. The suitable loaded liposomes will be then incorporated into the transdermal patches.
- To study the *in vitro* characterization of the transdermal drug delivery patches
- To investigate effectiveness of the selected formulation for estrogen positive breast cancer cell lines
- To investigate the selected formulation for in *vivo* physical and biochemical parameters in DMBA induced breast cancer.

2. REVIEW OF LITERATURE:

Drug data: Tamoxifen Citrate (TC)[7, 8, 30,31]

Tamoxifen citrate is the treatment of choice for the patients with all stages of estrogen receptor (ER) positive breast cancer.

Description

Physically TC is a synthetic white powder and is hygroscopic at high relative humidity and is sensitive to UV light. Chemically, it is the trans-isomer of a triphenylethylene derivative. The chemical name is (Z)-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-N,N-dimethylethanamine-2-hydroxy-1,2,3-propanetricarboxylate 1:1); trans-1-(p-ß-Dimethy laminoethoxypheny l)-1,2-Diphenylbut-1-ene citrate. TC is stable for at least two years when stored desiccated at 2°-8°C in the dark.

Physicochemical properties

TC has a molecular weight of 563.6, molecular formula ($C_{26}H_{29}NO.C_6H_8O_7$), melting point is 140-142°C, pKa is ~8.85, solubility in water is 0.3 mg/L at 20°C and 50 mg/100 ml at 37°C and 0.2 mg/ml in 0.02 N HCl at 37°C, soluble in methanol, ethanol and slightly soluble in acetone and in chloroform.

Pharmacokinetics

Administration of tamoxifen 10 mg twice daily and 20 mg once daily has demonstrated similar bioavailability. Well absorbed from the gastrointestinal tract. TC is metabolized by hydroxylation to glucuronides, other conjugates, and unidentified polar metabolites. Tamoxifen is extensively metabolized to a major metabolite N-desmethyl tamoxifen, 4-hydroxy tamoxifen, 4-hydroxy-N-desmethyl tamoxifen,

which have similar activity to tamoxifen. A single oral dose of 20 mg tamoxifen, an average peak plasma concentration of 40 ng/ml (range 35-45 ng/ml) occurred approximately 4-7 h after dosing. Administration of 10 mg tamoxifen given twice daily for 3 months resulted in an average steady state plasma concentration of 120 ng/ml (range 67 to 183 ng/ml. Administration of 20 mg tamoxifen once daily for 3 months resulted in average steady-state plasma concentration of 122 ng/ml (range 71 to 183 ng/ml). Steady-state concentrations for tamoxifen are achieved in approximately 4 weeks. 65% of the oral dose is excreted over a period of 2 weeks with fecal excretion being the primary route of elimination. Approximately 60 fold higher levels of tamoxifen and its metabolites relative to serum levels were found in the liver. Distribution half-life is 7 to 14 h. Elimination half life 7-11 days.

Mechanism of action

The TC is a drug of class Type I antiestrogens that competitively inhibits the binding of estradiol to the intracellular estrogen receptor.TC exhibits antiestrogenic activity by forming tamoxifen-estrogen receptor complex that is converted incompletely to the fully activated form. As a result of the imperfect changes in the tertiary structure of the protein, the complex is only partially active in initiating the programmed series of events necessary to orchestrate gene activation.Receptor complex binds with DNA and can alter or block subsequent mRNA transcription and lead to cellular apoptosis. Tamoxifen is also thought to induce a tumoricidal effect on estrogen receptor-negative cells by increasing the secretion of inhibitory growth factors.

Dosage and Administration

For patients with breast cancer, the recommended daily oral tablet 10 to 20 mg for 5 years. Doses greater than 20 mg daily should be given in divided doses (morning and evening). Current data from clinical trials support 5 years of adjuvant tamoxifen therapy for patients with breast cancer. There is no evidence that doses larger than 20 mg daily are more efficacious. The recommended dose of tamoxifen for ductal carcinoma in-situ (DCIS) is 20 mg daily for 5 years. In case of infertility dose administered is 10 mg of tamoxifen twice daily on days 2, 3, 4 and 5 of cycle, increasing to 20 mg twice daily and 40 mg twice daily in successive cycles if ovulation does not occur for regular menstruation. Dose recommended for amenorrhea is 10 mg twice daily on 4 successive days, increasing to 20 mg twice daily and 40 mg twice daily after intervals of 45 and 90 days if ovulation does not occur.

Contraindications

Concomitant coumarin type anticoagulant therapy in women at high risk for breast cancer

History of deep vein thrombosis in women at high risk for breast cancer or in women with ductal carcinoma in situ.

History of pulmonary embolus in women at high risk for breast cancer or in women with ductal carcinoma in situ

Hypersensitivity to tamoxifen

Pregnancy

Precautions

The potential benefits versus the potential risks of tamoxifen therapy prior to initiating therapy in women at high risk for breast cancer should be assessed.

Patients with leucopenia or thrombocytopenia.

Pregnancy should be avoided for up to 2 months following discontinuation of TC therapy.

Patients with bone metastasis hypocalcaemia may occur.

Adverse reactions

Thrombocytopenia, neutropenia, pancytopenia, anemia, thromboembolism, granulocytosis, myocardial infarction, hypercalcemia, vitamin deficiency, endometrial cancer, impotence, menstrual changes, ovarian cysts, vaginal discharge, hepatotoxicity, asthma and hot flashes.

EXCIPIENT PROFILE[32]

EUDRAGIT RL 100

Chemical Name: poly (ethyl acrylate methyl methacrylate triammonioethyl methacrylate chloride).

Functionl Category: film former, tablet binder, tablet diluent

Applications

Polymethacrylates are primarily used in oral capsule and tablet formulations as film-coating agents. It is used to form water-insoluble film coats for sustained-release products. *Eudragit RL* films are more permeable. Polymethacrylates are also used as

binders in both aqueous and organic wet-granulation processes. Polymethacrylate polymers may additionally be used to form the matrix layers of transdermal delivery systems and have also been used to prepare novel gel formulations for rectal administration.

Description

Polymethacrylates are synthetic cationic and anionic polymers of dimethylaminoethyl methacrylates, methacrylic acid, and methacrylic acid esters in varying ratios. Several different types are commercially available and may be obtained as the dry powder, as an aqueous dispersion, or as an organic solution. A (60: 40) mixture of acetone and propan-2-ol is most commonly used as the organic solvent.

Solubility

Soluble in 60:40 ratio of acetone & alcohol (95% ethanol, methanol, propan 2-ol), DCM, ethyl acetate. Insoluble in petroleum ether & water. It has 10% functional quaternary ammonium group. The ammonium group are present as salts & give rise to pH dependent permeability of the polymers. Solutions are colorless or slightly yellow in colour, may be clear or turbid, they have an odor characteristic of solvents.

Stability and Storage

Dry powders are stable for at least 3years if stored in a tightly closed container at less than 30°C. Dispersions are sensitive to extreme temperatures and phase separation occurs below 0°C. Dispersions should therefore be stored at temperatures between 5 and 25°C and are stable for at least 18 months after shipping from the

manufacturer's warehouse if stored in a tightly closed container at the above conditions.

EUDRAGIT RS 100

Chemical Name: poly (ethyl acrylate methyl methacrylate triammonioethyl methacrylate chloride) (1:2:0.1)

Functionl Category: film former, tablet binder, tablet diluent

Applications

Polymethacrylates are primarily used in oral capsule and tablet formulations as film-coating agents. It is used to form water-insoluble film coats for sustained-release products. *Eudragit RS* films are less permeable. Polymethacrylates are also used as binders in both aqueous and organic wet-granulation processes. Polymethacrylate polymers may additionally be used to form the matrix layers of transdermal delivery systems and have also been used to prepare novel gel formulations for rectal administration.

Description

Polymethacrylates are synthetic cationic and anionic polymers of dimethylaminoethyl methacrylates, methacrylic acid, and methacrylic acid esters in varying ratios. Several different types are commercially available and may be obtained as the dry powder, as an aqueous dispersion, or as an organic solution. A (60: 40) mixture of acetone and propan-2-ol is most commonly used as the organic solvent.

Solubility

Soluble in 60:40 ratio of acetone &alcohol (95% ethanol, methanol, propan 2-ol), DCM, ethyl acetate. Insoluble in petroleum ether and water. It has 5% functional quaternary ammonium group. The ammonium group are present as salts & give rise to pH dependent permeability of the polymers. Solution are colourless or slightly yellow in colour, may be clear or turbid, they have an odour characteristic of solvents.

Stability and Storage

Dry powders are stable for at least 3years if stored in a tightly closed container at less than 30°C. Dispersions are sensitive to extreme temperatures and phase separation occurs below 0°C. Dispersions should therefore be stored at temperatures between 5 and 25°C and are stable for at least 18 months after shipping from the manufacturer's warehouse if stored in a tightly closed container at the above conditions.

ETHYL CELLULOSE

Chemical Name: Cellulose ethyl ether

Molecular Formula: $C_{12}H_{23}O_6(C_{12}H_{22}O_5)_nC_{12}H_{23}O_5$ where *n* can vary to provide a wide variety of molecular weights.

Functional Category

Coating agent, flavouring fixative, tablet binder, filler, viscosity increasing agent

Applications in Pharmaceutical Formulation or Technology:

The main use of ethyl cellulose in oral formulations is as a hydrophobic coating agent for tablets and granules. Ethyl cellulose, dissolved in an organic solvent or solvent mixture, can be used on its own to produce water-insoluble films. Higher-viscosity ethyl cellulose grades tend to produce stronger and more durable films. Ethyl cellulose films may be modified to alter their solubility, by the addition of hypromellose or a plasticizer; High-viscosity grades of ethyl cellulose are used in drug microencapsulation. In tablet formulations, ethyl cellulose may additionally be employed as a binder, the ethyl cellulose being blended dry or wet-granulated with a solvent such as ethanol (95%). Ethyl cellulose produces hard tablets with low friability, although they may demonstrate poor dissolution. In topical formulations, ethyl cellulose is used as a thickening agent in creams, lotions, or gels, provided an appropriate solvent is used. Ethyl cellulose has been studied as a stabilizer for emulsions.

Description:

Ethyl cellulose is a tasteless, free-flowing and white to light tan-colored powder.

Stability and Storage

Ethyl cellulose is a stable, slightly hygroscopic material. It is chemically resistant to alkalis, both dilute and concentrated, and to salt solutions, although it is more sensitive to acidic materials than are cellulose esters. Ethyl cellulose is subject to oxidative degradation in the presence of sunlight or UV light at elevated temperatures. This may be prevented by the use of antioxidant and chemical additives

that absorb light in the 230–340 nm range. Ethyl cellulose should be stored at a temperature not exceeding 32°C (90°F) in a dry area away from all sources of heat.

HYDROXY PROPYL METHYL CELLULOSE

Chemical Name: Cellulose Hydroxy propyl methyl ether

Functional Category

Coating agent, stabilizing agent, viscosity increasing agent, film former(2-20%), rate controlling polymer for sustain release (10-80%), suspending agent, tablet binder (2-5%)

Description

Hypromellose is an odorless and tasteless, white or creamy-white fibrous or granular powder.

Applications

It is used as topical, oral, ophthalmic pharmaceutical formulation. coating agent, stabilizing agent, viscosity increasing agent, film former(2-20%), rate controlling polymer for sustain release (10-80%), suspending agent, tablet binder (2-5%).

Solubility

Soluble in cold water; practically insoluble in chloroform, ethanol (95%), ether, but soluble in mixture of ethanol & DCM, mixture of methanol & DCM &mix of watre & alcohol.

Stability and Storage

Hypromellose powder is a stable material, although it is hygroscopic after drying. Solutions are stable at pH 3–11. Increasing temperature reduces the viscosity of solutions. Hypromellose undergoes a reversible sol–gel transformation upon heating and cooling, respectively. The gel point is 50–90°C, depending upon the grade and concentration of material. Aqueous solutions are comparatively enzyme-resistant, providing good viscosity stability during long-term storage. However; aqueous solutions are liable to microbial spoilage and should be preserved with an antimicrobial preservative: when hypromellose is used as a viscosity-increasing agent in ophthalmic solutions, benzalkonium chloride is commonly used as the preservative. Aqueous solutions may also be sterilized by autoclaving; the coagulated polymer must be redispersed on cooling by shaking. Hypromellose powder should be stored in a well-closed container, in a cool, dry place.

CARBOPOL 934

Carbopol range is a wide derivative of synthetic high molecular weight cross-linked water-soluble polyacrylic acids, which confirms to USP/NF specification as carbomer. Carbopol have an average equivalent weight of 76. The general molecular structure can be as follow:

Description: It is available as a white free flowing powder. Carbopol is water soluble, macro-molecular compound having high affinity to water, alcohol and glycol. When dissolved in such solvents, optionally followed by neutralization with an alkali it gives, a high transparent, highly viscous, gel like, thixotropic liquid having a high yield value even in a low concentration. Carbopol is available in different grades, which can be used for specific applications as per requirements.

Solubility: Swellable in water and glycerin and, after neutralization, in ethanol (95%). Carbomers do not dissolve but merely swell to a remarkable extent.

Stability and Storage

Carbomers are stable, hygroscopic materials that may be heated at temperatures below 104ºC for up to 2 hours without affecting their thickening efficiency. However, exposure to excessive temperatures can result in discoloration and reduced stability. Complete decomposition occurs with heating for 30 minutes at 260ºC. Dry powder forms of carbomer do not support the growth of molds and fungi. In contrast, microorganisms grow well in unpreserved aqueous dispersions, and therefore an antimicrobial preservative such as 0.1% w/v chlorocresol, 0.18% w/v methylparaben–0.02% w/v propylparaben, or 0.1% w/v thimerosal should be added. The addition of certain antimicrobials, such as benzalkonium chloride or sodium benzoate, in high concentrations (0.1% w/v) can cause cloudiness and a reduction in viscosity of carbomer dispersions. Similarly, dispersion viscosity is maintained, or only slightly reduced, at elevated storage temperatures if an antioxidant is included in the formulation or if the dispersion is stored protected from light. Exposure to light causes oxidation that is reflected in a decrease in dispersion viscosity. Carbomer powder should be stored in an airtight, corrosion-resistant container and protected from moisture. The use of glass, plastic, or resin-lined containers is recommended for the storage of formulations containing carbomer.

Applications

The readily water-swellable carbopol polymers are used in a diverse range of pharmaceutical applications to provide: controlled release in tablets. Carbopol polymers offer consistent performance over a wide range of desired parameters (from

pH-derived semi-enteric release to near zero-order drug dissolution kinetics) at lower concentrations than competitive systems. Bioadhesion in buccal, ophthalmic, intestinal, nasal, vaginal, and rectal applications. Noveon AA-1 USP polycarbophil is the recognized industry standard for bioadhesion.

POLY (SEBECIC-CO-RECINOLIC ACID) (POLY (SA: RA) 50:50)[33, 34]

Poly (SA: RA) 50:50 is a polyanhydride biodegradable polymer for controlled drug delivery. Polyanhydrides have been considered to be useful biomaterials as carriers of drugs to various organs of human body such as brain, bone, blood vessels and eyes. They can be prepared easily from available low cost resources and can be manipulated to meet desirable characteristics. Polyanhydrides have been synthesized by various techniques viz., melt condensation, ring opening polymerization, interfacial condensation, dehydrochlorination and dehydrative coupling agents. A variety of catalysts have been used in the synthesis of a range of polyanhydrides by melt condensation. Particularly, coordination catalysts facilitate anhydride interchange in polymerization and enhance the nucleophilicity of carbonyl carbon. Significantly higher molecular weights in shorter reaction time were achieved by utilizing cadmium acetate and earth metal oxides.

Biodegradation of poly (sebecic-co-recinolic acid) poly (PSRA) 50:50[33]

Polyanhydrides undergo surface and bulk erosion, which is also termed heterogeneous erosion, by cleavage of hydrolytically sensitive bonds in the polymer that finally leads to polymer erosion type degradation.

LITERATURE REVIEW

1. Breast cancer represents a major health problem, with more than 1,000,000 new cases and 370,000 deaths yearly worldwide. In the last decade, in spite of an increasing incidence, breast cancer mortality has been declining in the majority of developed countries. This is the combined result of better education, widespread screening programmes and more efficacious adjuvant treatments. Better knowledge of breast cancer biology now allows the cosmetic, physical and psychological consequences of radical mastectomy to be spared in the majority of breast cancer patients. Use of the sentinel node technique is rapidly expanding and this will further reduce the extent and the consequences of surgery. Several clinico-pathological factors are used to discriminate between patients at low (<10%), average (10-40%) and high risk of relapse. Nodal status, tumour size, tumour grade and age are accepted universally as important factors to define risk categories. Newer factors such as uPA/PAI-1, HERer2-neu, proliferative indices and gene expression profile are promising and will allow better discrimination between patients at different risk. Endocrine manipulation with tamoxifen, ovarian ablation or both is the preferred option in the case of endocrine-responsive tumours. Tamoxifen administered for 5 years is the standard treatment for postmenopausal patients; tamoxifen plus ovarian ablation is more effective than tamoxifen alone for premenopausal women[35].
2. Hiremath JG, Devi KV, Devi K investigated that Tamoxifen citrate, 2-[4-(1,2-diphenyl-1-butenyl) phenoxy]-N, N-dimethyl ethanamine 2-hydroxy-1,2,3-propanetricarboxylate, the non-steroidal antiestrogen is a highly liopophilic drug. Currently, it is used as an endocrine therapeutic agent of choice for all stages of

breast cancer and has also been approved in the United States for use as a chemopreventive agent in women at high risk for the disease.[36]

3. Hiremath et al., formulated the tamoxifen citrate loaded cylindrical polymeric implants for application at tumor sites. The implant was based on poly (sebacic acid-co-ricinoleic-ester anhydride) 70: 30 w/w [poly (SA-RA) 70: 30 w/w], a low-melting, biodegradable, and biocompatible polymer. Implants were prepared by a standardized melt manufacturing method. Differential scanning calorimetry and scanning electron microscopy were used for implant characterization. In vitro drug release studies were performed in phosphate-buffered saline (pH 7.4) at 37^{o}C. The drug content was estimated by high-performance liquid chromatography. The differential scanning calorimetry studies showed that the tamoxifen citrate in the implants was in the amorphous state. The cumulative percentage of drug release from 10 and 20 wt % drug-loaded poly (SA-RA) 70: 30 w/w implants after 30 days was found to be 42.36 and 62.60%, respectively.[37]
4. Coppi and Iannuccelli revealed and investgated on oral administration of the nonsteroidal anti-estrogen tamoxifen (TMX) is the treatment of choice for metastatic estrogen receptor-positive breast cancer. Improved TMX oral bioavailability and decrease its side effects, crosslinked alginate microparticles for the targeting to the lymphatic system by Peyer's patch (PP) uptake was developed and *in vitro* characterized. TMX was molecularly dispersed inside the microparticles and an electrostatic interaction involving the TMX tertiary amine was detected by rheological and FT-IR assays. Microparticles showed a size less than 3µm, then suitability to be taken up by M cells in PP and a positive surface charge. Moreover, TMX loading level as well as *in vitro* release behavior was

affected by the polymer network connected with the mannuronic/guluronic ratio of the alginate chains[38].

5. Delivery of drugs into systemic circulation via skin has generated lot of interest during the last decade. Transdermal drug delivery systems (TDDS) offer many advantages over the conventional dosage forms or controlled release peroral delivery systems. TDDS provides; constant blood levels (l-7 days), avoids first-pass metabolism, increased patient compliance, and dose dumping never occurs. The choice of drugs delivered transdermally, clinical needs, and drug pharmacokinetics are some of the important considerations in the development of TDDS. In addition to methods to enhance transdermal absorption of drugs such as sorption promoters and prodrugs, the physicochemical and biological factors affecting transdermal permeation of drugs are discussed. Although, novel approaches like iontophoresis and ultrasound are gaining importance as a means to increase drug permeation into systemic circulation, clinical products based on these approaches are still far away. The importance of appropriate animal model selection in the development and evaluation of TDDS cannot be ignored. The cost per milligram of drug delivered transdermally is more expensive than peroral route. The added cost could be justified, if TDDS improve patient compliance and reduces toxic/side effects.[39]
6. The effect of penetration enhancer (i.e., 1, 2, 3 and 5% menthone in combination with 50% ethanol (EtOH)) was investigated on the in vitro percutaneous absorption of tamoxifen, and post-recovery epidermal permeability after removal of the above enhancer. The flux of tamoxifen with menthone in combination with 50% EtOH was significantly greater (*P*_0.05) than the control (50% EtOH). The flux of tamoxifen increased with increasing concentrations of menthone. The post-

recovery flux through enhancer exposed epidermis was significantly decreased (*P*_0.05) as compared to pre-recovery. However, post-recovery flux of tamoxifen through the enhancer-exposed epidermis did not completely recover to the baseline (i.e., post-recovery flux through phosphate buffered saline, pH 7.4 treated epidermis).[40]

7. Singh J and Gao S conducted a study on chemical penetration enhancers offer an approach to enhance the transdermal transport of drugs by partitioning into and interacting with skin constituents, inducing a temporary reversible increase in skin permeability. The effect of penetration enhancers (e.g. oleic acid: ethanol and oleic acid: propylene glycol) was investigated on the in vitro percutaneous absorption of a hydrophilic (5-fluorouracil) and a lipophilic (tamoxifen) anticancer drug through porcine epidermis. In vitro transepidermal water loss (TEWL) was undertaken to investigate the effect of the above enhancers on the macroscopic barrier properties of the epidermis. Oleic acid:ethanol and oleic acid:propylene glycol significantly enhanced the permeability coefficient of 5-fluorouracil (5-FU) and tamoxifen in comparison to their controls. In vitro TEWL was significantly greater (PB0.01) through epidermis treated with the above enhancers in comparison with control (epidermis that was not treated). However, neither oleic acid:ethanol nor oleic acid:propylene glycol enhanced (P\0.05) TEWL in comparison with ethanol and propylene glycol alone. Thus, changes in the permeability of 5-FU and tamoxifen caused by oleic acid:ethanol or oleic acid:propylene glycol could not be correlated with the in vitro TEWL.[41]
8. The purpose of this research was to develop a matrix-type transdermal therapeutic system containing carvedilol with different ratios of hydrophilic and hydrophobic polymeric combinations by the solvent evaporation technique. The

physicochemical compatibility of the drug and the polymers was studied by infrared spectroscopy and differential scanning calorimetry. The results suggested no physicochemical incompatibility between the drug and the polymers. In vitro permeation studies were performed by using Franz diffusion cells. The results followed Higuchi kinetics (r = 0.9953-0.9979), and the mechanism of release was diffusion mediated. Based on physicochemical and in vitro skin permeation studies, patches coded as F3 (ethyl cellulose:polyvinylpyrrolidone, 7.5:2.5) and F6 (Eudragit RL:Eudragit RS, 8:2) were chosen for further in vivo studies. The bioavailability studies in rats indicated that the carvedilol transdermal patches provided steady-state plasma concentrations with minimal fluctuations and improved bioavailability of 71% (for F3) and 62% (for F6) in comparison with oral administration. The antihypertensive activity of the patches in comparison with that of oral carvedilol was studied using methyl prednisolone acetate–induced hypertensive rats. It was observed that both the patches significantly controlled hypertension from the first hour (P G .05). The developed transdermal patches increase the efficacy of carvedilol for the therapy of hypertension.[42]

9. Transdermal patches of carvedilol with a HPMC-drugreservoir were prepared by the solvent evaporation technique.In this investigation, the membranes of EudragitRL100 and Eudragit RS100 were cast to achieve controlledrelease of the drug. The prepared patches possessedsatisfactory physicochemical characteristics. Thickness,mass and drug content were uniform in prepared batches.Moisture vapour transmission through the patchesfollowed zero-order kinetics. *In vitro* permeation studieswere performed using a K-C diffusion cell across hairlessguinea pig skin and followed the super case II transportmechanism. The effects of non-ionic surfactants Tween 80and Span 80 on drug permeation were

studied. The non--ionic surfactants in the patches increased the permeationrate, Span 80 exhibiting better enhancement relativeto Tween 80. The patches were seemingly free of potentiallyhazardous skin irritation.[43]

10. Sarmah JKet.al., prepared guar gum nanospheres containing tamoxifen citrate (TC) and characterized using guar gum as a model polymer for using it as a carrier for targeted drug delivery. Single step emulsion in situ polymer cross linking technique was employed to prepare polymer coated drug nanoparticles. Four-different drug loading solvents were tried and dichloromethane provided the best drug loading result. Cross-linking was made by the use of cross linker glutaraldehyde during the process. A core shell type particles were observed. Drug load was confirmed by FT-IR and quantitated by HPLC. Nanoparticles were further characterized for particle size and morphology. Influence of process variables on the size of nanoparticles were studied and it was observed that the concentration of polymer and stabilizer determined the size of nanoparticles.[44]
11. Zong-Hui M et al investigated tamoxifen microcapsules and drug loaded medicated fabrics using complex coacervation procedure involving gelatin B and acacia gum. The morphology, particle size, drug loading capacity and *in vitro* release characteristics of the drug microcapsules were optimized for coating tamoxifen microcapsules onto the cotton fabrics. Infrared (IR) spectra and SEM were used to characterize the medicated fabrics and air permeability and laundering testing to determine the efficiency and effectiveness of the system. Results showed that optimum condition for the microcapsules was at drug/polymer ratio 1:4, polymer concentration 3%, and rate of stirring 1000 rpm. *In vitro* release assays demonstrated that the tamoxifen was liberated over 10 h after an initial burst rate period. These observations demonstrated that they could

design and fabricate a medicated system that potentially could be applied within a transdermal drug delivery system and so act in a system for the treatment of breast cancer.[45]

12. Opatrny L, Dell'Anello S, Assouline S, Suissa S prepared sustained release tablets of tamoxifen by wet granulation and direct compression, using HPMC(K4M) as major retarded-release controller. The releasing characteristics of sustained release and conventional tablets were compared to clarify the sustained effect of the former. The sustained release tablet of tamoxifen citrate demonstrated a continuous and stable releasing profile and lasted for over 12 h. It had a significantly retarded effect in comparison with the conventional one and could be a new choice of regimen in its clinical application.[46]
13. Yosra SR, Elnaggar, Magda AE, Ossama YA prepared tamoxifen citrate self-nanoemulsifying drug delivery systems (SNEDDS). An optimum system composed of tamoxifen citrate (1.6%), Maisine 35-1 (16.4%), Caproyl 90 (32.8%), Cremophor RH40 (32.8%) and propylene glycol (16.4%) was selected. The drug release from the selected formulation was significantly higher than other SNEDDS and drug suspension, as well. Realizing drug incorporation into an optimized nano-sized SNEDD system that encompasses a bioactive surfactant, their results proposed that the prepared system could be promising to improve oral efficacy of the tamoxifen citrate.[47]
14. Takashi K, Fumie K, Yoshihisa M, Reiko T, Shuji K prepared tamoxifen hemicitrate hydrate. The crystalline form was identified and characterized by powder and single crystal X-ray diffractometries, differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA), and hot-stage microscopy, and its physicochemical stability was also evaluated. The physicochemical stability of

tamoxifen citrate forms A and B suspended in water and of form A during kneading and drying suggested that tamoxifen citrate was transformed into tamoxifen hemicitrate hydrate in water within 24 h, whereas tamoxifen citrate in a mixture with microcrystalline cellulose was quite stable during kneading. These results suggested that water and a mixture of water and organic solvent should be used for the manufacturing process with special attention paid to the transformation to tamoxifen hemicitrate sesquihydrate, because it showed a different stoichiometry from the active ingredient, tamoxifen citrate.[48]

15. Zhao.K, Singh S, Singh J studied the effect of penetration enhancer (i.e., 1, 2, 3 and 5% menthone in combination with 50% ethanol (EtOH)) on the in vitro percutaneous absorption of tamoxifen, and post-recovery epidermal permeability after removal of the above enhancer. The studies showed that the flux of tamoxifen increased with increasing concentrations of menthone. However, post-recovery flux of tamoxifen through the enhancer-exposed epidermis did not completely recover to the baseline (i.e., post-recovery flux through phosphate buffered saline, pH 7.4 treated epidermis).[49]
16. Barry BW reviewed that optimization of drug delivery through human skin is important in modern therapy. This review considered drug vehicle interactions (drug or prodrug selection, chemical potential control, ion pairs, coacervates and eutectic systems) and the role of vesicles and particles (liposomes, transferosomes, ethosomes, niosomes). It is possible to modify the stratum corneum by hydration and chemical enhancers, or bypass or remove this tissue via microneedles, ablation and follicular delivery. Electrically assisted methods (ultrasound iontophoresis, electroporation, magnetophoresis, photo chemical waves) also

show considerable promise. Of particular interest is the synergy between chemical enhancers, ultrasound, ionotphoresis and elcetroporation.[50]

17. Schreier H, Bouwstra J analysed that topical liposomes or niosomes may serve as solubilization matrix, as a local depot for sustained release of dermally active compounds, as penetration enhancers or as rate limiting membrane barrier for the modulation of systemic absorption of drugs. The mechanism of vesicle-skin interaction and drug delivery are being extensively investigated using radioactive or florescence labeled marker molecules and drugs, and various electron and light microscopic visualization techniques, and different models describing the interaction with and fate of vesicles in the skin have been proposed. It was concluded that liposomes and niosomes may become a useful dosage form for variety of dermally active compounds, specifically due to their ability to modulate drug transfer and serve as non-toxic penetration enhancers[51].
18. Shahriar S., Hamid M., Mohammad I.,Zimei R.,Ahmad J., M.,et al prepared and characterized an injectable and in situ forming drug delivery system based on photocrosslinked poly(ε-caprolactone fumarate) (PCLF) networks loaded with tamoxifen citrate (TC). Networks were made of PCLF macromers, a photoinitiation system (comprising initiator and accelerator) and the active ingredient N-vinyl-2-pyrrolidone (NVP) as a crosslinker and reactive diluent. Shrinkage behavior, equilibrium swelling and sol fraction ratios of photocrosslinked PCLF gels were determined as functions of NVP content. It was shown that the crosslinking is facilitated up to a certain concentration of NVP and most of NVP remained unreacted above this value. In vitro drug release, biocompatibility evaluation and activity against MCF-7 breast cancer cell line were also investigated. Accurate but simple bipartite expressions were also

derived that enable rapid determination of effective diffusion coefficients of TC in photocrosslinked PCLF/NVP disks. Cytotoxicity assay showed that while the photocrosslinked PCLF network with optimum NVP content exhibits no significant cytotoxicity against MCF-7 and L929 cell lines, 40–60% of the MCF-7 cells were killed after incubation with TC-loaded devices.[52]

19. Sarmah JK, Bhattacharjee KS, Mahanta R, Mahanta R prepared and chareacterized guar gum nanospheres containing tamoxifen citrate (TC) for using it as a carrier for targeted drug delivery. Single step emulsion in situ polymer crosslinking technique was employed to prepare polymer coated drug nanoparticles. Model polymer used in the study was guar gum, which is commonly used for colon specific drug delivery in the pharmaceutical industry. During preparation four-different drug loading solvents were tried and dichloromethane provided the best drug loading result. Cross-linking was made by the use of cross linker glutaraldehyde. A core shell type particles were observed. Drug load was confirmed by FT-IR and quantitated by HPLC. Nanoparticles were further characterized for particle size and morphology. Particle size between 200 and 300 nm were obtained. It was observed that the concentration of polymer and stabilizer determined the size of nanoparticles.[53]
20. Emilie A., Catherine P., Emmanuel G., Pascal Pigeon., Anne V showed that Ferrocenyl diphenol tamoxifen derivative (Fc-diOH) is one of the most active molecules of a new class of organometallic drugs, showing *in vitro* antiproliferative effects on both hormone-dependent and independent breast cancer cells. For the first time, Fc-diOH was tested on a 9L glioma model according to two encapsulation strategies: lipid nanocapsules (LNC) and swollen micelles. LNC showed a higher drug loading capacity because of a larger oily core

in their structure and were able to be up taken by glioma cells. The large amount of PEG present at the micellar interface prevented interaction with cytoplasm membrane which led to a low level of micelle cell uptake and no biological activity. Also, Fc-diOH-loaded LNC showed low toxicity levels when in contact with healthy cells, conferring a functional specificity of this compound on tumour cells. Fc-diOH LNC treatment was able to lower significantly both tumour mass and volume evolution after 9L-cell implantation into rats which evidenced for the first time the *in vivo* efficacy of this new kind of organometallic compound.[54]

21. Reddy HL; K. BakshiVN; R. S. R. Murthy RSR prepared Solid lipid nanoparticles (SLN) by emulsification and high pressure homogenization technique and characterized by size analysis and differential scanning calorimetry. The influence of experimental factors such as homogenization pressure, time, and surfactant concentration on the nanoparticle size and distribution were investigated to optimize the formulation. The SLN were loaded with an anticancer agent, tamoxifen citrate (TC). Short term stability studies indicated a significant increase in size of nanoparticles when stored at 50°C, compared to those stored at 30°C and 4°C. The particle destabilization upon storage in case of all the types of nanoparticles studied was in the order of day light > artificial light > dark. An ultraviolet (UV) spectrophotometric method of estimation of tamoxifen in rat plasma was developed and validated. The TC-loaded TSSLN was administered to the rats intravenously and the pharmacokinetic parameters in the plasma were determined. The $t_{1/2}$ and mean residence time of TC-loaded TSSLN in plasma was about 3.5-fold ($p< 0.001$) and 3-fold ($p< 0.001$) higher, respectively, than the free tamoxifen, indicating the potential of TC-loaded TSSLN as a long circulating system in blood. Thus the above mentioned solid lipid nanoparticles can be a

beneficial system to deliver tamoxifen to cancer tissues through enhanced permeability and retention (EPR) effect.[55]

22. Anna Wokovich *et al*.,Their article provides an overview of types of transdermal, their anatomy, the role of adhesion failure modes and how adhesion can be measured to improve transdermal adhesive performance.[56]
23. Claudia Valenta *et al*., reviewed the use of polymers for skin preparations they had reported the most applied polymers on skin belongs to various classes like Cellulose derivatives, chitosan, carageenan, polyacrylates, polyvinylalcohol, polyvinylpyrrolidone and silicones, the authors also discussed the promising futures trends of polymeric systems.[57]
24. Guyot and Fawaz investigated the influence of three polymers (HPMC, Polyisobutylene and Ucecryl MC808) on different factors like polymeric material, matrix thickness, drug content, thickness of the adhesive layer and presence of dissolution enhancer using an water soluble drug Propranolol hydrochloride. The authors carried out *in vitro* dissolution studies and reveled that, the release from HPMC matrices without adhesive coating was fast. Release from these matrices become more regular and slow when they are coated with Ucecryl layer (reduction of the burst effect). Release from Polyisobutylene matrices was too slow to be suitable as TDDS for propranolol. In all matrices types, proplyne glycol accelerated propranolol release rate.[58]
25. Sastry SP., J. S. V. M. Rao L., Rao KR developed three simple and sensitive spectrophotometric methods for the determination of tamoxifen citrate. They were based on the formation of an ion-association complex between the drug and a dye, Erioglaucine A, which is extractable into chloroform and has an absorption maximum at 625 nm (method A), oxidation with excess potassium permanganate

and the determination of unconsumed permanganate using Fast Green FCF (method B), or by the formation of a coloured cobalt thiocyante coordination complex which is extracted into benzene and measured at 635 nm (method C). Beer's law limits for methods A, B, and C are 0.5–3.0 μg ml^{-1}, 1.0–6.0 μg ml^{-1} and 100–500 μg ml^{-1}, respectively. No interference was observed from tableting additives and the applicability of the methods was examined by analysing tablets containing tamoxifen. The quantities determined were 99.0–100.03% of the expected values.[59]

26. El Maghraby, et al., have reviewed on liposomes drug delivery through skin. This article critically reviewed the relevance of using different types of vesicles as a model for human skin in permeation enhancement studies, concentrating primarily on liposomes after briefly surveying older models. The validity of different types of liposome was considered and traditional skin models are compared to vesicular model membranes for their precision and accuracy as skin membrane mimics.[60]
27. Qiu, et al., have developed and prepared microneedle based liposomes to increase skin permeation of drugs with high molecular weight and poor water solubility. Docetaxel (DTX, MW=807.9) was chosen as a model drug. DTX liposomal systems with and without elastic properties were prepared and characterized. The effect of the developed formulations on the permeation of DTX across both rat and porcine skin was investigated in vitro. The combination effect of microneedle pretreatment and elastic liposomes on the permeability of DTX was evaluated using porcine skin in vitro. Their obtained results suggested that elastic liposomes loaded with DTX can enhance transdermal delivery of DTX without microneedle treatment; an enhanced transdermal flux was reported (1.3-1.4 μg/cm2/h) for DTX from all liposomal formulations was observed after microneedle treatment.

Importantly, the lag time obtained following the application of elastic liposomes through microneedle-treated skin was decreased by nearly 70% compared with that obtained from conventional liposomes. Their results suggested that the combination of elastic liposomes with microneedle pretreatment can be a useful method to increase skin permeation of drugs with high molecular weight and poor water solubility.[61]

28. Arora, et al., have reviewed on recent trends and developments in the field of micro-scale devices for transdermal macromolecular delivery. These include liquid jet injectors, powder injectors, microneedles and thermal microablation. The historical perspective, mechanisms of action, important design parameters applications and challenges are discussed.[62]
29. Heiskanen et al., investigated on transdermal fentanyl patch for the treatment of both malignant and non-malignant chronic pain. The absorption of fentanyl from the patch was governed by the surface area of the patch, skin permeability and local blood flow. Their objective of the study was to find out whether absorption of fentanyl in cachectic patients with cancer-related pain is different from that of normal weight cancer patients. They have recruited ten normal weight (mean body mass index (BMI) 23 kg/m2) and ten cachectic (mean BMI 16 kg/m2) cancer pain patients. A transdermal fentanyl patch with a dose approximately equianalgesic to the patients' previous opioid dose was administered to the upper arm of the patient for 3 days. Prior to patch application, the height, weight and BMI of the patient, as well as upper arm skin temperature, local sweating, thickness of skin fold and local blood flow were measured. Plasma fentanyl concentrations were analyzed. Their results suggested that absorption of transdermal fentanyl is impaired in cachectic patients compared with that of normal weight cancer pain patients.[63]

30. Nie, et al., have conducted a study on tamoxifen citrate (TAM)-loaded polyacrylonitrile (PAN) fibers were prepared by using an improved wet spinning technique. TAM is used as a model drug to evaluate the potential application of the loaded fiber system for drug delivery. PAN was first homogeneously dissolved in the N,N-dimethylacetamide (DMAc) solution containing TAM and then the co-dissolving solution was solidified to prepare the fibers using a wet-spinning method. Chemical, morphological and mechanical property characterizations and drug release properties. The diameter of drug-loaded fiber was in the range of 40-60μm. The best values of the tensile strength at 2.968 cN/dtex and breaking elongation at 14.9% of drug-loaded fibers were obtained when the drug loading content was 23.1 wt.%. The *in vitro* release experiment indicated that constant drug release from the fiber was observed for a long duration of time. Kinetic studies demonstrated that the system followed the Higuchi kinetics. These findings demonstrate that controlled release of drugs from PAN fibers could be potentially useful in drug delivery systems in the development of transdermal drug delivery system**.**[64]
31. Gao, et al., investigated on sustained release matrix type transdermal patch containing gestodene (GEST) and ethinylestradiol (EE) using blends of different polymeric combinations. The multiple-layer technique was adopted in order to maintain a steady permeation flux for 7 days. The effects of polymer types, polymer ratios, permeation enhancers, drug loadings and drug ratios in different layers on the skin permeation of the drugs were evaluated using excised mice skin. Polariscope technique was used drug distribution behavior. The formulation with the mixture of polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP) (7:1) was provided the regular release and propylene glycol (PG) could enhanced the

permeation fluxes of drugs. The developed transdermal delivery system containing GEST and EE could be a promising non-oral contraceptive method.[65]

32. Ibrahim, et al., have conducted a study on chemical enhancers for topical pharmaceuticals such as aerosols, topical sprays, and hydro-alcoholic and polymer based gels. Their overall objectives was to determine the enhancement effects by enhancers deposited from a volatile solvent on human epidermal membrane (HEM). In their study, HEM was treated with enhancer/ethanol (enhancer dissolved in ethanol). After evaporation of ethanol, passive transport experiments were conducted using corticosterone (CS) as the model permeant. The uptake of another model corticosteroid, estradiol (E2β), into the intercellular lipid domain of stratum corneum after enhancer/ethanol treatment was also determined. Their data revealed that the CS transport rate limiting domain was likely the same as the intercellular lipid domain probed by E2β uptake. The correlation between steady-state permeation enhancement and uptake enhancement into the intercellular lipid domain suggested that the permeation enhancement mechanism was primarily due to enhancement of permeant partitioning into the transport rate limiting domain[66]
33. Ren, et al.,have developed and evaluated a novel drug-in-adhesive transdermal patch system for indapamide. Initially, they have conducted in *vitro* experiments to optimize the formulation parameters prior to transdermal delivery in rats. The effects of the type of adhesive and the content of permeation enhancers on indapamide transport across excised rat skin were evaluated. Their results indicated that DURO-TAK® adhesive 87-2852 is a suitable and compatible polymer for the development of transdermal drug delivery systems for indapamide. The final formulation contained 4% N-dodecylazepan-2-one, 6% *l*-menthol and 3% isopropylmyristate. In their *in vivo* studies patch systems were

administered transdermally to rats while orally administered indapamide in suspension was used as a control. The pharmacokinetic (PK) parameters, compared with oral administration. In contrast to oral delivery, a sustained activity was observed over a period of 48 h after transdermal administration. Their overall results suggested that sustained activity was due to the controlled release of drug into the systemic circulation following transdermal administration.[67]

34. Bhardwaj, et al., have investigated and evaluated the physicochemical and release properties of non-extruded 'multilamellar' and small sonicated and extruded 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) liposomes containing hydrophobic drug dexamethasone. Non-extruded liposomes had similar diameter, however dexamethasone encapsulation decreased with increase in lipid chain length. Cholesterol incorporation decreased drug encapsulation in both extruded and non-extruded DMPC liposomes which is due to structural similarities between cholesterol and dexamethasone. Incorporation of dexamethasone and cholesterol in the same DMPC liposomes caused a marked perturbation in the phase transition. Dexamethasone released from extruded liposomes was fast, while non-extruded liposomes showed slower release.[68]
35. Subedi, et al., have reviewed and summarized various physical and chemical approaches for transdermal flux enhancement, including the application of electricity, ultrasound, microneedle and chemical enhancers. Pressure sensitive adhesive such as acrylics, rubbers and silicones are described together with recent developments. Factors affecting dosage form design, particularly for drug in adhesive system, like adhesion and crystallization are also discussed.[69]

36. **Yanli et al.,** have designed a weekly sustained release matrix type double-layer transdermal patch containing gestodene (GEST) and ethinylestradiol (EE) using blends of different polymeric combinations for shown advanced contraception effect and lower side effect. They studied the effects of polymer types, polymer ratios, permeation enhancers, drug loadings and drug ratios in different layers on the skin permeations of the drugs using excised mice skin. Polariscope examination was carried out to observe the drug distribution behavior. The formulation with the mixture of polyvinyl alcohol and polyvinyl pyrrolidone (7:1) was found to provide the regular release and propylene glycol could enhance the permeation fluxes of drugs. This patch could sustain the steady permeation flux of drugs for 7 days when the ratio of drug in drug release layer and drug reservoir layer was 1:4 with the identical total drug amount.[70]
37. Madishetti et al., prepared bilayered matrix type transdermal drug delivery systems (TDDS) of domperidone by film casting technique using hydroxypropyl methyl cellulose as primary and eudragit RL 100 as secondary layers. Brij-35 was incorporated as a solubilizer, d-limonene and propylene glycol were employed as permeation enhancer and plasticizer respectively. The results were satisfactory for *in vitro* release, moisture absorption, moisture content, water vapor transmission, *ex vivo* permeation through rat abdominal skin, mechanical properties and stability studies. There was no physicochemical interaction between drug and polymers when investigated by DSC and FTIR. A shelf life of 2 years is predicted for the TDDS.[71]
38. **Darshan et al.,** have developed and evaluated a matrix-type transdermal drug delivery (TDD) system of a combination of ethinylestradiol (EE) and medroxyprogesterone acetate (MPA) for interception using various film-forming

polymers such as eudragit RL 100 and eudragit RS 100 with and without n-dibutyl phthalate as plasticizer and with glycerol and sodium lauryl sulphate as penetration enhancer. A slow and controlled release of drug through rat skin and human cadaver skin was indicated by the plot of drug release against square root of time, which was found to be linear, thus supporting transdermal film formulation. This delivery system proved to be promising for administration of EE and MPA in combination, as there has been no interaction between the drugs and polymer and it showed 100% antiimplantation activity.[72]

39. Shashikant et al.,have reported ketoprofen transdermal patches by mercury substrate method using polymers Eudragit RS100, Eudragit RL100, HPMC K100M, HPMC E5 and HPMC K4M. Propylene glycol and oleic acid used as permeation enhancer and dibutyl phthalate and polyethylene glycol-400 used as a plasticizer. The patches were evaluated for thickness, folding endurance, tensile strength, drug contain uniformity, *in-vitro* permeation study. Drug polymer interactions determined by FTIR. *In vitro* release study was performed by using Franz-diffusion cell. The formulated transdermal patch by using EudragitRS-100, EudragitRL100, HPMC K100M, HPMC E5 and HPMC K4M showed good physical properties. It was observed that the formulation containing HPMC E5 showed ideal zero-order release kinetics.[73]
40. Mamatha et al., had prepared matrix type TDDS of lercanidipine hyrochlrode by solvent evaporation technique composed of Eudragit RL100 and hydroxypropyl methyl cellulose in 1.5:8.5, 3:7, 4:6, 6:4, 7:3 and 8.5:1.5 ratios respectively containing d-limonene as a penetration enhancer, propylene glycol as plasticizer in methanol and dichloromethane as solvent system. The prepared TDDS were evaluated for physicochemical characteristics, in-vitro release, ex-vivo permeation

and skin irritation. The ex-vivo permeation studies were carried out across excised rat skin using Franz diffusion cell. All formulations exhibited satisfactory physicochemical characteristics.[74]

41. Petersen et al., have developed a high-throughput methodology to rapidly assess the effects of polymer chemistry and the various steps during protein delivery (i.e. encapsulation, storage and release) from polyanhydride nanoparticles on the stability of a bovine serum albumin as a model protein. They have also investigated additional factor microenvironment pH for protein stabilization. Their results showed that protein stability was affected by microenvironment pH which is due to acidic polymer degradation products. The nanoparticles were kept for over a range of temperature for 1 month for the determination of protein structure and protein antigenicity.[75]
42. Reinhard and Neubert, have reviewed on nanocarriers (NCs) are colloidal systems having structures below a particle or droplet size of 500 nm. In the previous years, the focus for the application of NCs was primarily placed on the parenteral and oral application. However, NCs applied to the skin are in the center of attention and are expected to be increasingly applied as the skin offers a lot of advantages for the administration of such systems. For the use of NCs to the skin, one has to differentiate between the desired effects: the local effect within the skin (dermal drug delivery) or a systemic effect accompanied by the permeation through the skin (transdermal drug delivery). Both for dermal and transdermal drug delivery, the stratum corneum (SC), the main barrier of the skin, has to be overcome. SC is one of the tightest barriers of the human body. Therefore, it is the primary goal of new NC to overcome this protective and effective barrier. For that purpose, new NCs such as microemulsions, vesicular (liposomes) and nanoparticular NCs are

developed and investigated. This article illustrated on potential use of these NCs for dermal and transdermal drug delivery.[76]

43. Santander-Ortega et al., have investigated on nanoparticles by using two different propyl-starch derivatives referred to as PS-1 and PS-1.45 with high degrees of substitution: 1.05 and 1.45 respectively. A simple o/w emulsion diffusion technique, avoiding the use of hazardous solvents such as dichloromethane or dimethyl sulfoxide, was chosen to formulate nanoparticles with both polymers, producing the PS-1 and PS-1.45 nanoparticles. They have characterized the nanoparticles including the evaluation of nanoparticles stability and applicability for lyophilization. Encapsulation and release properties of these nanoparticles were studied showed high encapsulation efficiency for three tested drugs (flufenamic acid, testosterone and caffeine); in addition a close to linear release profile was observed for hydrophobic drugs with a null initial burst effect. Finally, the potential use of these nanoparticles as transdermal drug delivery systems was also tested; displaying a clear enhancer effect for flufenamic acid was reported.[77]
44. Taner et al.,investigated on clobetasol-17-propionate (CP) loaded lecithin/chitosan nanoparticles were prepared with special attention to the transport of the active agent across the skin *in vitro*. Nanoparticles were characterized by measuring particle size, zeta potential, polydispersity index and encapsulation efficiency. The morphology of nanoparticles was evaluated by transmission electron microscopy. Their results demonstrated that suitability of lecithin/chitosan nanoparticles to induce epidermal targeting and improved the risk–benefit ratio for topically applied CP.[78]
45. Ramesh et al., conducted a experiment on Box–Behnken design was used to optimize hydrogels containing lisinopril as drug and carbopol 971P as gelling

agent. Independent variables selected were Carbopol 971P (X1), menthol (X2), and propylene glycol (X3) to evaluate their separate and combined effects on permeation of LSP in 24 h (Q24) across rat abdominal skin, flux, and lag time as dependent variables. Their conducted experiment suggested that Box–Behnken design approach helped in identifying the critical formulation parameters in the transdermal delivery of lisinopril from hydrogels.[79]

46. Dhaval et al., have prepared and developed transdermal films of the furosemide employing ethyl cellulose and hydroxypropyl methylcellulose as film formers. The effect of binary mixture of polymers and penetration enhancers on physicochemical parameters including thickness, moisture content, moisture uptake, drug content, drug polymer interaction, and in vitro permeation was evaluated. Stability studies conducted as per International Conference on Harmonization guidelines did not show any degradation of drug. Based on the results and observations, EC–HPMC polymers and propylene glycol are better suited for the development of transdermal delivery system of furosemide.[80]
47. Bharthi et al., describes the potential mechanisms of action of penetration enhancers include disruption of intercellular lipid and/or keratin domains and tight junctions. Their results showed enhancement in drug partitioning into tissue, altered thermodynamic activity/solubility of drug etc. Terpenes are included in the list of Generally Recognized As Safe (GRAS) substances and have low irritancy potential. Their mechanism of percutaneous permeation enhancement involved increasing the solubility of drugs in skin lipids, disruption of lipid/protein organization and/or extraction of skin micro constituents that are responsible for maintenance of barrier status. This article illustrated the mechanisms responsible

for percutaneous permeation enhancement activity of terpenes in developing transdermal formulations.[81]

48. Melero, et al., investigated on influence of propylene glycol (PG), ethanol, and oleic acid (OA) on nortriptyline hydrochloride (NTH) penetration through human epidermis was studied *in vitro* at two different pH values (5.5 and 7.4). They have also studied the influence of lactic acid and polysorbate 80 at different pH conditions. Permeation studies through Heat Separated Epidermis, as well as the enhancing effect of the different vehicles, showed a pH dependency. A pH value of 5.5 in the donor solution decreases significantly the permeability coefficient (Kp) with respect to a pH value of 7.4 was determined. Finally, they have concluded NTH-TDS based on the combination of ethanol/PG/OA showed an enhancement ratio with respect to control of 2.09 and the addition of polysorbate 80 to the matrix of 5.82.[82]
49. El-Laithy, et al., developed novel sustained release proniosomal system was designed using sugar esters (SEs) as non-ionic surfactants in which proniosomes were converted to niosomes upon skin water hydration following topical application under occlusive conditions. Different *in vitr*o aspects (encapsulation efficiency, vesicle size and shape, effect of occlusion, in vitro release, skin permeation and stability) were studied leading to an optimized formula that was assessed clinically for transdermal pharmacokinetics and skin irritation studies.[83]
50. Prow, et al., reviewed on skin is a widely used route of delivery for local and systemic drugs and is potentially a route for their delivery as nanoparticles. The skin provides a natural physical barrier against particle penetration, but there are opportunities to deliver therapeutic nanoparticles, especially in diseased skin and to the openings of hair follicles. The nanoparticle drug delivery has been touted as

an enabling technology, its potential in treating local skin and systemic diseases has yet to be realised. Most drug delivery particle technologies are based on lipid carriers, i.e. solid lipid nanoparticles and nanoemulsions of around 300 nm in diameter, which are now considered microparticles. Current chemistry limits both atom by atom construction of complex particulates and delineating their molecular interactions within biological systems. In this review we discuss the skin as a nanoparticle barrier, recent work in the field of nanoparticle drug delivery to the skin, and future directions currently being explored. This review provides the basic concepts on nanoparticulate drug delivery system towards skin.[84]

51. Xi, et al., investigated on anastrozole is a potent aromatase inhibitor and there is a need for an alternative to the oral method of administration to target cancer tissues. The purpose of their study was to prepare a drug-inadhesive transdermal patch for anastrozole and evaluate this for the site-specific delivery of anastrozole. Different adhesive matrixes, permeation enhancers and amounts of anastrozole were investigated for promoting the passage of anastrozole through the skin of rats *in vitro*. Their findings showed that anastrozole transdermal patches are an appropriate delivery system for application to the breast tumor region for site-specific drug delivery to obtain a high local drug concentration.[85]
52. Schulz et al., conducted a study on levonogestrel release from medium molecular weight polyisobutene patches containing adsorbates. Increasing the adsorbate's drug loading increased the drug release up to a crospovidone content of 15% (w/w). Patches were crystal free for crospovidone contents P10% (w/w), which corresponds to a drug loading of crospovidone of 12% (w/w). They have concluded that the incorporation of drug adsorbates onto crospovidone into patches based on polyisobutene significantly increased the drug release

(approximately 9.1 times for ethinyl estradiol and 15.4 times for levonorgestrel) and prevented drug recrystallization was reported.[86]

3. MATERIALS AND METHODS

Drug and chemicals

Equipments

METHODS

- **Development of UV Spectrophotometric method for the analysis of Tamoxifen citrate**
- **Differential scanning calorimetry (DSC) of TC**
- **X-ray diffraction studies of TC**
- **Preparation of transdemal drug delivery systems.**
- **Physicochemical evaluation of formulations**
- **Evaluation of transdemal drug delivery systems.**
- ***In vitro* skin permeation study**
- **Determination of flux, diffusion coefficient and permeability coefficient of formulations.**
- ***In Vivo* Studies**

MATERIALS

The drug, chemicals and equipments used for the present research work are mentioned. Drug and chemicals used were of analytical grade and were procured either as gift samples or purchased.

List of drug and chemicals used for present research work with source

SL NO	MATERIAL	GRADE	SOURCE
1	Tamoxifen Citratet(TC)	Pharma	Cipla laboratories pvt. Ltd., Mumbai
2	Eudragit RL	Pharma	Leo chem. Bangalore
4	Ethyle cellulose	Pharma	SD Fine chemicals, Bangalore
5	HPMC K50	Pharma	SD Fine chemicals, Mumbai
8	Carbopol 934	Pharma	SD Fine chemicals, Bangalore
11	IPA	Analytical	SD Fine chemicals, Bangalore
12	$CHCl_3$	Analytical	SD Fine chemicals, Bangalore
13	DMSO	Analytical	SD Fine chemicals, Bangalore
14	DBP	Analytical	SD Fine chemicals, Mumbai
15	Disodium hydrogen phosphate	Analytical	SD Fine chemicals, Bangalore
16	Potassium dihydrogn phosphate	Analytical	SD Fine chemicals, Bangalore
17	Sodium chloride	Analytical	SD Fine chemicals, Bangalore

List of equipments used for present research work with source

Sl. No.	*Equipments*	Source
1	Electronic balance	Shimadzu AUX220, Japan
2	Magnetic stirrers	Remi Magnetic stirrers, Remi equipment Pvt. Ltd., Mumbai
3	Centrifuge	Remi centrifuge, Remi equipment Pvt. Ltd., Mumbai
4	UV-Visible Spectrophotometer	UV-1800 Shimadzu, Japan
5	Scanning electron microscope	JSM-848 SEM, Jeol, Japan
6	Vaccum filtration unit	Millipore india
7	Sonicater	Remi india
8	High performance liquid chromatography	Shimadzu-1800, Japan

METHODOLOGY

Development of UV Spectrophotometric method for the analysis of Tamoxifen citrate

Stock solution (100µg/ml) of tamoxifen citrate (TC) was prepared by dissolving accurately weighed 10 mg of the pure TC in 100 ml of methanol and PBS pH 7.4 (5:5). This stock solution was scanned over the wavelength range of 400-200 nm against methanol and PBS pH 7.4 (5:5) solutions as a blank by using double beam UV-spectrophotometer (UV-1800 Shimadzu). The spectrum of absorbance versus wavelength was recorded using double beam UV-spectrophotometer and spectrum was analyzed for the absorbance maximum (λ_{max}). The obtained UV spectrum of TC is shown in Figure 1. For further studies 277.0 nm was fixed and considered as λ_{max} of TC.

Preparation of standard calibration curve of TC

Stock solution (100µg/ml) of TC was prepared by dissolving accurately weighed 10 mg of the pure TC in 100 ml of methanol and PBS pH 7.4 (5:5). From this stock solution, aliquots of 1.0 to 13.0 ml were transferred to 25 ml volumetric flasks and diluted up to 25 ml with same solvent to prepare subsequent solutions containing 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 48 and 52µg/ml of TC, respectively. The absorbance of subsequent solutions was measured at 277.0 nm (λ_{max}) using double beam UV-Spectrophotometer against methanol and PBS pH 7.4 mixtures as blank. A graph of concentration versus absorbance was plotted and the data was subjected to linear regression analysis in Microsoft excel-2007. The obtained results are given in Table 4 and Figure 2.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) was conducted using Mettler Toledo Star system, Metallurgy Department, Indian Institute of Science Bangalore, India. Samples were weighed (5.00-8.00 ± 0.5 mg) and placed in sealed aluminum pans. The coolant was liquid nitrogen. The samples were scanned at 10^{o}C/ min from 20 ^{o}C to 160 ^{o}C. DSC thermogram of pure tamoxifen citrate was obtained as shown in Figure3.

X-ray diffraction studies

X-ray diffraction patterns was determined using a diffractometer equipped with a rotating target X-ray tube and a wide-angle goniometer in department of Physics, Indian Institute of Science (IISC), Bangalore, India. The X-ray source was Kα radiation from a copper target with graphite monochromater. The X-ray tube was operated at a potential of 50 kV and a current of 150 mA. The range (2θ) of scans was from 0 to 70^{o} and the scan speed was 2^{o} per minute at increments of 0.02^{o}. The X-ray diffraction patterns of pure tamoxifen was obtained as shown in Figure 3.

Preparation of transdemal drug delivery systems.

1. **Transdermal films of tamoxifen citrate with HPMC-E15 and EC**.

Transdermal films of tamoxifen citrate (5.0 mg/3.14 cm^2) containing different ratio of

Eudragit-RL, hydroxypropyl methyl cellulose (HPMC K-50), ethyl cellulose were prepared on mercury surface. The required amount of drug and polymers were dissolved in methanol-dichloromethane (1:1) solvent system. Di-n-butyl phthalate (20 and 30 % w/w of polymer) was used as plasticizer. Isopropyl myristate (IPM), and DMSO were added to the polymer drug solution. The resultant homogeneous solution was poured into a circular plane with uniform surface on mercury substrate. The films

were dried for a period of 24 h, and the rate of evaporation was controlled by inverting funnel over the petridish. The dry films were wrapped in aluminum foil and kept in desiccators. Compositions of prepared formulations were tabulated in Table.2 and photographs of the drug containing patches are shown in Fig.6, respectively.

Table. 1. Formulation composition of tamoxifen citrate containing matrix transdermal systems.

Formulations	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**
TAMOXIFEN CITRATE (mg)	50	50	50	50	50	50
EUDRAJIT RL (mg)	150	150	60	90	96	95
HPMC-E15 (mg)	--	--	90	60	--	--
EC (mg)	--	--	--	--	4	5
DMSO (w/w)	10%	--	10%	10%	10%	10%
IPM (w/w)	--	10%	--	--	--	--
DBT µl/w/w	45 (30%)	45 (30%)	45 (30%)	45 (30%)	20 (20%)	20 (20%)
METHANOL:DCM (1:2) (ml)	12	12	12	12	12	12

All the formulation contains 30% DBT except F5 & F6 containing 20% DBT.

HPMC: hydroxypropyl methyl cellulose, EC: ethyl cellulose, DMSO: dimethyl sulfoxide, IPM: isopropyl myristate, DBT: dibutyl phthalate, DCM: dichloromethane

2. **Transdermal films of tamoxifen citrate with HPMC-K15 and HPMC-K50.**

Transdermal films of tamoxifen citrate (5.0 mg/3.14 cm^2) containing different ratios of polymers, hydroxypropyl methyl cellulose (HPMC K-15), carbopol-934, eudragit-RL, hydroxypropyl methyl cellulose (HPMC K-50) were prepared on mercury surface by solvent casting technique[1]. The required amount of drug and polymers were dissolved in methanol-dichloromethane (1:1) solvent system. Di-n-butyl phthalate (30 % w/w of polymer) was used as plasticizer. DMSO was added to the polymer drug solution. The resultant homogeneous solution was poured into a circular plane with uniform surface on mercury substrate. The films were dried for a period of 24 h, and the rate of evaporation was controlled by inverting funnel over the petri dish. The dried films were wrapped in aluminum foil and kept in desiccators. Compositions of prepared formulations were tabulated in Table.2 and photographs of the drug containing patches are shown in Fig.1, respectively.

Table. 2. Formulation composition of tamoxifen citrate containing matrix transdermal systems

Formulations	F7	F8	F9	F10	F11	F12
TAMOXIFEN CITRATE (mg)	50	50	50	50	50	50
HPMC-K-15 (mg)	60	90	60	40	85	85
CARBOPOL-934 (mg)	90	60	40	60	40	25
EUDRAGIT RL (mg)	--	--	50	50	--	--
HPMC-K- 50 (mg)	--	--	--	--	25	40
DMSO (w/w)	10%	10%	10%	10%	10%	10%
DBT µl/w/w	45 (30%)	45 (30%)	45 (30%)	45 (30%)	45 (30%)	45 (30%)
METHANOL:DCM (1:2) (ml)	12	12	12	12	12	12
All the formulation contains 30% DBT.						
HPMC: hydroxypropyl methyl cellulose, DMSO: dimethyl sulfoxide, DBT: dibutyl phthalate, DCM: dichloromethane						

Physicochemical evaluation of formulations

Thickness

The thickness of film before and after the permeation study was determined using micrometer gauge (Mitoyoto, Japan). Film was measured at different places and mean value was calculated[66].

Drug content analysis

The uniformity of drug distribution in the transdemal films was determined by taking known area of the films at different places of the film. The films were dissolved in 2 ml of methanol, sonicated for 10 min and subsequently diluted with phosphate buffer saline (PBS) pH 7.4. After appropriate dilution, solutions were analyzed spectrophotometrically (UV Shimadzu-1700, Japan) for tamoxifen citrate at 274 nm using solution of films prepared without drug as reference to neglect the absorption of components of the formulation if any.

Moisture Content

The prepared films were weighed individually and kept in desiccators containing activated silica at room temperature (30°C) for 24 h till a constant weight was attained. The percentage of moisture content was calculated as the difference between initial and final weight with respect to final weight.[57, 58, 66, 67]

Moisture Uptake

A weighed film kept in desiccator at room temperature (30°C) for 24 h was taken out and exposed to 84% relative humidity (RH) in a stability chamber (Lab Care, Mumbai, India) until a constant weight of film was obtained. The percentage moisture uptake was calculated as the difference between final and initial weight with respect to initial weight.[57, 58, 66, 67]

Folding endurance

A strip of film (2× 2 cm) was cut evenly and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the value of the folding endurance.

Determination of tensile strength

The tensile strength was determined by using dynamic mechanical analyzer (computerized, EPLEXOR 500 N, IISC, Bangalore). 2 cm^2 patches of all the formulation were subjected and determined.

Determination of flux, diffusion coefficient and permeability coefficient

Flux of drug permeated in case of *in vitro* was calculated from slope of the steady-state portion of permeation profile by linear regression analysis[73,74, 80]. Lag time was calculated from back extrapolation of steady-state portion of the graph. Diffusion coefficient (*D*/*h*2) and permeability coefficient (*K*p) was also calculated for the *in vitro* studies using mentioned below equations respectively,

***D/h*2= 1/6 × *T*Lag, *K*p = *J*SS/*C*D,** Where, *T*Lag is the lag time, J_{SS} the flux at steady state, *C*D is concentration in donor compartment, *D* the, diffusion coefficient and *h* is the diffusion path length.

***In vitro* skin permeation study**[73,74, 86, 87]

Female Albino rats weighing 150-200 gm were selected for permeation studies. The animals were sacrificed using anesthetic ether. The hair of the test animals was carefully trimmed short with a pair of scissors and the full thickness skin was removed from the abdominal region. The epidermis was prepared surgically by heat separation technique, which involved soaking of the entire abdominal skin in water at 60°C for 45 sec, followed by careful removal of the epidermis. The epidermis was washed with water and used for permeability studies.

Permeation studies were performed for different formulations across female rat skin in modified Keshary-Chein diffusion cell at 32±0.5°C. The diameter of the

donor compartment cell, providing 3.14 cm^2 effective constant area. The films with area 3.14 cm^2 were applied to the skin using adhesive tape (cellophane) as backing layer. The phosphate buffer pH 7.4 (20 ml) was used as receptor compartment medium to ensure sink conditions and stability of the drug. This whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a magnetic bead. The samples were withdrawn at different time interval and replaced with equal volume of diffusion medium. Samples were analyzed spectrophotometrically at 274 nm. To ascertain whether the components of the skin or other excipients of the film interfere in the drug analysis; blank experiment (films without drug) was run using skin as barrier membrane using phosphate buffer saline pH 7.4. When the solution was analyzed at 274 nm for any interfering constituents, the released constituents were amounting to an average of 0.04±0.02%.

Formulation of TC containing Solid lipid-nanoparticles

The formulae for the TC biodegradable Solid lipid nanoparticles are given in Table 4. Formulation composed of tamoxifen citrate (TC), polymer poly (sebacic-co-ricinoleic acid) 50:50 and cry protectants mannitol and trehalose.

Table 3. Composition of formulations

Formulation	Drug	Polymer	Cry protectant	
	Tamoxifen citrate (mg)	**Poly(SA:RA) 50:50** (mg)	**Glucose** (mg)	**Mannitol** (mg)
LN-1	10	190	14	14
LN-2	20	180	14	14
LN-3	40	160	14	14
LN-4	60	140	14	14

Preparation of TC containing polymeric Solid lipid-nanoparticles[34]

Drug free and TC loaded poly (sebacic acid-co-ricinoleic acid) 50:50 (Procured from, Sigma Aldrich, Bangalore, India) lipid Solid lipid nanoparticles were prepared by solvent evaporation method. Four different types of formulations LN-1 to LN-4 were prepared, differing in the theoretical loading of TC by 5, 10, 20 and 30% w/w of polymer, poly(SA:RA) 50:50, respectively. The exact amount of polymer and drug used for the preparation of each type of system are indicated in Table 4. Known amount of poly(SA:RA) was dissolved in 10 ml acetone and magnetically stirred for 15 min separately, after which TC was dissolved in this organic phase and further stirred for 15 min. The 40 ml of ethanol and water mixture of 1:1 ratio was added to the organic phase containing drug and polymer and stirred at 1000±5 rpm for 20 min. This system was stabilized for 30 min. The organic solvent from the tri-phasic system was removed by rapid evaporation using rotary flash evaporator; where drug and polymer displacement takes place in sequence as acetone, ethanol and water. The

resulting aqueous system was frozen in liquid nitrogen and lyophilized using Christ alpha 1-4 LD plus lyophilizer (Indian Institute of Science, IISc, Bangalore) to obtain free flowing fluffy poly(SA:RA) 50:50 lipid Solid lipid nanoparticlesof TC using glucose and mannitol (7% w/w of polymer and drug) as cryoprotectants, added prior to lyophilization. Drug free Solid lipid nanoparticleswere prepared in the same manner without adding drug.The lipid Solid lipid nanoparticleswere collected and stored in an amber coloured screw capped bottle in a cool and dark place. The 1% w/v of HPMC (Hydroxy propyl methyl cellulose (METHOCEL™ K15M Premium CR Grade) gel was prepared 20% v/v ethanol:water with 10% w/w of DMSO as penetration enhancer, under constant stirring until complete gel formation.[81,83,89]

Thereafter, the gels were allowed to stabilize at room temperature for 24 h, respectively[2]. Equivalent 10 to 60 mg containing (formulations LN-1 to LN-4 theoretical loading of TC by 5, 10, 20 and 30% w/w of polymer, poly (SA:RA) 50:50) lipid Solid lipid nanoparticleswere mixed in the above prepared gel (1 gm containing equivalent 10 to 60 mg drug loaded lipid Solid lipid-nanoparticles) slowly stirred by using magnetic stirrer with tiny magnetic bead for a period of 10 min, respectively.

Differential scanning calorimetric analysis (DSC)[34,36, 88,90,91]

Differential scanning calorimetry (DSC) analysis of pure TC, poly(SA:RA), physical mixture and TC loaded nanoparticulate formulations was conducted to determine the compatibility of drug with the polymer using Mettler Toledo DSC 822e (IISC, Metallurgy Department). Lyophilized lipid nanoparticlulate samples were weighed (4.00-6.00±0.1 mg) and placed in crimped copper pan. The samples were scanned from 10 to 160^{o}C at rate of 10^{o}C/min. The coolant used was liquid nitrogen. An empty copper pan was used as reference.

X-ray diffraction analysis (XRD)[34,36, 88,90,91]

X-ray diffraction patterns of pure TC, pure polymer poly(SA:RA) 7:3, physical mixture and drug loaded lipid nanoparticulate formulations were determined using a X-ray diffractometer (Bruker AXS D8 Advance) equipped with a rotating target X-ray tube and a wide-angle goniometer (IISC, Metallurgy Department). The X-ray source was K_α radiation from a copper target with graphite monochromater. The X-ray tube was operated at a potential of 40 kV and a current of 30 mA. The range (2θ) of scans was from 0 to 70^{o}C and the scan speed was 0.04^o per min at increments of 0.02^o.

Evaluation of TC Solid lipid-nanoparticles[88,90,91]

The obtained TC Lipd-Solid lipid nanoparticles formulations LN-1 to LN-4 were evaluated for percentage yield, drug loading, percentage entrapment efficiency, particle size, polydispersity index and *in vitro* release and *in vitro* drug permeation by using Albino rat skin.

Percentage yield

The lyophilized Solid lipid nanoparticlesfrom each formulation were weighed and the percentage yield was calculated using the following formula.[88,90,91]

Percentage yield = weight of lipid-Solid lipid nanoparticlesobtained/weight of drug, polymer, cryoptectants used X 100

Drug loading[88,90,91]

Accurately weighed 10.0 mg of each TC loaded lipid-nanoparticulate formulations were dissolved in 25.0 ml of methanol and PBS pH 7.4 (9:1) solvent.

From the above solution, 2.0 ml was transferred to 10 ml volumetric flask and diluted upto the mark with same media. The resulting solutions were analyzed for TC content using double beam UV-spectrophotometer and drug loading was calculated using below mentioned equation.

% Drug Loading = weight of drug in solid lipid-nanoparticles/weight of solid lipid nanoparticlestaken X 100

Percentage entrapment efficiency [88,90,91]

To determine TC entrapment in poly (SA:RA) nanoparticulate, accurately weighed about 10.0 mg of each solid lipid nanoparticles were suspended in 10.0 ml PBS pH 7.4. Solid lipid nanoparticlessuspensions were subjected to cold centrifugation at ~4 ^{0}C with 14,000 rpm by using Remi centrifuge for 15 min. From the supernatant, 2.5 ml clear solution was transferred into 25 ml volumetric flask and diluted upto the mark with methanol. The resulting solutions were analyzed for TC content using double beam UV-spectrophotometer and percentage entrapment efficiency was calculated using below mentioned equation.[47]

% EE = 1- free drug/theoretical drug loaded X 100

Particle size analysis[88]

In order to analyze particle size drug loaded lyophilized Solid lipid nanoparticleswere dispersed in deionized water, vortexed for 10 min and sonicated for 5 min before sampling. Particle size and polydispersity index of nanoparticulate formulations were determined by laser scattering light using Malvern Laser Analyzer Instrument (IISc, Bangalore, India).

Formualtion and Measurement of viscosity of gels[89]

In preformualtion studies, 0.5-1.5% w/v HPMC gels (20% v/v ethanol:water) were prepared with varying the concentration of 10-30% w/w of dimethyl sulfoxide (DMSO), respectively. The prepared formulations were subjected for viscosity studies. The viscosity of gel formulations was measured using a Nano Rheometer (Bob and Cup type, Malvern Instruments, Malvern, Worcestershire, UK, Department of Materials IISC Bangalore). Steady shear measurements were made in triplicate at 25° C using a controlled rate mode with increasing shear rates from 0.01 to 1001/s. Among all the three different concentration of polymer and penetration enhancer, 1% w/v HPMC and 10 % w/w of DMSO gel was standardized, in respect to viscosity and manual spreadability on regenerated dialysis membrane and albino rat skin. Other two different formulations had either low or high viscosity or also problems associated in spreadibilty aspects. Thus, the gel with 1% w/v HPMC and 10 % w/w of DMSO was used for loading solid-lipid nanoparticles.

Determination of drug content in the prepared gel formulations[43,89,97]

For analysis of TC content in the gel, ~1 g of the HPMC gel containing TC was weighed in volumetric flask drug was extracted by shaking 6h with 50 mL of methanol and PBS pH 7.4 (9:1) solvent (experiment was conducted in triplicate). A 5 mL aliquot of the extracted sample was filtered through 0.22 μm (Millipore India), suitably diluted and TC was analyzed by UV-VIS spectroscopic method at 274 nm, respectively.

In-vitro release studies of gels containing Solid lipid-nanoparticles[31,43,54,77]

Release of TC from HPMC gels (10% w/w of DMSO as penetration enhancer) was measured using regenerated cellulose dialysis membranes (10 K MWCO, Himedia. Pvt. Ltd, Bangalore, India). The membranes were washed and equilibrated with 0.1M PBS (phosphate buffer saline), and then mounted on Keshary-Chein diffusion cells (receptor volume 20 ml, permeation area 3.14 cm^2) by clamping them between the donor and receptor compartments. The receptor compartments were filled with phosphate buffer pH 7.4 maintained at 37±0.5°C and constantly stirred at 100 rpm. 1gm of gel containing (10-60 mg of drug) was spread uniformly on the donor film and sampling ports were covered with Parafilm M® (Fisher Scientific, Bangalore India). Samples were collected from the receptor fluid at pre-determined time points and replaced with an equivalent amount of buffer. The drug content in the withdrawn samples was analyzed by UV-VIS method as described above. All release studies were conducted in triplicate.

In vitro skin permeation studies

Albino rats of female sex weighing 150-200 gm were selected for permeation studies. The animals were sacrificed using anesthetic ether. The hairs of the test animals were carefully trimmed short with a pair of scissors and the full thickness skin was removed from the abdominal region. The epidermis was prepared surgically by heat separation technique, which involved soaking of the entire abdominal skin in water at 60°C for 45 sec, followed by careful removal of the epidermis from dermis. The epidermis was washed with water and used for permeability studies.[39, 50, 66, 73,74]

Permeation studies were carried out for different formulations through rat skin using Keshary-Chein diffusion cell, consisting of donor and receptor compartment at

32±0.5°C. The diameter of the donor compartment cell, providing 3.14 cm^2 effective constant area. The 1 gm of gel containing (10-60 mg F1-F4) formulations (containing 10% w/w of DMSO as penetration enhancer) was uniformly spread on the skin and it is covered with Parafilm M® (Fisher Scientific, Bangalore India). Samples were collected from the receptor fluid at pre-determined time points and replaced with an equivalent amount of buffer. The drug content in the withdrawn samples was analyzed by UV-VIS method as described above. All release studies were conducted in triplicate.

Development of HPLC method development for TC Analysis [37,93]

Analytical procedures should have reproducibility when carried out by the same or different person, in same or different laboratories using different reagents, different equipments, etc. The objective of any analytical procedure should be clearly understood and this will govern the validation characteristics, which need to be evaluated. Typical validation characteristics that should be considered for evaluation listed are listed below:

Accuracy

It is a closeness of agreement between the values obtained by the method and the true value

Accuracy (%)= 100-[(100*(standard concentration-observed concentration)]/ standard concentration).

Precision

It expresses the reproducibility between a series of measurements obtained from multiple sampling. Precision is often expressed as the standard deviation or relative standard deviation of replicate measurements.

Precision, coefficient of variation (C.V.), is estimated by the following equation: C.V. (%)=100*(standard deviation of observed concentration/average of observed concentration).

Determination of LOD and LOQ

The LOD is defined as the lowest concentration of drug that the developed assay can reliably differentiate from background noise (Signal/Noise greater or equal to three). The LOQ is determined as drug at the lowest calibrated concentration. The signal noise ratio is equivalent to 10 times the standard deviation of the noise.

Linearity

The range of concentrations of analyze for which the procedure provides test results that are in direct correlation to the amount of analyze in the sample.

Range

The same as linearity, except the test results must also be accurate and precise over the concentrations tested

Specificity

The ability to analyze in the presence of components, which are expected to be present in the sample matrix.

Robustness

Measure of a method's capacity to remain unaffected by small but deliberate variations in method parameters.

Tamoxifen citrate[37,93]

HPLC procedure for the estimation of tamoxifen citrate was developed for estimation of the drug in blood (plasma) samples.

Details of the HPLC procedure:

Sample : Tamoxifen citrate

Analytical column : Phenomenex C8 (250 x 4 .6 mm) 5µ

Guard column : (4.0 x 3.0 mm, 5µ)

Mobile Phase : Methanol: 90%, Water: 10% and Triethyl amine: 0.1%

Detection : 265 nm

Detector : UV-Visible-SPD-10AVP

Injection Volume : 50 µl

Preparation of tamoxifen citrate stock solution for determination of drug in plasma samples.

Stock solution of tamoxifen citrate was prepared in methanol in a concentration of 1mg/ml. These stock solutions were diluted with mobile phase 90: 10: 0.1% v/v% of methanol: deionzed water: triethyl amine to get concentrations required for preparation of standard working solutions prior to use. For tamoxifen citrate concentrations of working solutions were 0.01, 0.020, 0.05, 0.1, 0.25, 0.5, 1, 2 and 3 µg/ml.

Plasma sample preparation

Plasma500 µlwas placed into 10 ml screw capped glass tubes. For working standard solutions the appropriate volume of diluted standard stock was added to each tube. Then, 0.5 ml of hexane (95%), 1ml of acetone and 1 ml of methanol (HPLC grade) were added to each tube. The solutions were mixed in a bath sonicator for 20 minutes followed by centrifugation for 5 min at 4000 rpm. The organic phase was

transferred into wide mouth glass culture tubes and evaporated to dryness under a stream of liquid nitrogen. The resulting residue was reconstituted with the mobile phase.

Linearity

Standard calibration curve was obtained by adding 500 µl of pure tamoxifen citrate at concentration of viz., 0.01, 0.020, 0.05, 0.1, 0.25, 0.5, 1, 2 and 3 µg/ml to 500µl drug free rat plasma. Thus the corresponding plasma concentrations for calibration standards were 10, 20, 50, 100, 200, 500, 1000, 2000, and 3000 ng/ml. These samples were extracted as described above. The calibration curve was plotted with conc (ng/ml) as x-axis versus area (m. Vs) as y-axis. The calibration curve and chromatogram are shown in Figure 1 and 2.

Recovery

For the determination of extraction yield, four different concentrations of tamoxifen citrate viz., 20, 100, 500, 1000 and ng/ml were added separately to 500 µl of plasma. The spiked plasma samples were then subjected to exaction procedure as described above. The absolute recovery was calculated by comparing the peak area of injected samples after extraction with those obtained on direct injection into column while considering the same amount of tamoxifen citrate dissolved in mobile phase. Each measurement was repeated six times.

Determination of LOD and LOQ[37,93]

The limit of detection (LOD) and limit of quantitation (LOQ) was determined.

Evaluation of precision and accuracy for HPLC analysis of tamoxifen citrate

The precision and accuracy were determined by analyzing plasma at four different concentrations ranging from 20 to 1000 ng/ml. The precision of an HPLC method was determined as the coefficient of variation (% RSD) of intra and inter-day.

The intra-day precision was determined by analyzing the spiked plasma samples prepared within a day. The inter-day precision was determined by analyzing the spiked plasma samples on five different days. The results are shown in Table 2.

***In vitro* cytotoxicity**[31,52, 54]

In vitro cytotoxicity test was performed in Radiant Research Laboratories, Bangalore, in our visual presence. Based on the *in* vitro physicochemical characterization, F1 was selected to carry out *in vitro* cytotoxicity due to its promising results. The monolayer cell culture of Estrogen positive -MCF-7 cells was trypsinized and the cell count was adjusted to 4.0 x 10^5 cells/ml using DMEM medium containing 10% FBS. To each well of the 6 wells plate, 3.8 cm^2 (SIAL0512-100EA, Sigma Aldrich) 0.5 ml of the diluted cell suspension was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium. The test samples of Group I (n=3): F1 and F2, Group II (n=3): F9 and F10, transdermal patches of 0.3 cm^2 to 1.5 cm^2 containing TC concentration of 500 mcg/ml to 2500 mcg/m/ and the other study group consisting of 100 mcg/ml to 2500 mcg/ml TC containing PSRA lipd-nanoparticle gel (LN-3 and LN-4) were placed on to the partial monolayer in cell culture plates. The plates were then incubated at 37^o C, for 24 hrs in 5% CO_2 atmosphere and microscopic visual examination was every 6 h interval and at the end of 24 h the TC containing patches in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and

incubated for 3 h at 37^{o} C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 440 nm. The percentage growth inhibition was calculated using the following formula and concentration of drug needed to inhibit cell growth by 50% (CT_{50}) values is generated from the dose-response curves for each cell line.[31,]

% growth of inhibition = 100-an OD of individual test group/Mean OD of control group X 100

***In Vivo* Studies** [92-100]

The ethics committee of the KLE University and Medical Center for animal welfare approved all animal study protocols. Animal experiments were conducted in full compliance with the approved protocols.

Animals were housed under normal 12-h light/dark cycle and a temperature of 21-22° C, with food and water freely available. Thirty female Albino rats 300-330 g in weight, were randomly divided into six groups (n=5). A day before the experiment the back area of 5 cm×6 cm was shaved using an electrical clipper.

Group I (n=5): animals received Eudragit RL and HPMC-15 combination based two different concentration based formulations (F1EH and F2EH) were selected and determined the pharmacokinetic studies.

Group II (n=5): animals received Eudragit HPMC-K-15; Carbopol-934 and Eudragit RL combination based two different concentration based formulations (F3HCE and F4HCE) were selected and determined the pharmacokinetic studies.

Group III (n=5): animals received (SA:RA) based two different concentration based formulations (LN3: 40:160 and LN-4: 60:140 were selected and determined the pharmacokinetic studies.

Group I and II formulations of 3.14 cm^2 of patch was placed on dorsal surface the female albino rats Parafilm M® (Fisher Scientific, Bangalore India) as backing adhesive layer was used. However, In case of SA: RA formulations equivalent to 5 mg drug containing nanoparticualte gel was spread on the dorsal surface of the female Albino rats and closed with Parafilm M® (Fisher Scientific, Bangalore India). The animals were restricted by hands for 5 min after application and then the rats were placed in individual cages. Blood samples of 500 µl were collected, at several pre-defined intervals after dosing (1, 2, 4, 6, 8, 12 and 24 h), by 250 µl heparinized glass capillary tubes into 1.5 ml vials. Following centrifugation at 4000 rpm for 10 min, plasma was separated and then frozen immediately at −20 ◦C until assayed. Before detection, the samples were thawed and then 50 µl of n-hexane, 200 µl of acetonitrile and 200 µl methanol were added. The mixture was centrifuged for 10 min at 4000 rpm. 50 µl of supernatant were injected into HPLC. Phenomenex C8 (250 x 4 .6 mm) 5µ column was used for the analysis. The samples were then prepared for analysis as described above. The other five (Group IV) rats received an oral dose of 4mg/kg TC aqueous solution by gavage needle. Pharmacokinetic parameters from *in vivo* experiments. The $AUC_{0–24}$ was calculated by the trapezoidal rule for the time interval 0 to the last measurable point, 24 h. The peak plasma concentration C_{max} and time to reach the maximum drug plasma concentration t_{max} were obtained from the concentration time plot.

***In vivo* analysis of tamoxifen‑loaded transdermal patch on physical and biochemical parameters in DMBA-induced breast cancer**

The study was performed after performing acute toxicity as OECD 423. A mixture containing 25 mg of DMBA was dissolved in 1 ml of vehicle and injected by subcutaneous route. Tumor yield was evaluated after the end of the study. Seven different groups which composed 12 female albino rats in each group were used. i. Group 1: The normal control ii. Group 2: The cancer group negative control iii. Group 3: The tamoxifen group positive control (PC) iv. Group 4: F-1 v. Group 5: F-2 vi. Group 6: F-3 vii.Group 7: F-4.

	Tamoxifen (mg)	**Poly (SA:RA) 50:50 (mg)**	**Glucose (mg)**	**Mannitol (mg)**
F1	10	190	14	14
F2	20	180	14	14
F3	40	160	14	14
F4	60	140	14	14

Test sample treatment

Tamoxifen was injected i. p. for PC. Normal group was injected with vehicle. Similarly, Groups 4, 5, 6, and 7 were treated with multiple formulations, and the study was carried for 30 days. At the end of the study, multiple physical and biochemical parameters were evaluated.

Parameters evaluated

Body weight was measured at the end of the study, and each group was compared to evaluate the role of formulation in tumor treatment. Similarly, hematological parameters such as hemoglobin, white blood cell (WBC), and red blood cell (RBC) counts were also determined in peripheral blood. Further, serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) were also quantified using commercially available kits. Institutional ethics approval was obtained from the institutional ethics committee Ref. No KLECOP/CPCSEA-Reg No 221/Res-13-2010.

Statistical data analysis

All the data of the animal studies are presented in the mean ± SEM. Data were analyzed using one way ANOVA followed by post hoc Tukey's test using Graphpad prism version 5. The difference among the means were considered as significant if $p<0.05$.

4. RESULTS

Development of UV Spectrophotometric method for the analysis of Tamoxifen citrate

The solution showed two absorption maxima at 237 and 275nm. It was found to obey Beer Lamberts law over a concentration range of 2 to 20 µg/ml. The coefficient of linear regression was found to be R^2 = 0.9992. Averages of three determinations ±SD were reported.

Table 4. Calibration curve of TC in 7.4 pH phosphate buffer.

S. No.	Concentration (µg/ml)	Absorbance at 277nm
1	2	0.045±0.002
2	4	0.093±0.004
3	6	0.138±0.003
4	8	0.182±0.002
5	10	0.228±0.003
6	12	0.272±0.002
7	14	0.314±0.002
8	16	0.358±0.005
9	18	0.406±0.006
10	20	0.442±0.002

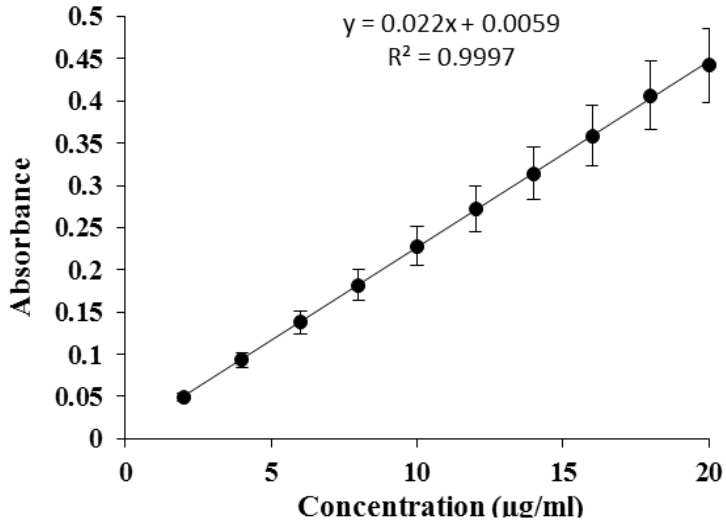

Figure 1: Calibration curve of TC in 7.4 pH PBS

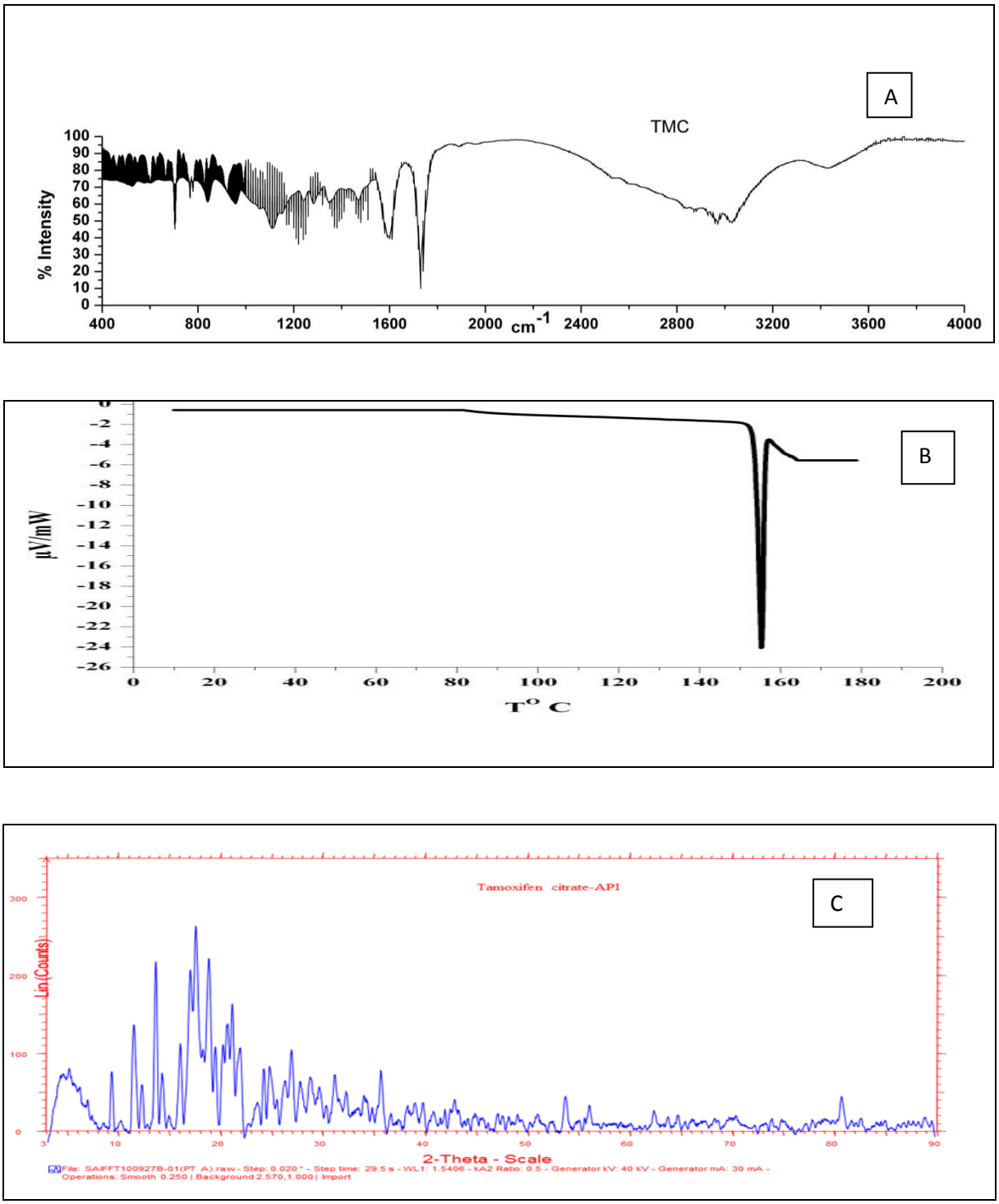

Figure. 2. The FT-IR (A), DSC thermogram (B) and XRD (C) spectra's of pure Tamoxifen citrate.

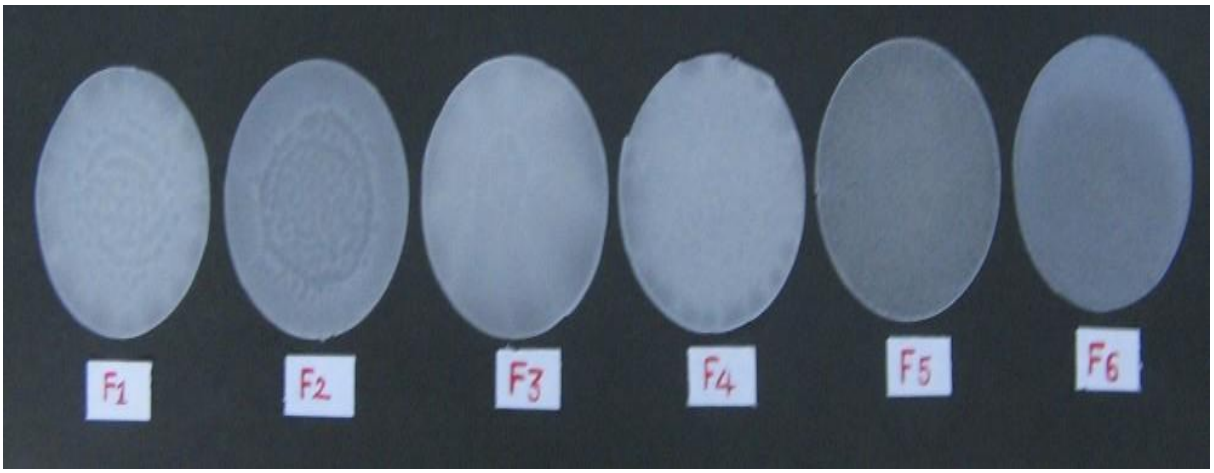

Figure 3. Photographs of TC containing matrix transdermal patches.

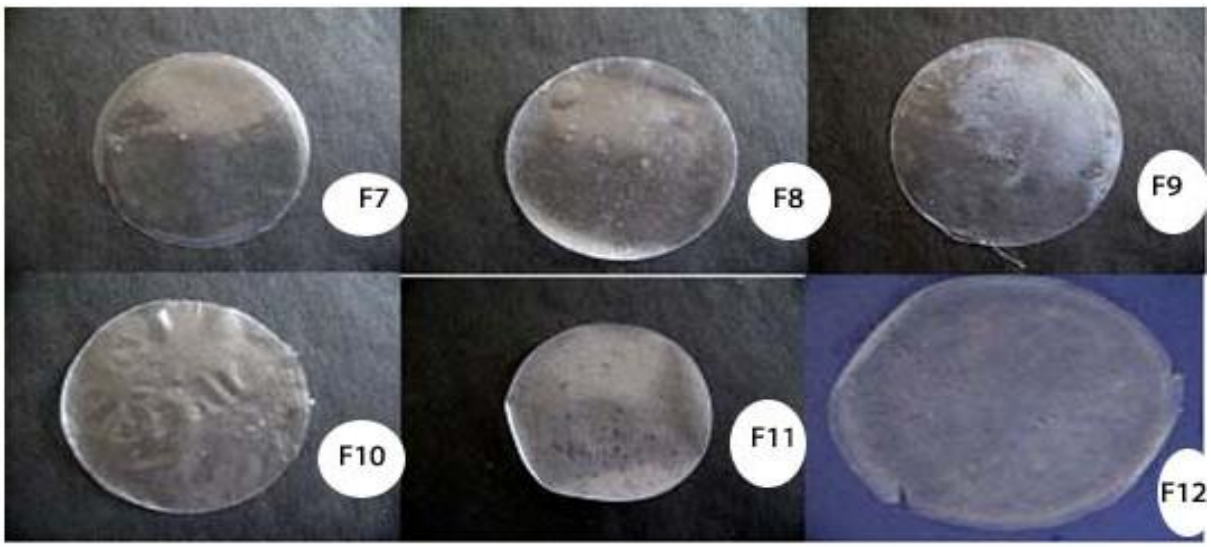

Figure.4. Photographs of tamoxifen citrate containing matrix transdermal patches.

Table. 5. Evaluation parameters of drug containing transdermal films with HPMC-E15 and EC

Evaluations	F1	F2	F3	F4	F5	F6
Thickness (mm)	0.11±0.01	0.116±0.01	0.12±0.02	0.14±0.03	0.12±0.01	0.14 ±0.02
Uniformity of weight	191.1±0.8	202.2±0.9	200.06±1.2	200.7±0.4	135.7±0.7	147.5±0.8
Content uniformity (%)	99.62±3.20	97.15±3.78	99.36±1.51	97.63±1.92	98.93±1.16	98.94±1.39
Moisture content (%)	2.61±0.38	2.04±0.08	3.06±0.15	3.36±0.06	3.62±0.10	4.93±0.22
Moisture uptake (%)	3.58±0.13	3.00±0.11	3.74±0.07	4.05±0.11	4.28±0.06	5.39±0.20
Folding endurance	39.50±2.65	38.50±1.29	39.25±1.26	31.75±0.96	44.50±1.73	46.00±2.16
Tensile strength gm/cm^2	12.91±0.15	12.24±0.10	12.73±0.04	12.82±0.09	12.52±0.05	13.07±0.09
Average of three determinations were reported (±SD, n=3)						

Table. 6. Evaluation parameters of drug containing transdermal films with HPMC-K15 and HPMC-K50

Evaluations	F7	F8	F9	F10	F11	F12
Thickness (mm)	0.10±0.03	0.11±0.01	0.13±0.02	0.15±0.03	0.19±0.04	0.19 ±0.01
Uniformity of weight	200.0±1.0	201.0±0.8	201.8±1.0	200.7±0.4	203.9±0.9	205.0±0.7
Content uniformity (%)	98.96±2.60	98.62±1.53	99.81±2.51	98.84±1.80	99.00±1.10	99.89±1.20
Moisture content (%)	2.78±0.18	2.98±0.10	3.85±0.18	3.46±0.12	4.48±0.12	4.46±0.19
Moisture uptake (%)	3.53±0.20	3.86±0.19	4.05±0.20	3.86±0.11	5.06±0.06	5.10±0.20
Folding endurance	47.25±2.25	48.50±1.75	49.50±1.45	51.75±1.75	55.50±2.15	58.50±1.75
Tensile strength gm/cm^2	12.60±0.12	12.18±0.18	12.20±0.04	12.25±0.09	12.85±0.04	12.85±0.09
Average of three determinations were reported (±SD, n=3)						

Table 7. Determination of flux, diffusion coefficient and permeability coefficient of formulations F1-F6

Formulations	(Jss)Flux mg/cm^2. hr	Permeation coefficient (cm/h)	Diffusion coefficient (cm/h)
F1	0.009±0.02	0.0017±0.001	0.0009±0.002
F2	0.007±0.03	0.0014±0.002	0.0001±0.003
F3	0.054±0.04	0.0116±0.003	0.0018±0.004
F4	0.063±0.05	0.0125±0.005	0.0020±0.002
F5	0.052±0.07	0.0102±0.004	0.0020±0.001
F6	0.053±0.06	0.0108±0.003	0.0021±0.002
Average of three determinations were reported (±SD, n=3)			

Table 8. Determination of flux, diffusion coefficient and permeability coefficient of formulations F7-F12

Formulations	(Jss)Flux mg/cm^2. hr	Permeation coefficient (cm/h)	Diffusion coefficient (cm/h)
F7	0.0248±0.02	0.0049±0.001	0.0703±0.002
F8	0.0236±0.03	0.0052±0.002	0.0806±0.003
F9	0.0281±0.04	0.0056±0.003	0.0761±0.004
F10	0.0222±0.05	0.0044±0.005	0.0703±0.002
F11	0.0236±0.07	0.0057±0.004	0.0864±0.001
F12	0.030±0.06	0.0060±0.003	0.0811±0.002
Average of three determinations were reported (±SD)			

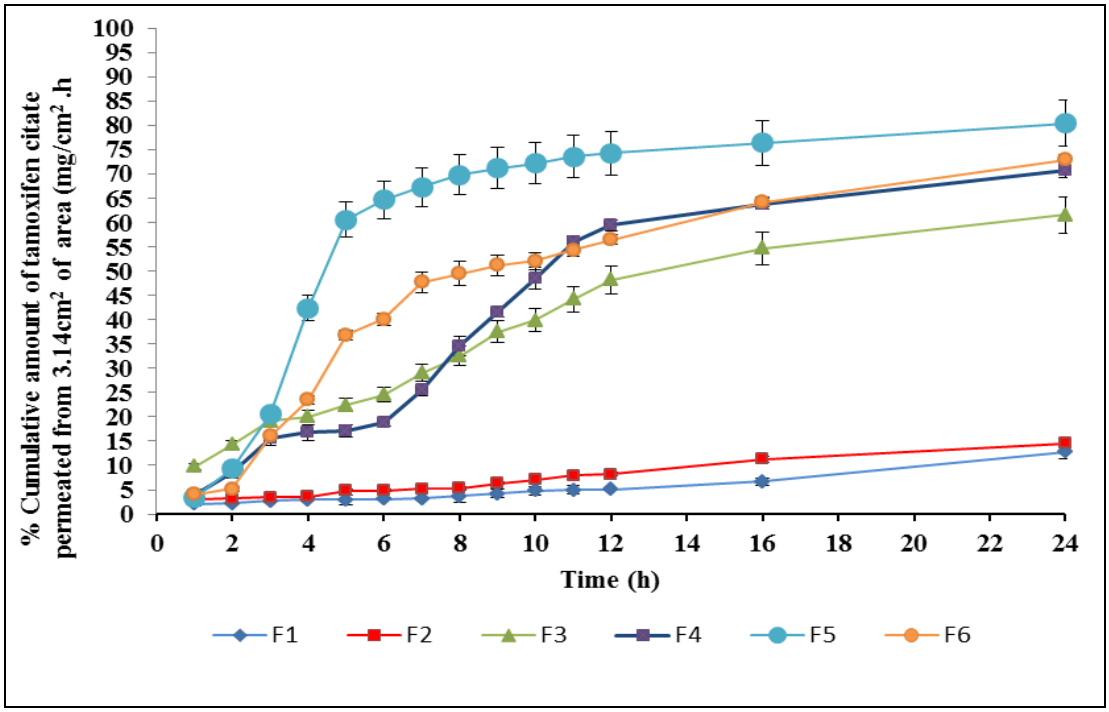

Figure. 5. Cumulative amount of TC permeation from formulations F1-F6 through female rat skin

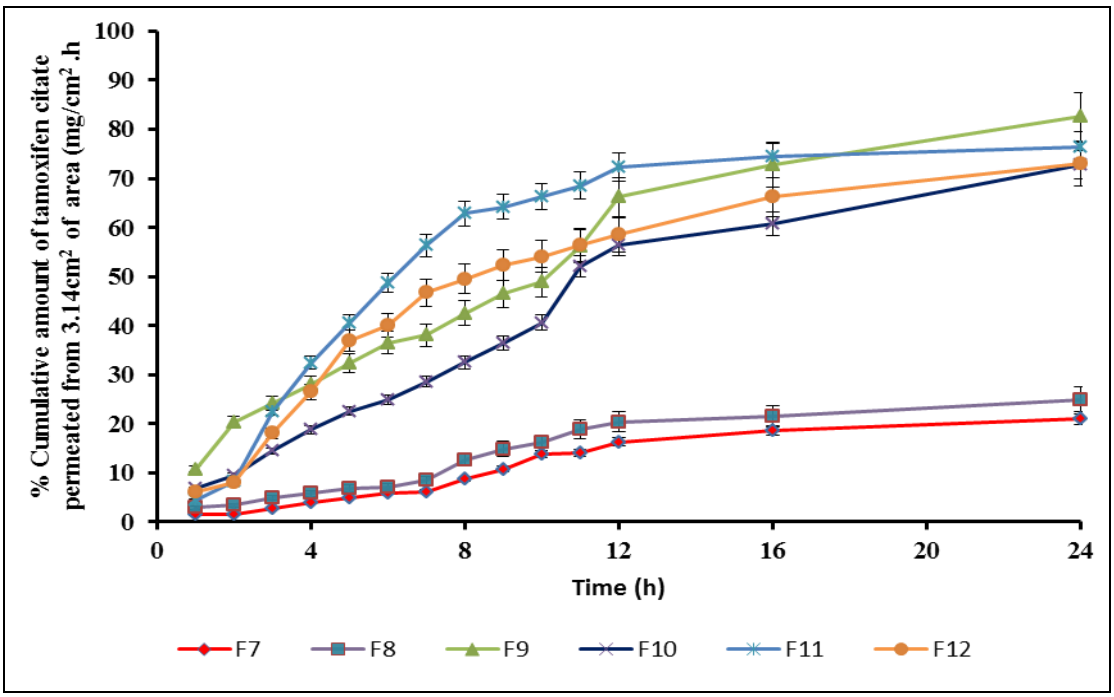

Figure. 6. Cumulative amount of TC permeation of formulations F7-F12 through female rat skin.

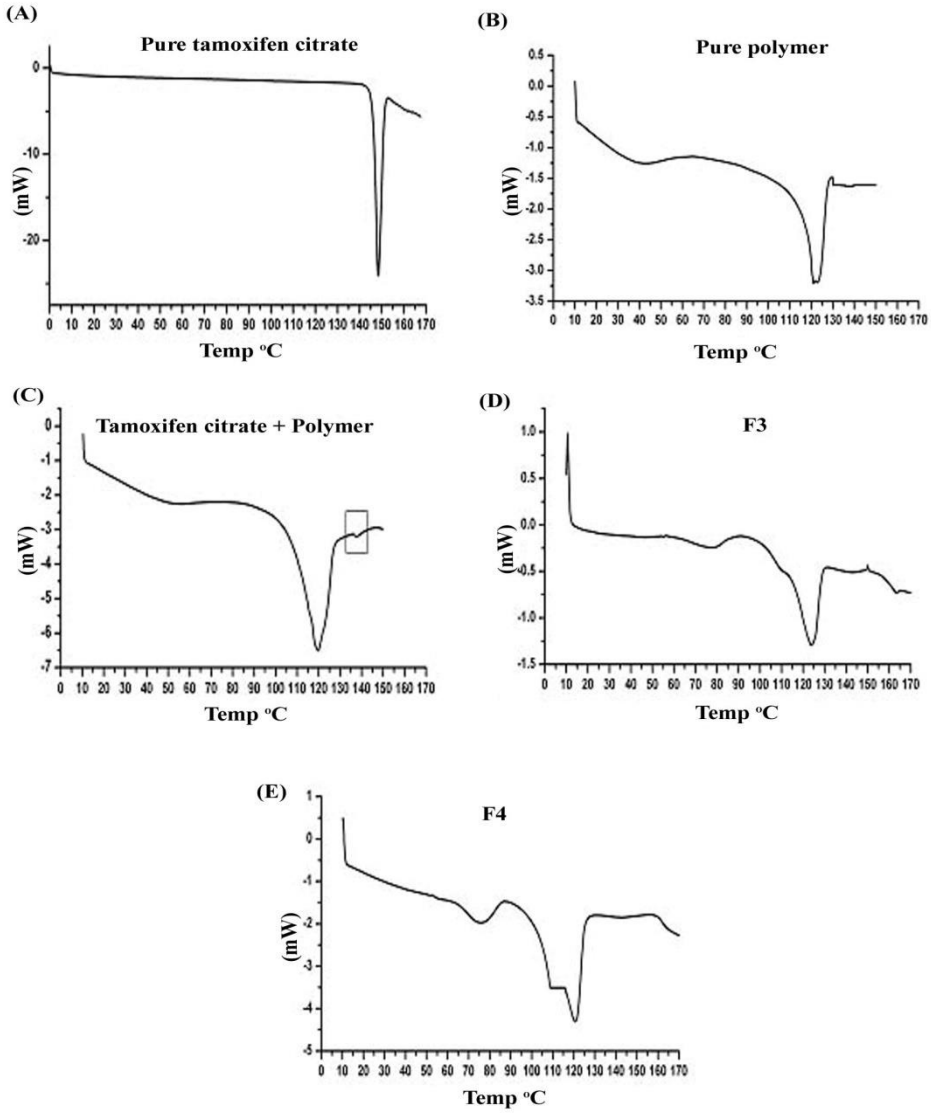

Figure 7: DSC thermograms of (A) pure tamoxifen citrate (B) pure polymer (C) physical mixture (D) F3 and (E) F4 of lipid PSRA nanoparticles.

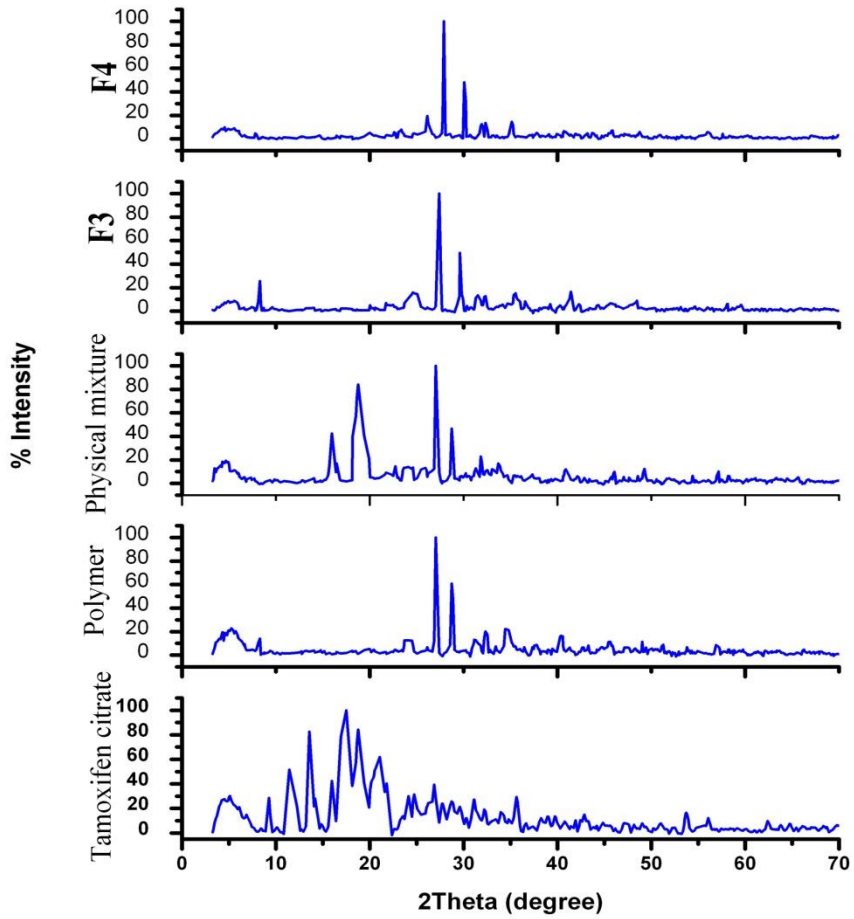

Figure 8: XRD pattern of tamoxifen citrate, pure polymer, physical mixture, F3 and F4.

Table 9: XRD pattern of tamoxifen citrate, pure polymer, physical mixture, F3 and F4, respectively.

Formulations	Degree (2θ)
Tamoxifen citrate	9.25°, 11.42°, 13.52°, 15.98°, 17.49°, 18.72°, 21.07°
Poly (SA:RA)5:5	8.29°, 24.12°, 27.04°, 28.78°
Physical mixture	15.96°, 18.72°, 24.12°, 27.04, 28.78°
F3	8.29°, 24.61°, 27.39°, 29.60°
F4	7.79°, 26.12°, 27.82°, 30.08°

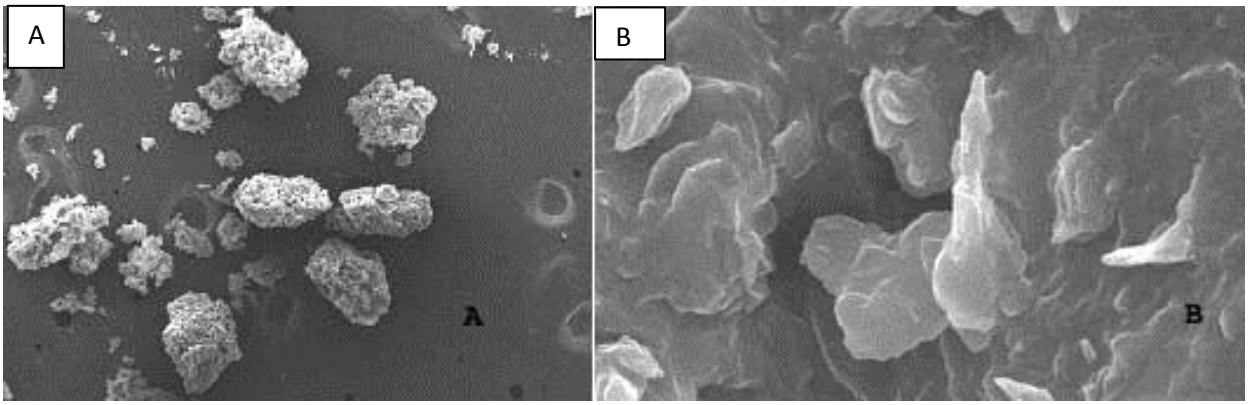

Figure 9. Shows the SEM studies of lipid nanoparticles (A) and typical F3 (B) drug loaded Solid lipid nanoparticle gel formulation.

Table 10: *In vitro* drug release and permeation studies by using cellulose dialysis membrane and albino rat skin.

Formulations	Drug content (%)	Viscosity (Pascal)	(Jss)Flux mcg/cm^2/hr *CDM/SKIN	Permeation coefficient (cm/h) *CDM/SKIN	Diffusion coefficient (cm/h) *CDM/SKIN
LN1	89-91	1.26	41.08/13.05	0.0041/0.0031	0.442/0.958
LN2	90-92	1.28	67.83/22.29	0.0034/**0.00**11	0.362/0.802
LN3	89-92	1.39	86.00/32.16	0.0022/0.0008	0.322/0.729
LN4	90-92	1.45	103.00/35.66	0.0017/0.00002	0.271/0.624
CDM: Cellulose dialysis membrane (*in vitro* release)/ SKIN: Albino rat skin (permeation)					

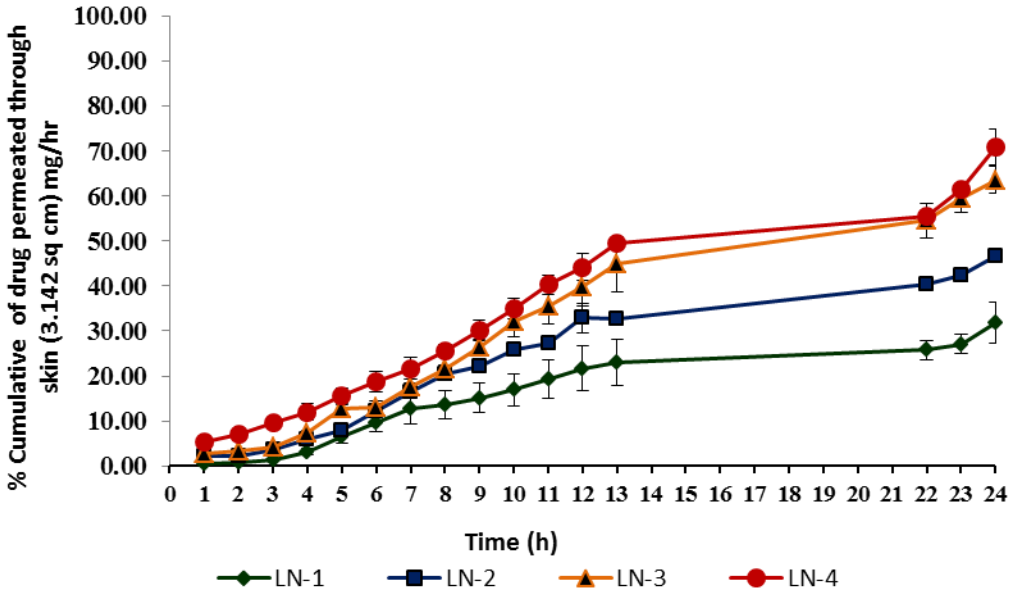

Figure 10. Cumulative amount of drug release of LN1-LN4 gel formualtions.

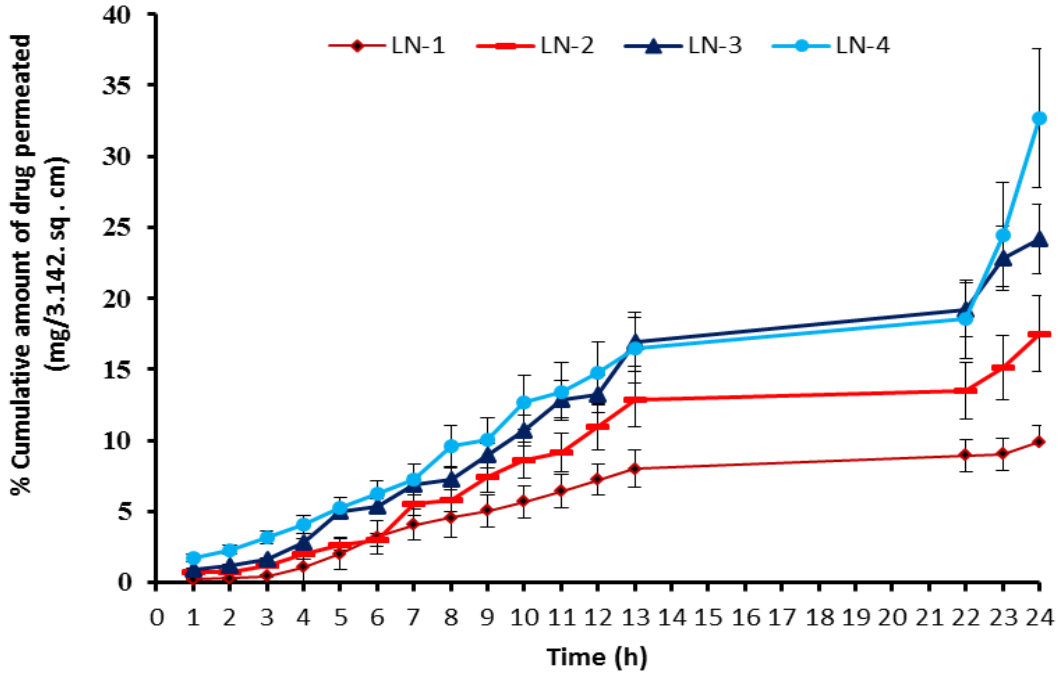

Figure 11. Cumulative amount of drug permeated thorugh Albino rat skin (LN1-LN4 gel formualtions).

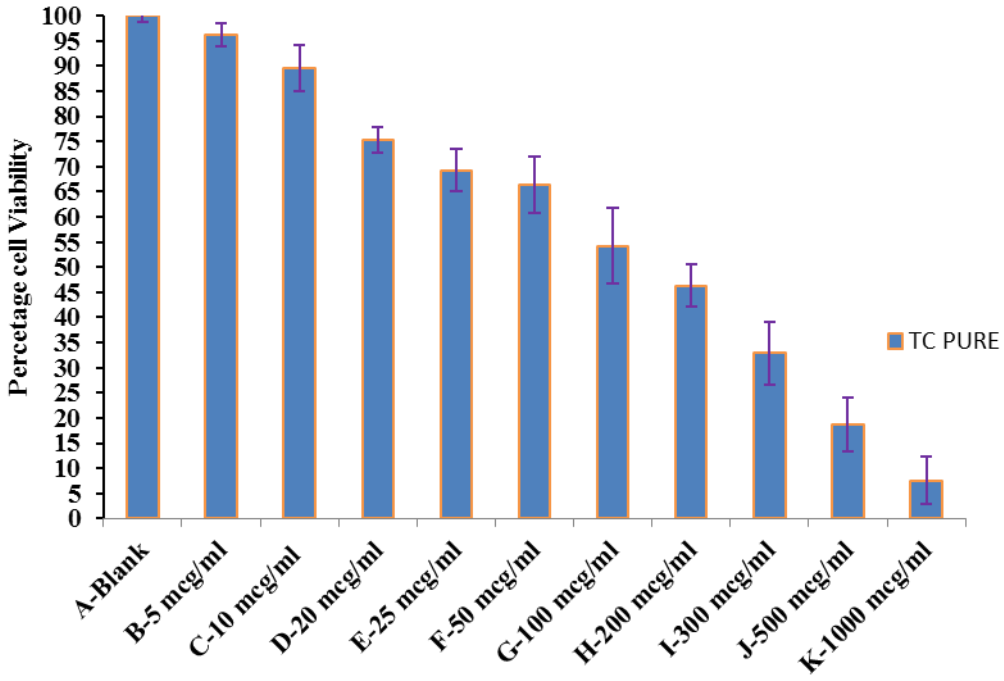

Figure. 12. Estrogen postive MCF-7 Cell lines treated with pure TC.

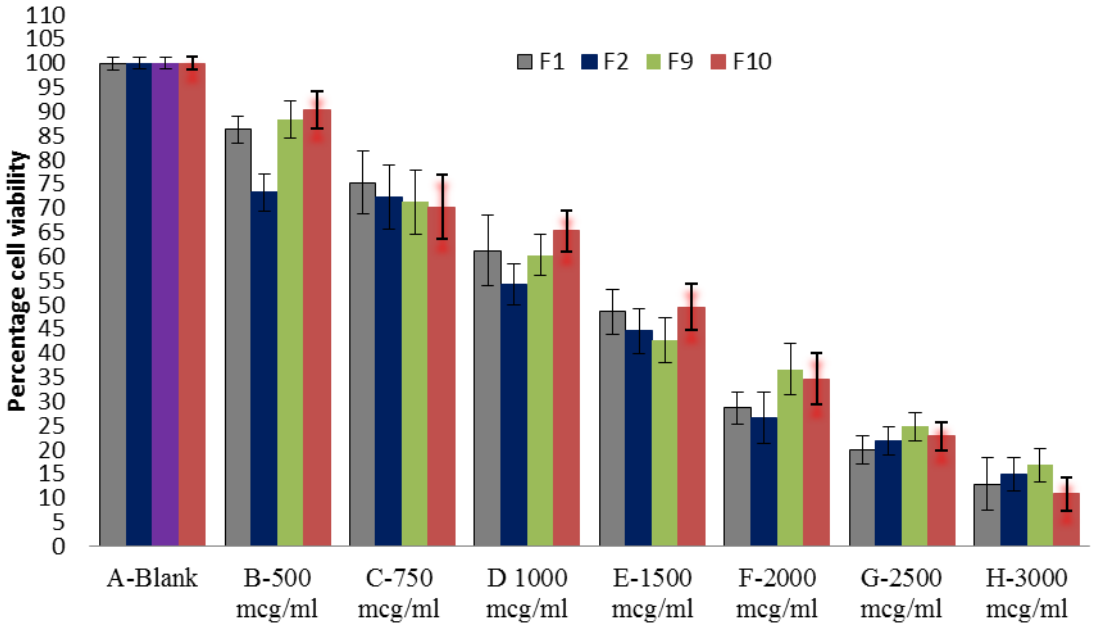

Figure. 13. Estrogen postive MCF-7 Cell lines treated with TC loaded transdermal patches.

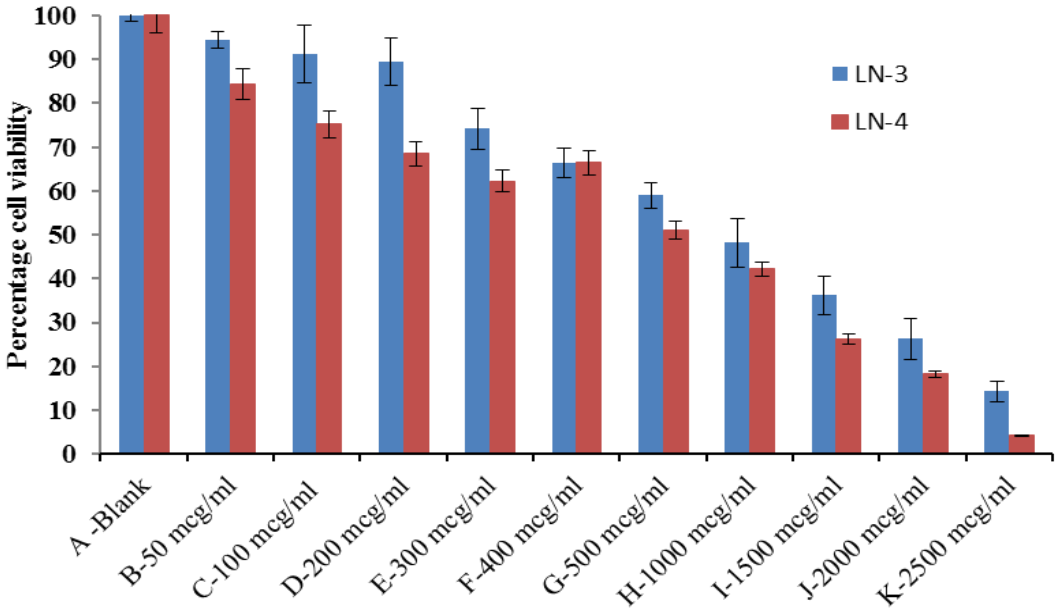

Figure. 14. Estrogen postive MCF-7 Cell lines treated with TC loaded lipid nanoparticle reservoir gel systems.

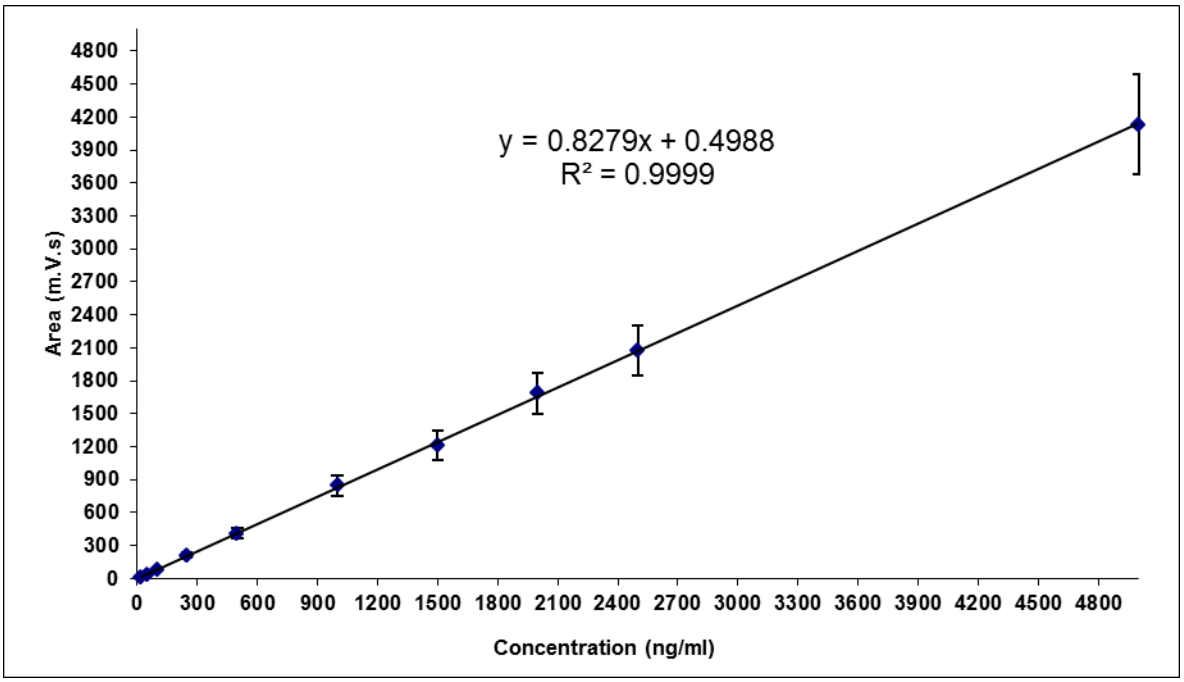

Figure 15. Calibration curve of tamoxifen citrate by HPLC method.

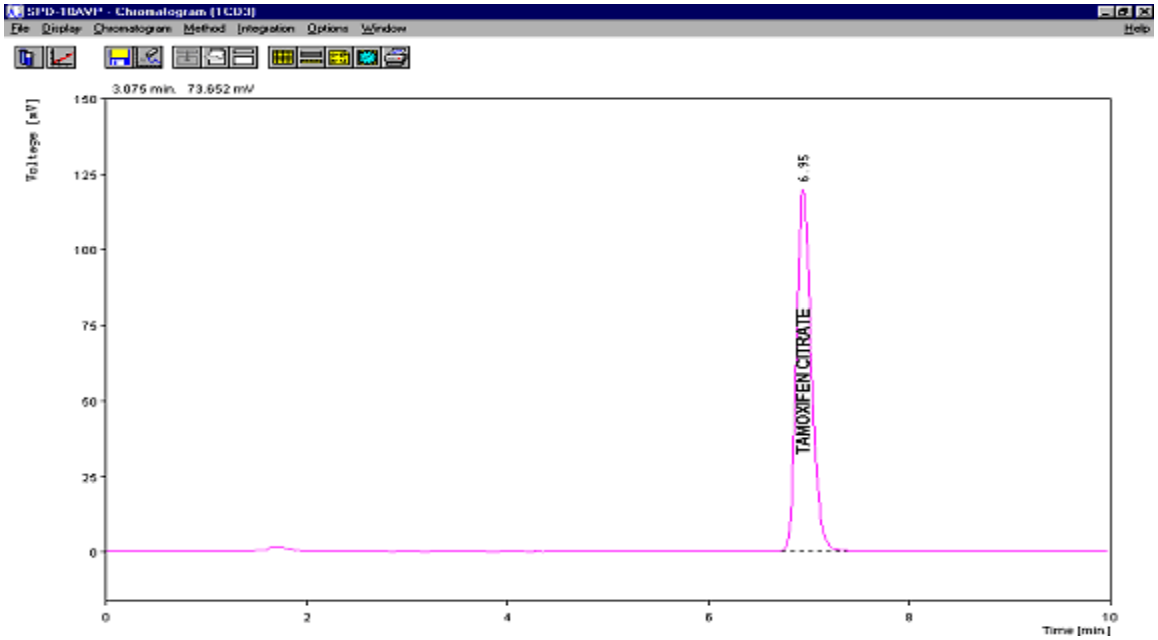

Figure 16. HPLC chromatogram of tamoxifen citrate (extracted from plasma)

Table. 11. Pharmacokinetic parameters after tamoxifen transdermal and oral single dose administration to rats.

Study design	Drug dose (mg/kg)	$t_{(max)}$ (h)	$C_{(max)}$ ng/ml	AUC_{0-24} ng/ml	F_{rel} Transdermal vs. oral
Group I (n=5):					
F1EH	16.6 mg	1.30	111.85±45	957±387	0.56
F2EH	16.6 mg	1.20	115.23±85	997±474	0.58
Group II (n=5):					
F3HCE	16.6 mg	0.90	112.63±87	1087±870	0.63
F4HCE	16.6 mg	0.90	108.58±12	1189±666	0.69
Group III (n=5):					
LN-3: 40:160	16.6 mg	0.80	116.23±33	1425±412	0.67
LN-4: 60:140	16.6 mg	0.60	120.89±78	1258±387	0.74
TC Oral solution	3 mg	2.00	097.85±88	308±145	

$P < 0.05$ significant difference from oral BH (unpaired t-test).

Note: Dose: All formulations containing 5.0 mg were applied to 300±5 gm of female albino rats; dose becomes 16.6±0.5 mg. In case of oral 5-10 mg/kg is reported. For 300±5 gm equivalent to 3 mg, respectively.

Effect on final body weight

In the disease group, there was a considerable increase in body weight compared to a normal group. Similarly, in the PC group, there was a considerable decrease in body weight compared to the cancer group. Similarly, formulation F1 showed a considerable decrease in body weight compared to the cancer group. Likewise, formulation F2 showed a considerable decrease in body weight compared to the cancer group. Further, the F3 group showed a considerable decrease compared to the cancer group. Similarly, the F4 group showed a considerable decrease compared to the cancer group [Figure 17].

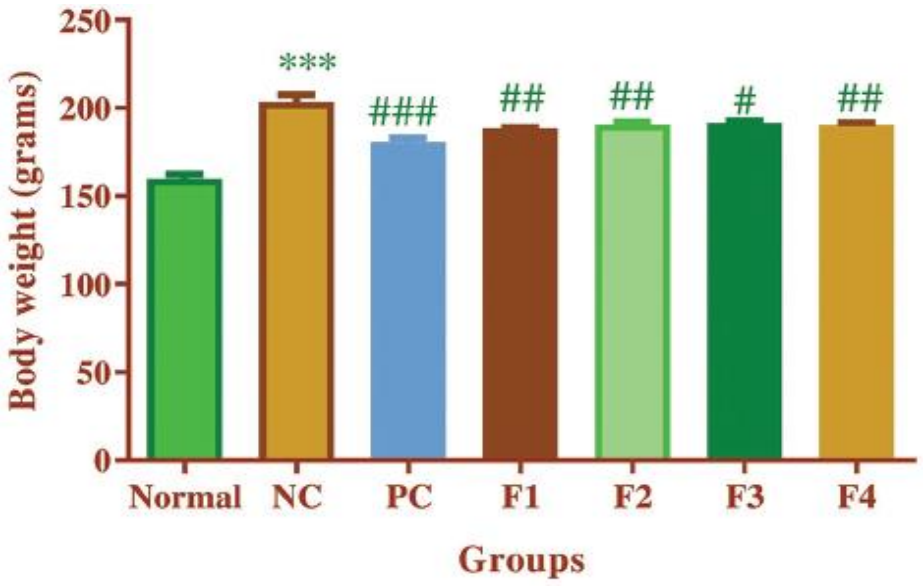

Figure 17: Effect of multiple formulations in body weight ***P < 0.001 compared to normal control, #P < 0.05, ##P < 0.01, ###P < 0.001 compared to cancer group

Effect on hemoglobin content

The hemoglobin content in the disease group was found to be considerably decreased in the disease group compared to normal control. Similarly, there was a

considerable increase in hemoglobin content in the PC compared to the disease group. Similarly, formulations F1, F3, and F4 also showed a considerable increase in hemoglobin content compared to the normal disease group. However, formulation F2 did not affect hemoglobin content compared to the disease group [Figure 18].

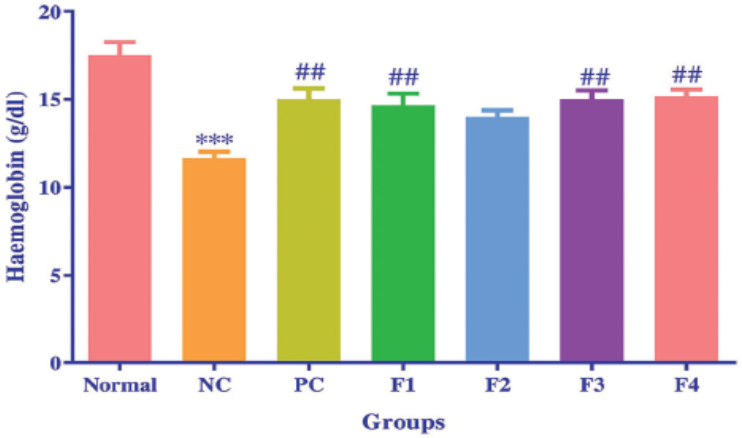

Figure 18: Effect of formulations on hemoglobin content (gm/dl) ***P < 0.001 compared to normal group, ##P < 0.01 compared to disease control group

Effect on red blood cell count

RBC count was considerably decreased in the disease group compared to a normal group and was increased in the PC compared to the disease group. There was an increase in RBC count in formulation treatments. However, the results were not considerable [Figure 19].

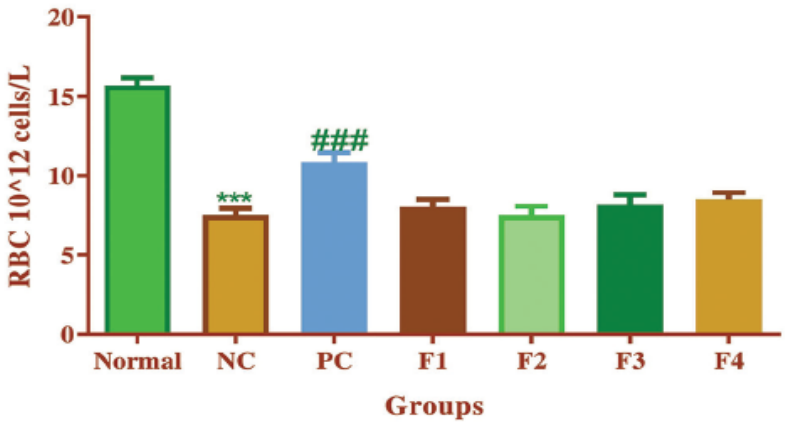

Figure 19: Effect of formulations in red blood cell ***P < 0.001 compared to normal, ###P < 0.001 compared to disease group

Effect on white blood cell count

There was a considerable decrease in WBC count in the disease group compared to the normal group. Similarly, there was a considerable increase in WBC count in PC compared to the disease group. There was an increase in WBC count in formulation treated groups; however, the results were not considerably different compared to the disease control group [Figure 20].

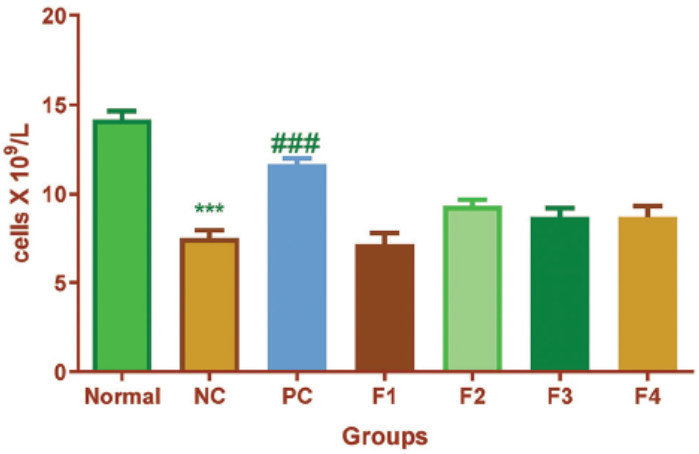

Figure 20: Effect of formulations in white blood cell ***P < 0.001 compared to normal, ###P < 0.001 compared to disease group

Effect on tumor weight

The PC group showed a considerable decrease in tumor weight in the PC group compared to the disease group. Likewise, treating with formulations F1 and F2 showed an equal level of a considerable decrease in tumor weight compared to disease control. Likewise, formulation F3 showed a considerable decrease in tumor weight compared to the disease group. However, formulation F4 had no effect in decreasing tumor weight compared to the disease group [Figure 21].

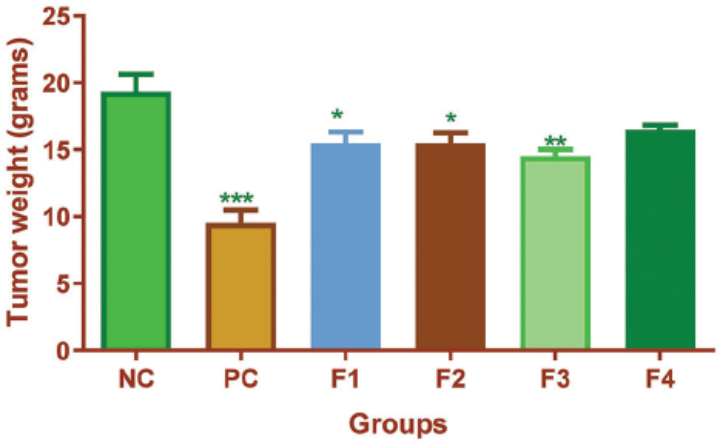

Figure 21: Effect of formulations in tumor weight *P < 0.05, **P < 0.01, ***P < 0.001 compared to disease group

Effect on SGPT

A considerable increase in the SGPT level was found in the disease group compared to normal. However, there was a considerable decrease in the SGPT level in tamoxifen and formulation (F1–F4)-treated groups. Furthermore, there was a decreased level in the SGPT level in formulations compared to the tamoxifen group [Figure 22].

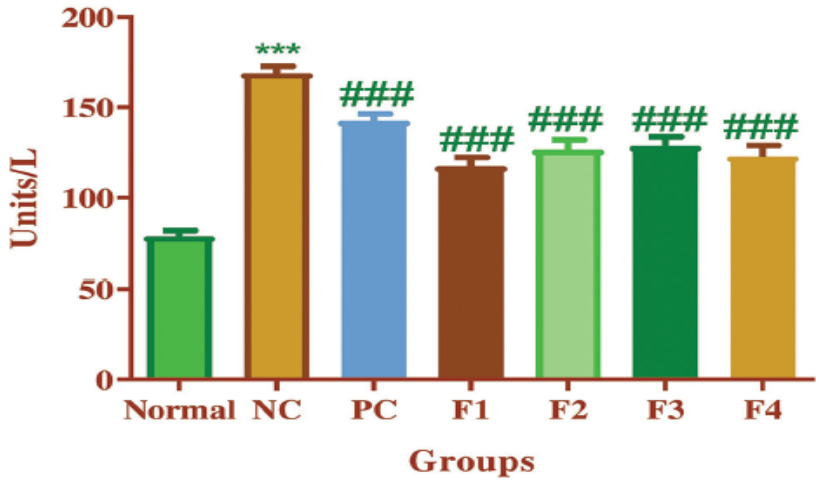

Figure 22: Effect on SGPT levels ***P < 0.001 compared to normal group, ###P < 0.001 compared to disease group

Effect on SGOT level

SGOT level in the disease group was found to be considerably higher compared to normal. However, there was a considerable decrease in the SGOT level in tamoxifen and formulation (F1–F4)-treated groups. Furthermore, there was a decreased level in the SGOT level in formulations compared to the tamoxifen group [Figure 23].

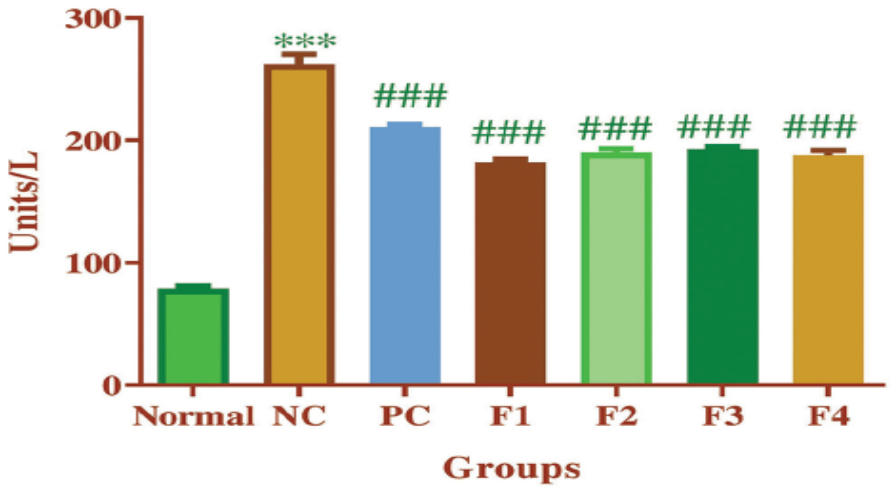

Figure 23: Effect on SGOT levels ***P < 0.001 compared to normal group, ###P < 0.001 compared to disease group

5. DISCUSSION:

The aim of the present research work is to prepare a matrix and reservoir type Transdermal drug delivery system containing tamoxifen citrate (TC), using different hydrophilic and lipophilic polymer (Table 1). TC containing matrix patches were prepared by using hydroxypropyl methyl cellulose, ethyl cellulose, Carbopol-934, Eurdragit-RL, hydroxyl propyl methyl cellulose-K-50. Dimethyl sulfaoxide and isopropyl myristate were used as a penetration enhancer (10-30% w/v). Dibutyl phthalate (DBT) as a plasticizer, formulations were prepared by plate casting method (Table 2). The reservoir transdermal equivalent 10 to 60 mg (formulations F1-F4 theoretical loading of TC by 5, 10, 20 and 30% w/w of polymer, poly (SA:RA) 50:50) of TC containing solid lipid-nanoparticle gel was prepared with 1% w/v of HPMC (Table 3).

TC in methanol : PBS pH 7.4 (5:5) was scanned under UV spectrophotometer in the range of 400-200 nm. The absorption peak was observed at 277.00 nm and 236.60 nm against reported value of 237.0 nm and 275.0 nm as $\lambda_{max}$of TC in methanol.[48] The maximum absorbance peak at 275.0 nm considered for further studies. The standard curve of TC was found to be linear over a concentration range of 2 to 20 μg/ml, with the correlation coefficient (R^2) value 0.9992, slope 0.019 and intercept -0.009 (Figure 1 and the generated data were tabulated in Table 1). The developed analytical method based on UV-Spectrophotometry for TC was found to be more sensitive, precise, accurate and reproducible.

Figure 2 displays the outcome of preformulation studies the TC was subjected for DSC and XRD to investigate the physical, thermal and crystal structure of the drug. The study revealed that TC showed an endothermic onset/peak/endset peak at 146.22/148.61/151.96^{o}C. It was observed that the peaks were at the same temperature

(no exothermic peaks were observed). Crystalline form can be described as an arrangement of molecular chains that results in ordered structure. The study revealed that TC was observed in the form of crystalline nature.

Figure 3 shows the physical examination; films appeared to be slightly translucent suggesting that the drug was not completely solubilized rather dispersed/suspended in the matrix. The studies revealed that addition of di-n-butyl phthalate at 30% w/w was fixed and standardized for formulation F1-F4 and incase of F5 and F6, 20% w/w, respectively. All the developed and prepared formulations of polymer were smooth, uniform and flexible films were obtained.

Figure4shows the transdermal patches appeared as flexible, smooth, non-sticky and translucent on visual inspection, indicating that might be drug is solubilized and dispersed in the matrix. The studies revealed that the addition of di-n-butyl phthalate at 30% w/w concentration was fixed and standardized for formulation F7-F12(Figure 4). All the developed and prepared formulations of polymer were smooth, uniform and flexible films were obtained.

Thickness of films of F1-F6 varied between 0.106 and 0.127 mm (Table.5) suggesting that formulation variables used in the study did not produce any significant effect on the thickness of films. The uniformity of weight varied between 135.7±0.7 to 202.2±0.9, as the Eudragit concentration decreased, decrease in the weight was obtained.

Thickness and weights of transdermal films of F7-F12 were found range in between 0.10±0.03-0.19±0.04 mm and 200.0±1.0 to 205.0±0.7 (Table.6) respectively, indicating that formulation variables and solvent casting method used in the study did not produce any significant effect on the thickness of films.

The drug content of all the formulations (F1-F12) was range in between 98.62±1.53 to 99.89±1.20 with a low standard deviation (≤0.61, Table 5 and 6). The results of drug content analysis have shown that the method employed to prepare films in this study was capable of giving films with uniform drug distribution with an insignificant batch variability ($p>0.001$).

Moisture content and moisture uptake studies of formulations F1-F6 provide information regarding stability of the formulation[57, 58, 66, 67].The results revealed that the moisture content and moisture uptake were found to increase with increasing concentration of hydrophilic polymer (HPMC). The presence of penetrations enhancers DMSO and IPM did not show any major changes in moisture content and moisture uptake values. In case of DMSO, slight increment in both parameters was observed. This may be due to the water affinity of DMSO. The small moisture content in the formulation helps them to remain stable and from being a completely dried and brittle films[66,67] and low moisture uptake protects the material from microbial contamination and bulkiness of the films[66]. Thus, the results of physicochemical studies conducted on different polymeric films containing tamoxifen favored the combination of these polymers for preparation of transdermal films, the obtained results are shown in Table.5.

Moisture content and moisture uptake studies of formulations F7-F12 provide information related to stability of the formulation in wet and dry conditions[72]. The results displayed that the moisture content and moisture uptake were found to increase with proportionate increasing concentration of polymers HPMC - K15 and HPMC - K50. This may be attributed due to the hydrophilicity of HPMC. The moisture content and moisture uptake values for formulations F7 and F8 found to be

2.78±0.18/3.53±0.20 and 2.98±0.10/3.86±0.19, respectively. The moisture content and moisture uptake values for formulations F3 and F4 found to be 3.85±0.18/4.05±0.20 and 3.46±0.12/3.86±0.11 respectively. The moisture content and moisture uptake values found to be higher for formulations F5 and F6 i.e., 4.48±0.12/5.06±0.06 and 4.46±0.19/5.10±0.20 respectively. This may be due to contribution of hydrophilicity of two hydrophilic polymers HPMC - K15 and HPMC - K50[7]. The presence of constant concentration (10% w/w) of penetration enhancer DMSO did not exhibit any potential changes in moisture content and moisture uptake values. The presence of minute moisture content in all patches may be due to the water affinity property of DMSO as well as hydrophilic characteristics of HPMC helps them to remain stable and from being a completely dried[66] and brittle films[66], and low moisture uptake protects the material from microbial contamination and bulkiness of the films[66, 67]. Thus, the results of physicochemical studies performed on different polymeric films containing tamoxifen favored the combination of these polymers for preparation of transdermal films, the obtained results were displayed in Table.6.

The transderamal patches of F1-F6 formulations showed very good tensile strength and folding endurance values ranging in between 12.91±0.15 to 13.07±0.09 kg/cm^2, and 38.50±1.29 to 46.00±2.16, respectively, Table.5.

The patches of F7-12 formulations exhibited very good tensile strength and folding endurance values ranged in between 12.18±0.18 to 12.85±0.04 gm/cm^2, and 47.25±2.25 to 58.50±2.16, respectively, Table.6.

The *in vitro* release profile is an important tool that predicts in advance how a drug will behave in vivo. The results of *in vitro* skin permeation studies of tamoxifen

citrate from transdermal patches of F1-F6 are shown in Figure 5 and Table 7. The cumulative amount of drug release from (2.0 cm^2,area of 3.14 cm^2), formulation from F3, F4, F5 and F6 was (4.278, 4.56, 4.224, 4.665 mg)high when compared to other formulations, this phenomenon attributed due the amount of combination hydrophilic and hydrophophic polymersused in the formulations.[66, 69, 73, 74] When the cumulative amount of drug permeated with an area of 3.14 cm^2 patches through rat skin was plotted against time. The flux, permeation coefficient and diffusion coefficient was high in formation F3, F4, F5 and F6 when compared to other two formulations due the hydrophilic nature of the polymer, absorption of water, swelling nature are contributed.[66, 69, 73, 74, 79-82]

The data of *in vitro* skin permeation studies of TC from transdermal patches are shown in Figure 6 and Table 8. Formulations F7-F12 has displayed the cumulative amount of drug permeated with the values 3.49, 3.33, 4.34, 3.36, 3.52 and 3.59 mg respectively. The ascribed results are attributed due to the combination of hindrance properties of hydrophilic and hydrophobic polymers in patches[73, 74].Formulation F9, has displayed the highest amount of drug diffusion than others. The Flux, permeation coefficient and diffusion coefficient was high in formulation F9, F11 and F12 than other formulations due the hydrophilic nature of the polymers, absorption of water, and swelling nature are contributed.[66, 69, 73, 74, 79-82]

Tamoxifen citrate loaded poly(SA:RA) solid lipid nanoparticles were prepared by solvent displacement method. Polyanhydride based lipid polymer poly (sebacic-co-ricinoleic acid) 5:5 was used as carrier. Poly(SA:RA)7:3 used in this study is a hydrophobic polymer, built of natural fatty acids, which may be used for release of both hydrophobic and hydrophilic drugs (Table 3). Glucose and mannitol and used as

cryoprotectants that are added prior to lyophilization to facilitate the particle formation and prevent shrinkage of lipid particles on lyophilization. Cryoprotectants used were polysaccharides and are non toxic to the human body that metabolizes within the body by glycolysis followed by Krebs cycle to produce carbon dioxide and water to generate a form of usable energy[33].

Formulations of F3 and F4 were finally selected DSC and XRD studies due to their prominent results in respect to particle size, entrapment efficiency, and this solid lipid nanopartcles dispersed into HPMC gels, respectively.

Using the DSC analysis of drug, polymer materials and produced lipid nanoparticles, the nature of the drug inside the polymer matrix can be assessed, which may emerge in solid solution, metastable molecular dispersion or crystallization. In order to identify the mechanism of sustained drug release, we first characterized the physical state of the drug within the nanoparticles. The DSC thermograms shown in Figure 7 indicates melting peak of TC was absent in DSC thermograms of lipid naoparticles containing TC, reveals that the drug was dispersed as an amorphous form or dissolution state. This amorphous nature of the drug may have pronounced pharmaceutical significance as it could lead to increased solubility and finally to an improved biological activity.

XRD is a powerful technique for the identification of crystalline solid phases[53,64], but there are numerous sources of error in quantitative XRD. X-ray lines are affected by preferred orientation of the particles in the sample. Variation in particle size can have a significant influence on the peak shape. Figure 8 shows the pure TC, poly(SA:RA)7:3, physical mixture, LN-4 and LN-5 were characterized by

prominent diffraction peaks in the range of 0-70° 2θ during XRD studies and their respective characteristic peaks are given in Table 9**.**

The drug peak did not appear in formulation and only the polymer peak was observed, which revealed that drug is in the form of amorphous/dissolution state in the formulations.

The particles of size range in between 400-600 nm were obtained. Particle size was increased slightly with increasing TC content. The particle size (mean diameter) variation observed in the TC loaded poly(SA:RA) nanoparticles might be due to the variations in drug & polymer concentration and also the method of preparation. Figure 9, Shows the scanning electron microscope (SEM) studies of lipid nanoparticles and typical LN-3 drug loaded lipid nanoparticle gel formulation. TC loaded lipid nanoparticles were more homogeneously dispersed in the HPMC gel with irregular and network like distribution was observed.

Dispersion of polymer-based nanoparticles in hydrophilic gels can further improve drug delivery to the skin. The gel can aid in creating a uniform dispersion of the carriers in the matrix and increase the contact time and deposition of the carriers on the skin, resulting in enhanced skin penetration of the payload.[3140,41,89] Gels can also accommodate additional ingredients or excipients for development of multicomponent formulations. To this end, hydrophilic gels have been shown to serve as an effective and inert environment for actives and carriers including nanocapsules, liposomes, solid lipid nanoparticles and microemulsions. Based on these concepts 1% w/v of HPMC is selected for the preparation of gel with varying the concentration of drug loading was optimized and standardized based on viscosity, uniformity of drug distribution and transfer of gel or spreadability on regenerated cellulose membrane

and skin was considered. A plot of the shear rate vs the resultant viscosity of the 1.0% w/v TC-HPMC gels were found to be in between 1.26-1.45 (Pa.s at 25^{o} C) demonstrated decrease in viscosity with increasing shear rates. This indicated that shear-thinning properties (pseudoplastic behavior) of these formulations were desirable property for topical preparations, as they should be thin during application and thick otherwise.[100, 101]

To know the effect of gel chemistry the *in vitro* release studies of the four different concentration of drug loaded (5, 10, 20 and 30% w/w of polymer) 1% w/v HPMC gels (10% w/w of DMSO as penetration enhancer) containing lipid nanoparticles was conducted on Keshary-Chein diffusion cells by using regenerated cellulose dialysis membrane. Visually TC containing gels were slightly opaque and were stable for 48h at room temperature was observed.

Figure 10 shows the cumulative amount of TC released from a fixed concentration of 1% wv HPMC gels, as we observed as the concentration of drug loading increased drug release was increased. This phenmenon can be exaplained as HPMC is hydrophilic but however, poly(SA-RA)50:50 hydrophobic, and its degrdartion (hydrolytic or enzymatic) in aqueous and bilogical media is slow[100,101]. Since both polymers complex network 65-70% of drug was released with 10% w/w of DMSO as penetration enhancer at the end of 24 h, through cellulose dilysis membrane (0.22µm). As the concentration of drug loading is incereased in the gels, drug relase, flux, permeation and diffusion coeffiecient values are enhanced (increased) is shown in the Table 10.

Figure 11 shows cumulative amount of TC permeated/released and their corresponding steady state fluxes, permeation and diffusion coefficient is illustrated in

Table 11.The *in vitro*skin permeation TC containg lipid nanopartciles containing HPMC gels revealed that, the amount of drug permeated/relaesd through the albino rat skin was very slow with penetration enhancer as compared to the *in vitro* release studies.

Figure 12 shows the inhibitory concentration (IC_{50}) of tamoxifen citrate in various concentration of tamoxifen citrate was compared with control (no drug). Inhibitory concentration (IC_{50}) of tamoxifen citrate on estrogen positive MCF-7 cell lines was 100 µg/ml after 48 hours[31], obtained results was statistically significant ($P<0.05$). The 500, 750, 1000, 1500, 2000, 2500, and 3000 µg loaded TC transdermal patches F1, F2, F9 and F10 showed Inhibitory concentration (IC_{50}) at 1500 µg the results are as follows, 48.58±4.68, 44.58±3.64, 42.58±6.21and 49.58±6.24% Figure 13, and in case of solid lipid nanoparticle gel formulations LN-4 and LN-5 showed (IC_{50}) at 1000 mg/ml the obtained results are as follows, 48.18±5.46 and 42.20±4.52% cell viability when compared to the blank after 48 hours, MTT assay technique was employed determination of cell viability and results was statistically significant ($p<0.05$) Figure 13. The cell lines were sensitive to the released drug when it was exposed continuously to tamoxifen citrate for 48 hours. The reduction rate of cell viability from TC loaded patches and lipid nanoparticle gel formulations could be explained with respect to the dependence on release of tamoxifen citrate from the hydrophilic and hydrophobic combination of polymers. Increasing the TC concentration in the patches which results in a proportionate increase in dose, resulted in increased reduction in cell proliferation. The cytotoxicity against the MCF-7 cell line was affected significantly by the released amount of tamoxifen citrate.

A standard curve was constructed for tamoxifen citrate by plotting the peak area as function of tamoxifen citrate concentration in plasma. The typical equation describing the calibration curve for plasma is y=1.075x-1.306, where y is the peak area of tamoxifen citrate, c is intercept and x is the concentration of tamoxifen citrate, with a mean correlation coefficient (R^2) of 0.9996 respectively. The obtained results are shown in Figure 14 and 15, respectively.

The recovery of tamoxifen citrate at four different concentrations of 20-1000 ng/ml from rat plasma was found to be 96.75±1.69%. The LOD for tamoxifen citrate in plasma was 4.6 ng/ml. The LOQ for tamoxifen in plasma was an acceptable precision and accuracy. The intra-day deviation was within values and the variations intra-day precision (R.S.D.) was obtained. The inter-day deviation was within 11.78% and precision (R.S.D) was between 1.19 and 6.11%, respectively.[92,95]

Table 11. Shows the results of our pharmacokinetic study, when administered transdermal, the drug was present in rat plasma for amuchlonger period compared to the oral administration, 18 h vs. 4 h, respectively. By providing a non-fluctuated and continuous deliveryof TC into the bloodstream, transdermal administration may offer sustained efficacy with reduced side effects. A relative bioavailability of formulation ranges in between 0.56-0.74 was estimated for transdermal vs. oral administration.[31,55]

6. SUMMARY

The aim of the present research work was to prepare and characterized matrix and transdermal lipid nanoparticle reservoir transdermal systems using hydroxypropyl methyl cellulose, ethyl cellulose, Carbopol-934, Eurdragit-RL, Hydroxyl propyl methyl cellulose-K-50. Dimethyl sulfaoxide and isopropyl myristate were used as a penetration enhancer (10-30% w/v). Dibutyl phthalate (DBT) as a plasticizer, formulations were prepared by plate casting method. The solid lipid-nanoparticles were prepared by solvent evaporation /displacement method using poly (SA:RA) 50:50. The reservoir transdermal equivalent 10 to 60 mg (formulations F1-F4 theoretical loading of TC by 5, 10, 20 and 30% w/w of polymer, poly (SA:RA) 50:50) of TC containing solid lipid-nanoparticle gel was prepared with 1% w/v of HPMC.

The UV-Visible and analytical method was developed and standardized for TC in bulk as well as in formulation.The standard curve of TC was found to be linear over a concentration range of 2 to 20 μg/ml, with the correlation coefficient (R^2) value 0.9992, slope 0.019 and intercept -0.009. The developed analytical method based on UV-Spectrophotometry for TC was found to be more sensitive, precise, accurate and reproducible.[48]

The high performance liquid chromatographic (HPLC) method was developed and standardized for TC in bulk and blood samples. A standard curve was constructed for tamoxifen citrate by plotting the peak area as function of tamoxifen citrate concentration in plasma. The typical equation describing the calibration curve for plasma is y=1.075x-1.306, where y is the peak area of tamoxifen citrate, c is intercept and x is the concentration of tamoxifen citrate, with a mean correlation coefficient (R^2) of 0.9996 respectively. The recovery of tamoxifen citrate at four different

concentrations of 20-1000 ng/ml from rat plasma was found to be 96.757±1.69%. The LOD for tamoxifen citrate in plasma was 4.6 ng/ml. The LOQ for tamoxifen in plasma was 15.64 ng/ml with an acceptable precision and accuracy. The intra-day deviation was within 15.16% for all values and the intra-day precision (R.S.D.) varied between 1.54 and 9.53%. The inter-day deviation was within 11.78% and precision (R.S.D) was between 1.19 and 6.11%, respectively.[92, 93]

The thermal and crystallinity was investigated for TC by using DSC and XRD. The study revealed that an endothermic peak was generated for TC. It was also observed that the peakwas at the same temperature. Crystalline form can be described as an arrangement of molecular chains that results in ordered structure. The study revealed that TC was observed in the form of crystalline nature.

The physical evaluation of formulations results revealed that slightly translucent suggesting that the drug was not completely solubilised rather dispersed/suspended in the matrix. The plasticizer di-n-butyl phthalate different concentration wasfixed and standardized. All the developed and prepared formulations of polymer were smooth, uniform and flexible films were obtained.In case of formulations F7-F12 the transdermal patches appeared as flexible, smooth, non-sticky and translucent on visual inspection, indicating that the drug was partially solubilised rather dispersed in the matrix. Variations in the thickness of the patches were investigated indicating that formulation variables and solvent casting method used in the study did not produce any significant effect on the thickness of films.

The developed UV-Visible spectrophotometric results of drug content analysis have shown that the method employed to prepare films in this study was capable of giving films with uniform drug distribution with an insignificant batch variability ($p>0.001$).

The generated moisture content and moisture uptake were found to increase with increasing concentration of hydrophilic polymer (HPMC). The presence of penetrations enhancers DMSO and IPM did not show any major changes in moisture content and moisture uptake values. In case of DMSO, slight increment in both parameters was observed. This may be due to the water affinity of DMSO. The small moisture content in the formulation helps them to remain stable and from being a completely dried and brittle films[5], and low moisture uptake protects the material from microbial contamination and bulkiness of the films[57, 58, 66, 67]. Thus, the results of physicochemical studies conducted on different polymeric films containing tamoxifen favored the combination of these polymers for preparation of transdermal films. Moisture content and moisture uptake studies of formulations F7-F12 provide information related to stability of the formulation in wet and dry conditions[66]. The results displayed that the moisture content and moisture uptake were found to increase with proportionate increasing concentration of polymers HPMC - K15 and HPMC - K50. This may be attributed due to the hydrophilicity of HPMC.The moisture content and moisture uptake values found to be higher for formulations F5 and F6. This may be due to contribution of hydrophilicity of two hydrophilic polymers HPMC - K15 and HPMC - K50[7].The transdermal patches exhibited very good tensile strength and folding endurance

The *in vitro* cumulative amount of drug release from (2.0 cm^2,area of 3.14 cm^2), formulation from F3, F4, F5 and F6 was (4.278, 4.56, 4.224, 4.665 mg) high when compared to other formulations, this phenomenon attributed due the amount of combination hydrophilic and hydophophic polymersused in the formulations[66, 69, 73, 74].When the cumulative amount of drug permeated with an area of 3.14 cm^2 patches through rat skin was plotted against time. The flux, permeation coefficient and

diffusion coefficient was high in formulations of F3, F4, F5 and F6 when compared to other two formulations due the hydrophilic nature of the polymer, absorption of water, swelling nature are contributed.[66, 69, 73, 74, 79-82]

Moreover, formulations F7-F12 has displayed the cumulative amount of drug permeated with the values 3.49, 3.33, 4.34, 3.36, 3.52 and 3.59 mg respectively. The ascribed results are attributed due to the combination of hindrance properties of hydrophilic and hydrophobic polymers in patches[73,74]. Formulation F9, has displayed the highest amount of drug diffusion than others. The Flux, permeation coefficient and diffusion coefficient was high in formulation F9, F11 and F12 than other formulations due the hydrophilic nature of the polymers, absorption of water, and swelling nature are contributed.[66, 69, 73, 74, 79-82]

Tamoxifen citrate loaded poly (SA: RA) Lipid-nanoparticualtes were prepared by solvent displacement method. Polyanhydride based polymer poly (sebacic-co-ricinoleic acid) 5:5 was used as carrier. Formulations of F3 and F4 were finally selected DSC and XRD studies based on *in vitro* physicochemical properties due to their prominent results in respect to particle size, entrapment efficiency, and this lipid nanopartcles dispersed into HPMC gels, respectively. In order to identify the mechanism of sustained drug release, we first characterized the physical state of the drug within the nanoparticles.The DSC thermograms indicatedthat melting peak of TC was absent in TC containing lipid nanoparticles, reveals that the drug was dispersed as an amorphous form or dissolution state. This amorphous nature of the drug may have pronounced pharmaceutical significance as it could lead to increased solubility and finally to an improved biological activity.XRD is a powerful technique for the identification of crystalline solid phases[53,56], the pure TC, poly(SA:RA)7:3, physical mixture, LN-4 and LN-5 were characterized by prominent diffraction peaks

in the range of 0-70° 2θ during XRD studies. The drug peak did not appear in formulation and only the polymer peak was observed, which revealed that drug is in the form of amorphous/dissolution state in the formulations.

The particle size analysis of lipid nanoparticles showed particles of size range in between 400-600 nm. Particle size was increased slightly with increasing TC content. The particle size (mean diameter) variation observed in the TC loaded poly(SA:RA) nanoparticles might be due to the variations in drug and polymer concentration and also the method of preparation. The scanning electron microscope (SEM) studies TC loaded lipid nanoparticles were more homogeneously dispersed in the HPMC gel with irregular and network like distribution was observed. The particle size analysis of lipid nanoparticles showed particles of size range in between 400-600 nm. Particle size was increased slightly with increasing TC content.

Dispersion of polymer-based nanoparticles in hydrophilic gels can further improve drug delivery to the skin. The gel can aid in creating a uniform dispersion of the carriers in the matrix and increase the contact time and deposition of the carriers on the skin, resulting in enhanced skin penetration of the payload[9,10]. Gels can also accommodate additional ingredients or excipients for development of multicomponent formulations. To this end, hydrophilic gels have been shown to serve as an effective and inert environment for actives and carriers including nanocapsules, liposomes, solid lipid nanoparticles and microemulsions. Based on these concepts 1% w/v of HPMC is selected for the preparation of gel with varying the concentration of drug loading was optimized and standardized based on viscosity, uniformity of drug distribution and transfer of gel or spreadability on regenerated cellulose membrane and skin was considered. The resultant viscosity of the 1.0% w/v TC-HPMC gels

were found to be in between 1.26-1.45 (Pa.s at 25^{o} C)demonstrated decrease in viscosity with increasing shear rates. This indicated that shear-thinning properties (pseudoplastic behaviour) of these formulations were desirable property for topical preparations, as they should be thin during application and thick otherwise.[31,40,41,89]

To know the effect of gel chemistry the *in vitro* release studies of the four different concentration of drug loaded (5, 10, 20 and 30% w/w of polymer) 1% w/v HPMC gels (10% w/w of DMSO as penetration enhancer) containing lipid nanoparticles was conducted on Keshary-Chein diffusion cells by using regenerated cellulose dialysis membrane. Visually TC containing gels were slightly opaque and were stable for 48h at room temperature was observed.

The cumulative amount of TC released from a fixed concentration of 1% wv HPMC gels, as we observed as the concentration of drug loading increased drug release was increased. This phenmenon can be exaplained as HPMC is hydrophilic but however, poly(SA-RA)50:50 hydrophobic, and its degrdartion (hydrolytic or enzymatic) in aqueous and bilogical media is slow[33]. Since both polymers complex network 65-70% of drug was released with 10% w/w of DMSO as penetration enhancer at the end of 24 h, through cellulose dilysis membrane (0.22µm). As the concentration of drug loading is incereased in the gels, drug relase, flux, permeation and diffusion coeffiecient values are enhanced.

The *in vitro* skin permeation TC containg lipid nanopartciles containing HPMC gels revealed that, the amount of drug permeated/relaesd through the albino rat skin was very slow with penetration enhancer as compared to the *in vitro* release studies.

Inhibitory concentration (IC_{50}) of tamoxifen citrate in various concentration of tamoxifen citrate was compared with control (no drug). Inhibitory concentration (IC_{50}) of tamoxifen citrate on estrogen positive MCF-7 cell lines was 100 µg/ml after 48 hours,[31] obtained results was statistically significant ($P<0.05$). The TC loaded transdermal patches F1, F2, F9 and F10 showed Inhibitory concentration (IC_{50}) at 1500 µg and in case of lipid nanoparticle gel formulations of LN-4 and LN-5 showed (IC_{50}) at 1000 mg/ml compared to the blank after 48 hours, MTT assay technique was employed determination of cell viability and results was statistically significant ($p<0.05$). The cell line was sensitive to the released drug when it was exposed continuously to tamoxifen citrate for 48 hours. The reduction rate of cell viability from TC loaded patches and lipid nanoparticle gel formulations could be explained with respect to the dependence on release of TC from the hydrophilic and hydrophobic combination of polymers. Increasing the TC concentration in the patches which results in a proportionate increase in dose, resulted in increased reduction in cell proliferation. The cytotoxicity against the MCF-7 cell line was affected significantly by the released amount of tamoxifen citrate.

A simple, accurate and precise high performance liquid chromatography (HPLC) method was developed the estimation TC in blood samples. A standard curve was constructed for tamoxifen citrate by plotting the peak area as function of tamoxifen citrate concentration in plasma. The typical equation describing the calibration curve for plasma is $y=1.075x-1.306$, where y is the peak area of tamoxifen citrate, c is intercept and x is the concentration of tamoxifen citrate, with a mean correlation coefficient (R^2) of 0.9996 respectively. The recovery of tamoxifen citrate at four different concentrations of 20-1000 ng/ml from rat plasma was found to be 96.75±1.69%. The LOD for tamoxifen citrate in plasma was 4.6 ng/ml. The LOQ for

tamoxifen in plasma was 15.64 ng/ml with an acceptable precision and accuracy. The intra-day deviation was within 15.16% for all values and the intra-day precision (R.S.D.) varied between 1.54 and 9.53%. The inter-day deviation was within 11.78% and precision (R.S.D) was between 1.19 and 6.11%, respectively.[92,95]

The pharmacokinetic studies revealed that the administered TC containing transdermal formulations, the drug was present in rat plasma for a much longer period compared to the oral administration, 18 h vs. 4 h. By providing a non-fluctuated and continuous delivery of TC into the bloodstream, transdermal administration may offer sustained efficacy with reduced side effects[31,55]. A relative bioavailability of formulation ranges in between 0.56-0.74 was estimated for transdermal vs. oral administration.

7. CONCLUSION

The present study clearly indicates tamoxifen citrate (TC) loaded polymeric transdermal matrix and solid lipid-nanoparticle gel based reservoir have a tremendous potential as novel local drug delivery systems investigated for estrogen positive breast cancer.

Initially as the first step of preformulation studies, drug excipient compatibility studies were carried out on tamoxifen citrate with polymers under investigation using DSC and XRD. As there was no difference in the thermograms and XRD pattern it was proved that there was no drug excipient incompatibilities. The UV-Visible and analytical method was developed and standardized for TC in bulk as well as in formulation. A simple and sensitive HPLC method was developed for estimation of tamoxifen citrate in bulk, tissue (mammary gland tumor) and plasma (blood). The developed assay methods for tamoxifen citrate provided excellent sensitivity, accuracy and precision.

Another contribution of the present study was the matrix and transdermal lipid nanoparticle reservoir transdermal systems using hydroxypropyl methyl cellulose, ethyl cellulose, Carbopol-934, Eurdragit-RL, Hydroxyl propyl methyl cellulose-K-50. Dimethyl sulfaoxide and isopropyl myristate were used as a penetration enhancer (10-30% w/v). Dibutyl phthalate (DBT) as a plasticizer, formulations were prepared by plate casting method. The reservoir transdermal systems of solid lipid nanoparticleswere prepared by solvent evaporation /displacement method using poly (SA:RA) 50:50. The physical evaluation of formulations results revealed that slightly translucent suggesting that the drug was not completely solubilized rather dispersed/suspended in the matrix. The generated moisture content and moisture uptake were found to increase with increasing concentration of hydrophilic polymer

(HPMC). The variations of flux, permeation coefficient and diffusion coefficient of TC loaded formulations due the hydrophilic nature of the polymer, absorption of water, swelling nature are contributed. Dispersion of polymer-based PSRA solid lipid nanoparticlesin hydrophilic gels can further improve drug delivery to the skin. The cumulative amount of TC released from a fixed concentration of 1% w/v HPMC gels, as we observed as the concentration of drug loading increased drug release was increased. The amount of drug permeated/released through the albino rat skin was very slow with penetration enhancer as compared to the *in vitro* release studies. The reduction rate of cell viability from TC loaded patches and lipid nanoparticle gel formulations could be explained with respect to the dependence on release of TC from the hydrophilic and hydrophobic combination of polymers. Increasing the TC concentration in the patches which results in a proportionate increase in dose, resulted in increased reduction in cell proliferation. The cytotoxicity against the estrogen positive MCF-7 cell line was affected significantly by the released amount of tamoxifen citrate. The pharmacokinetic studies revealed that the administered TC containing transdermal formulations, the drug was present in rat plasma for a much longer period compared to the oral administration, 18 h vs. 4 h. A relative bioavailability of formulation ranges in between 0.56-0.74 which was estimated for transdermal vs. oral administration.These results prove that the formulations developed in this study could be used for clinical trials. Thus, this type of sustained/controlled drug delivery system will definitely be helpful to improve the local treatment of operable and inoperable estrogen positive breast tumors. Thus it is concluded that **'Local transdermal matrix and reservoir Drug Delivery Technology'** is a usefull tool for the local targeting of anticancer drugs to breast cancer.

8. FUTURE SCOPE OF THE STUDY:

In order to achieve the clinical impact for the developed transdermal patches, further efforts should be directed to perform *in vivo* permeation study, pharmacokinetic and bioavability studies in human volunteers.

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10. ANNEXURES

Animal ethical committee approval letter

KLE UNIVERSITY'S

COLLEGE OF PHARMACY.

JNMC Campus, Nehru Nagar, **Belgaum**-590 010. Karnataka, India

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Ref. No. KLEUCOP : _____ *Date :* 31-07-201

CERTIFICATE

This is to certify that the research project (~~M.Pharm~~/Ph.D.) ✓

PREPARATION AND CHARACTERIZATION OF TAMOXIFEN LOADED TRANSDERMAL DRUG DELIVERY SYSTEM FOR ESTROGEN RECEPTOR POSITIVE BREAST CANCER

Submitted by Mr./Ms. ✓ Anjana Adhyapak

Has been approved by the Institutional Animal Ethics Committee meeting held

On 31-07-10, resolution No. 13.

No of animals Permitted (Rats) - 24

MEMBER SECRETARY
Institutional Animal Ethical Committee,
KLES's College of Pharmacy,
BELGAUM - 590010

Dr. S. Shishupal
CPCSEA Nominee
Institutional Animal Ethics Committee
KLES's College of Pharmacy,
BELGAUM.

ORAL PRESENTATION AND PUBLICATIONS

ORAL PRESENTATION				
Sl. No.	Title of the paper	Conference/FDP	Venue	Date
1.	Importance and applications of solid state in pharmaceutical formulations	UGC Sponsored Faculty Development Program	UGC-SAP Approved Department of Pharmaceutical sciences; Sourashtra University; Rajkot, Gujrat.	23rd November 2013
PUBLICATIONS				
Sl. No.	Title of the paper	Details of Journal (name, year, vol., issue, page no.)	Indexed/ UGC specified journal/	
1.	Preparation and in vitro characterization of the transdermal drug delivery system containing tamoxifen citrate for breast cancer	Asian Journal of Pharmaceutics Vol- 5 Issue 1, Jan To Mar 2011	UGC Care List	
2.	Effect of tamoxifen‑loaded transdermal patch on physical and biochemical parameters in DMBA‑induced breast cancer	Indian Journal of Health Sciences and Biomedical Research KLEU 05- October 2020	UGC Care List	

Re-Accredited
Grade B by NAAC
CGPA (2.93)

CERTIFICATE

ज्ञान-विज्ञान विमुक्तये
University Grants Commission

This is to certify that

Mrs. Anjana Adhyapak

has delivered Oral Presentation under Faculty Development Program on

"Importance and Application of Solid State in Pharmaceutical Formulation"

at

UGC-SAP approved Department of Pharmaceutical Sciences, Saurashtra University, Rajkot
from 11th November 2013 to 23rd November 2013

Dr. Navin R. Sheth
Convener

Dr. Mihir K. Raval
Coordinator

Department of Pharmaceutical Sciences, Saurashtra University, Rajkot, Gujarat, India

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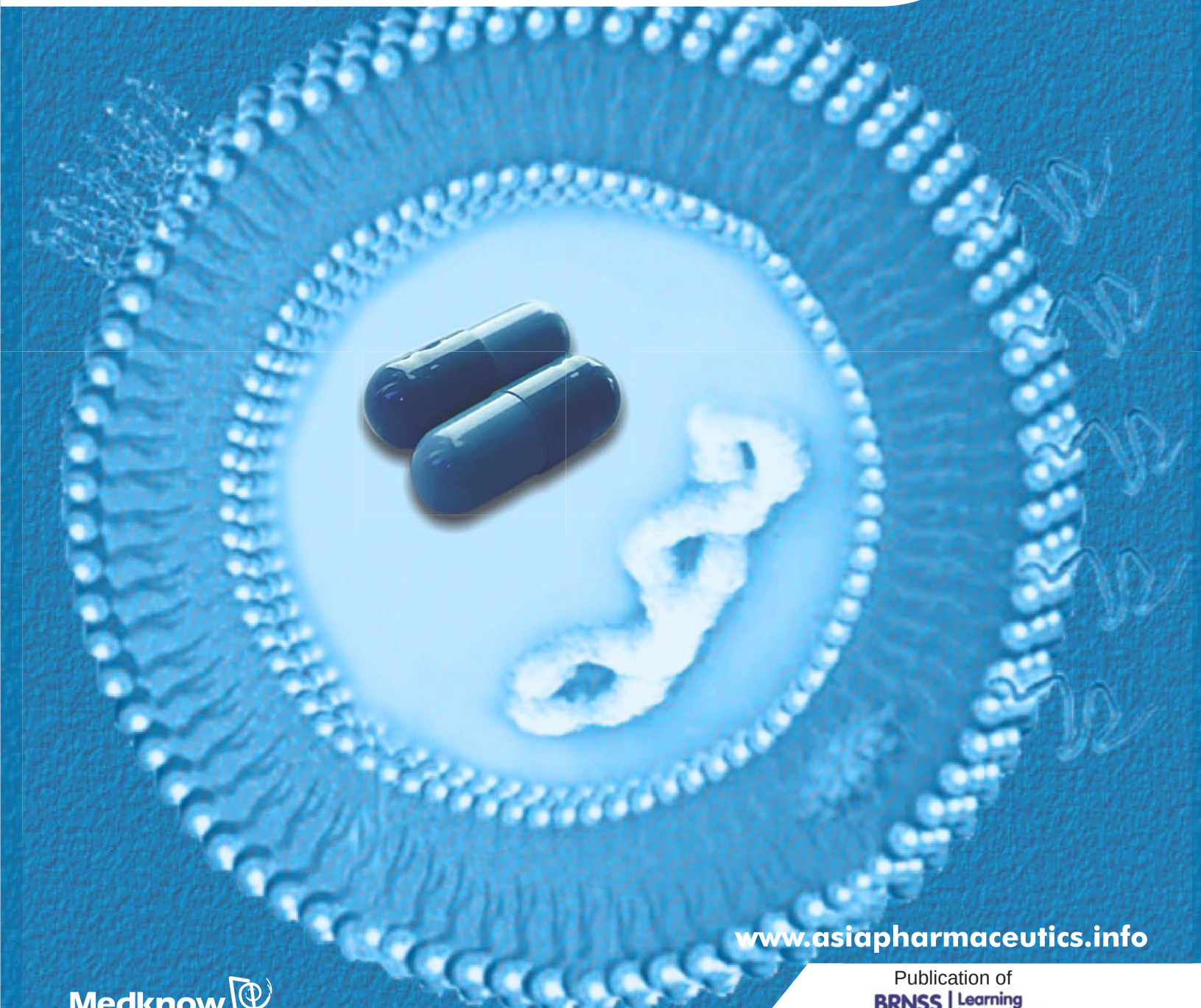

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RESEARCH ARTICLE

Preparation and *in vitro* characterization of the transdermal drug delivery system containing tamoxifen citrate for breast cancer

Anjana Adhyapak, Desai B G[1]

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Department of Pharmaceutics, KLE University's College of Pharmacy, Nehru Nagar, Belgaum, Karnataka, India

A matrix-type transdermal drug delivery system of tamoxifen citrate was developed by using a different ratio of eudragit-RL100, hydroxypropyl methyl cellulose (HPMC-K15), and ethyl cellulose (EC), by the solvent evaporation technique. The effect of the binary mixture of polymers with a penetration enhancer on the physical chemical parameters including, thickness, folding endurance, uniformity of drug content, moisture content, moisture uptake, tensile strength, and *in vitro* drug permeation were evaluated. The *in vitro* drug permeation studies were conducted by using modified Keshary-Chein diffusion cells through female Albino rat skin using pH 7.4 phosphate buffer saline (PBS). The selected formulation's stability studies were conducted as per the International Conference on Harmonization (ICH) guidelines, and did not show any degradation of the drug.

Key words: *Breast cancer, transdermal, tamoxifen citrate, skin permeation*

INTRODUCTION

Transdermal drug delivery systems (TDDS) encompass a wide array of non-invasive or minimally invasive technologies for delivering drugs and vaccines across the skin.[1-3] Applications of transdermal delivery include easy accessibility of the skin, which aids in high patient compliance, avoidance of the gastrointestinal tract, and the ability to achieve sustained / controlled release.

During the past decade, women had been looking forward to alternatives for oral hormonal chemotherapy. Transdermal drug delivery has been developed for contraception and hormonal therapy. Tamoxifen citrate has been a clinical choice for the treatment of advanced breast cancer and is often an adjuvant therapy after surgical resection. The drug has also been used in treating menopause. However, one of the side effects of the drug is the proliferative effect on the endometrium.[4] Tamoxifen citrate is a highly lipophilic drug, with poor water solubility.[5] Furthermore, its oral bioavailability is mainly affected by the first-pass metabolism and P-glycoprotein (P-gp) pump efflux in the liver and intestine.[6] Hence, there is a need for the development of a controlled / sustained delivery device, which is desired for successful local hormonal chemotherapy.

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MATERIALS AND METHODS

Materials

Tamoxifen citrate was a gift sample from Dabur Pharmaceutical Ltd., Ghaziabad, India. Beta-cyclodextrin, eudragit-RL-100, ethyl cellulose, and hydroxypropyl methylcellulose (K-15) were procured from Lab Care Ltd., Bangalore, India. All other chemicals and solvents were of analytical grade, purchased from Merck Pvt. Ltd., Bangalore, India.

Methods

Preparation of the transdermal drug delivery system

Transdermal films of tamoxifen citrate (5.0 mg / 3.14 cm^2) containing a different ratio of, eudragit-RL, hydroxypropyl methyl cellulose (HPMC K-50), and ethyl

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Table 1: Formulation composition of tamoxifen citrate containing matrix transdermal systems

Formulations	F1	F2	F3	F4	F5
Tamoxifen citrate (mg)	50	50	50	50	50
Eudrajit-RL-100 (mg)	150	60	90	96	95
HPMC-E15 (mg)	-	90	60	-	-
EC (mg)	-	-	-	4	5
DMSO (w/w) %	10	10	10	10	10
DBT (w/w) %	30	30	30	20	20
Methanol:DCM (1:2) (ml)	12	12	12	12	12

All the formulation contain 30% DBT except F4 and F5, which contain 20% DBT; HPMC: hydroxypropyl methyl cellulose, EC: ethyl cellulose, DMSO: dimethyl sulfoxide, IPM: isopropyl myristate, DBT: dibutyl phthalate, DCM: dichloromethane

cellulose were prepared on the mercury surface. The required amount of drug and polymers were dissolved in the methanol-dichloromethane (1 : 1) solvent system. Di-n-butyl phthalate (20 and 30% w/w of polymer) was used as a plasticizer. Isopropyl myristate (IPM) and Dimethyl sulfoxide (DMSO) were added to the polymer drug solution. The resultant homogeneous solution was poured into a circular plane, with a uniform surface, on a mercury substrate. The films were dried for a period of 24 hours, and the rate of evaporation was controlled by inverting a funnel over the petri dish. The dry films were wrapped in aluminum foil and kept in desiccators. Compositions of the prepared formulations are tabulated in Table 1 and photographs of the drug containing patches are shown in Figure1, respectively.

Evaluation of prepared transdermal patches

Thickness

The thickness of the film was determined using a micrometer gauge (Mitoyoto, Japan). The film was measured at different places and the mean value was determined.[7]

Weight uniformity

The films of different batches were dried at 40°C, for six hours, before testing. Six patches from each batch were accurately weighed on a digital balance.[8] The average weight and the standard deviation values were calculated from the individual weights.

Drug content analysis

The uniformity of drug distribution in the transdemal films was determined by taking a known area of the films at different places of the film. The films were dissolved in 2 ml of methanol, sonicated for 10 minutes, and subsequently diluted with phosphate buffer saline (PBS), pH 7.4. After appropriate dilution, the solutions were analyzed spectrophotometrically (UV Shimadzu-1700, Japan) for tamoxifen citrate, at 274 nm,[9] using a solution of films prepared without the drug as a reference, to neglect the absorption of components of the formulation if any.

Moisture content

The prepared films were weighed individually and kept in desiccators containing activated silica at room temperature

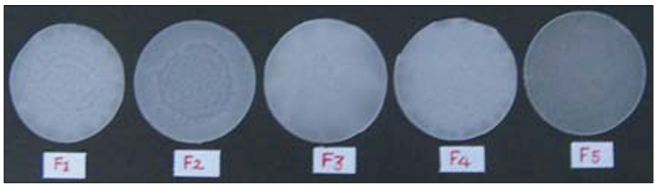

Figure 1: Typical photographs showing drug containing transdermal patches

(30°C) for 24 hours, until a constant weight was attained. The percentage of moisture content was calculated as the difference between the initial and final weight with respect to the final weight.[7]

Moisture uptake

A weighed film kept in the desiccator at room temperature (30°C), for 24 hours, was taken out and exposed to 84% relative humidity (RH) in a stability chamber (Lab Care, Mumbai, India) until a constant weight of the film was obtained. The percentage moisture uptake was calculated as the difference between the final and initial weights, with respect to the initial weight.[7]

Folding endurance

A strip of film (2 × 2 cm) was cut evenly and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the value of the folding endurance.

Determination of tensile strength

The tensile strength was determined by using a dynamic mechanical analyzer (computerized, EPLEXOR 500 N, IISC, Bangalore). Patches of 2 cm^2, of all the formulations were subjected and determined.

Determination of flux, diffusion coefficient and permeability coefficient

The flux of drug permeated in case of *in vitro* was calculated from the slope of the steady-state portion of the permeation profile by linear regression analysis.[10,11] The lag time was calculated from the back extrapolation of the steady-state portion of the graph. The diffusion coefficient (*D*/*h*2) and permeability coefficient (*K*p) were also calculated for the *in vitro* studies using the equations mentioned herwith, respectively,

$$D/h2 = 1/6 \times TLag,$$

$$Kp = JSS/CD,$$

Where, *T*Lag is the lag time, J_{SS} the flux at steady state, *C*D the concentration in the donor compartment, *D* the diffusion coefficient, and *h* the diffusion path length.

In vitro skin permeation study

Female Albino rats weighing 150 – 200 g were selected

for the permeation studies (the study was approved by the Animal Ethical Committee, KLE University, Department of Pharmacology, Belgaum, Karnataka, India). The animals were sacrificed using anesthetic ether. The hair of the test animals was carefully trimmed short with a pair of scissors and the full thickness skin was removed from the abdominal region. The epidermis was prepared surgically by the heat separation technique, which involved soaking of the entire abdominal skin in water at 60°C for 45 seconds, followed by careful removal of the epidermis. The epidermis was washed with water and used for permeability studies.[10,11] The permeation studies were performed for different formulations across female rat skin in a modified Keshary-Chein diffusion cell at 32±0.5°C. The diameter of the donor compartment cell provided an effective constant area of 3.14 cm^2. The films with an area of 3.14 cm^2 were applied to the skin using adhesive tape (cellophane) as the backing layer. The phosphate buffer pH 7.4 (20 ml) was used as the receptor compartment medium, to ensure sink conditions and stability of the drug. This whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a magnetic bead. The samples were withdrawn at different time intervals and replaced with an equal volume of diffusion medium. The samples were analyzed spectrophotometrically at 274 nm. To ascertain whether the components of the skin or other excipients of the film interfered in the drug analysis; a blank experiment (films without drug) was run, using the skin as a barrier membrane, with phosphate buffer saline pH 7.4. When the solution was analyzed at 274 nm for any interfering constituents, the released constituents amounted to an average of 0.04±0.02%.

Stability aspects

Stability studies were conducted according to the International Conference on Harmonization (ICH) guidelines by storing the TDDS in a stability chamber at 40±2°C / 75% RH (Thermo Lab., Mumbai, India). The samples were withdrawn at 0, 30, 60, and 90 days, and the physical and the drug content were analyzed by a UV spectrophotometer method.[7]

RESULTS AND DISCUSSION

The formulations were subjected to physical examination; the films appeared to be slightly translucent suggesting that the drug was not completely solubilized, but rather dispersed / suspended in the matrix. The studies revealed that addition of di-n-butyl phthalate at 30% w/w was fixed and standardized for formulations F1 – F3 and in the case of F4 and F5, 20% w/w, respectively. All the developed and prepared formulations of the polymer were smooth and uniform, and flexible films were obtained.

Thickness and uniformity of weight

The thickness of films varied between 0.106 and 0.127mm, suggesting that the formulation variables used in the study did not produce any significant effect on the thickness of films. The uniformity of weight varied between 135.7±0.7 and 202. 2±0.9, as the eudragit concentration decreased, and decrease in the weight was obtained. The obtained results are shown in Table 2.

Drug content analysis

The drug content of all the formulations [Table.2] was in between 97.15 to 99.62 with a low standard deviation (≤0.61). The results of drug content analysis have shown that the method employed to prepare films in this study was capable of giving films with uniform drug distribution with an insignificant batch variability ($p>0.001$).

Moisture content and moisture uptake

Moisture content and moisture uptake studies provide information regarding stability of the formulation.[13] The results revealed that the moisture content and moisture uptake were found to increase with increasing concentration of hydrophilic polymer (HPMC). The presence of penetrations enhancers DMSO and IPM did not show any major changes in moisture content and moisture uptake values. In case of DMSO, slight increment in both parameters was observed. This may be due to the water affinity of DMSO. The small moisture content in the formulation helps them to remain stable and from being a completely dried[14] and brittle films[14], and low moisture uptake protects the material from microbial contamination and bulkiness of the films[3,7,17]. Thus, the results of physicochemical studies conducted on different polymeric films containing tamoxifen favored the combination of these polymers for preparation of transdermal films, the obtained results were shown in Table 2.

Determination of tensile strength and folding endurance

All the formulations showed very good tensile strength, and

Table 2: Evaluation parameters of drug containing transdermal patches

Evaluations	F1	F2	F3	F4	F5
Thickness (mm)	0.11±0.01	0.12±0.02	0.14±0.03	0.12±0.01	0.14±0.02
Uniformity of weight	191.1±0.8	200.06±1.2	200.7±0.4	135.7±0.7	147.5±0.8
Content uniformity (%)	99.62±3.2	99.36±1.51	97.63±1.92	98.93±1.16	98.94±1.39
Moisture content (%)	2.61±0.38	3.06±0.15	3.36±0.06	3.62±0.10	4.93±0.22
Moisture uptake (%)	3.58±0.13	3.74±0.07	4.05±0.11	4.28±0.06	5.39±0.20
Folding endurance	39.50±2.65	39.25±1.26	31.75±0.96	44.50±1.73	46.00±2.16
Tensile strength (gm/cm^2)	12.91±0.15	12.73±0.04	12.82±0.09	12.52±0.05	13.07±0.09

Average of three determinations were reported (±SD, n = 3)

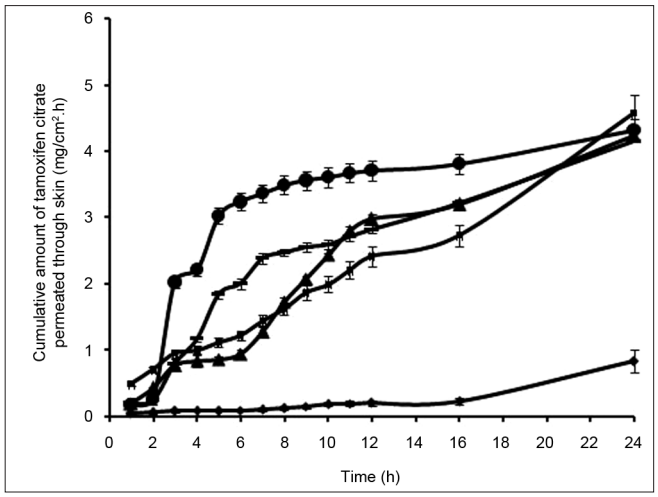

Figure 2: Cumulative amount of tamoxifen citrate permeated through the skin, F1 (♦), F2 (■), F3 (▲), F4 (–), F5 (•)

the folding endurance values ranged between 12.91±0.15 and 13.07±0.09 kg/cm^2 and 38.50±1.29 and 46.00±2.16, respectively, [Table 2].

Determination of flux, diffusion coefficient and permeability coefficient

The *in vitro* release profile is an important tool that predicts in advance how a drug will behave *in vivo*. The results of *in vitro* skin permeation studies of tamoxifen citrate from transdermal patches are shown in Figure 2 and Table 3. The cumulative amount of drug release (area of 3.14 cm^2) from formulations F2, F3, F4, and F5 was (4.278, 4.561, 4.224, and 4.665 mg) high when compared to other formulations; this phenomenon was attributed to the amount of the combination, of hydrophilic and hydophophic polymers,[14-17] used in the formulations. When the cumulative amount of drug permeated with an area of 3.14 cm^2 patches through rat skin was plotted against time. The flux, permeation coefficient, and diffusion coefficient were high in formations F2, F3, F4, and F5, when compared to the F1 formulation, due the hydrophilic nature of the polymer, absorption of water, and its swelling nature.[14-17]. From these obtained results it could be revealed that the usual dose of tamoxifen was in the range of 10 – 20 for a single dose and 20 – 40 mg for the daily dose, respectively. However, only 60% of the bioavaibility could be predicted, due to its first pass metabolism. Administration through the transdermal route made the drug available directly to the blood stream; hence, a lesser dose was required compared to the oral dose. Application of a patch having a surface area of more than 3.14 cm^2 or with a suitable large loading dose would provide the effective systemic concentration of tamoxifen. Moreover, once the tamoxifen reached the systemic circulation after transdermal administration; it underwent metabolism by a biological process, to produce hydroxytamoxifen, similar to the one administered orally. This metabolite was also very important in the therapeutic activity of estrogen positive receptor binding, respectively.[18]

Table 3: Determination of flux, diffusion coefficient, and permeability coefficient

Formulations	(Jss)Flux mg/cm^2. hr	Permeation coefficient (cm/h)	Diffusion coefficient (cm/h)
F1	0.009±0.02	0.0017±0.001	0.0009±0.002
F2	0.054±0.04	0.0116±0.003	0.0018±0.004
F3	0.063±0.05	0.0125±0.005	0.0020±0.002
F4	0.052±0.07	0.0102±0.004	0.0020±0.001
F5	0.053±0.06	0.0108±0.003	0.0021±0.002

Average of three determinations were reported (±SD)

Stability aspects

All the samples of formulations when subjected to stability studies, at a periodic interval of days, were observed for changes in color, appearance, flexibility, and drug content. The patches were analyzed at an interval of 30 days for a period of three months. No physical changes were observed, however, a negligible decrease in drug content (2 – 4%) after three months was observed at a temperature of 40±2°C/75% RH.

CONCLUSION

The matrix-type of transdermal containing tamoxifen citrate has been successfully formulated, which has brought a new modality delivery system for local chemotherapy, for breast cancer. The model patch formulation defines a positive outcome based on both qualitative observations and quantitative measurements of different parameters. The study demonstrated the possibility of developing an efficacious and acceptable transderaml drug delivery system for tamoxifen citrate, other than the conventional tablets. The study also concluded that the tamoxifen citrate transdermal patch could be a novel drug delivery of choice in the field of local chemotherapy for breast cancer.

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Effect of tamoxifen-loaded transdermal patch on physical and biochemical parameters in DMBA-induced breast cancer

Anjana Adhyapak, B. G. Desai[1]

Abstract:

BACKGROUND: The current study assessed the effect of tamoxifen-loaded transdermal patch in Dimethylbenz(a)anthracene (DMBA)-induced breast cancer.

MATERIALS AND METHODS: Different formulations composed of various concentrations of tamoxifen citrate, poly (SA: RA), glucose, mannitol were formulated and were evaluated in DMBAinduced breast cancer in female albino Wistar rats. Multiple parameters such as body weight, hemoglobin content, red blood cell, white blood cell, SGPT, and SGOT were evaluated.

RESULTS: Treatment with formulations showed a decrease in body weight contrast to disease. Equally, a considerable increase in hemoglobin was observed in the formulation treated group over disease grouping. Likewise, there decrease in SGPT and SGOT in formulation compared to disease.

CONCLUSION: The present study revealed a transdermal patch loaded with tamoxifen showed promising antitumor activity.

Keywords:

Anticancer, nanoformulation, tamoxifen, transdermal patch

Introduction

Patients with carcinoma are prescribed with tamoxifen for prophylactic care in pre- and postmenopausal females.[1] It is a triphenylethylene derivative (nonsteroidal) and competes with steroids for steroid receptor positive in carcinoma cells. However, this agent is associated with multiple side effects such as multifocal viscous, fatty infiltration, hepatotoxicity, viscous sphacelus, and blood disorders.[2,3]

Formulation loaded with tamoxifen in the transdermal patch would possess lower hepatotoxicity and hemolytic carcinoma possessing higher patient adherence to treatment. This system plays an important role in promoting pharmacokinetic profile, enhancing efficacy, followed by minimization of toxicity[4] which could be the outcome of minimal particle size possessing them to penetrate biological barriers.[5,6] Nanoparticles can be helpful as controlled release system, applied for cancer medical care and decrease the exploding of multiple side effects. Hence, the present study aimed to investigate the tamoxifen-loaded transdermal patch against breast carcinoma. Formulations were composed of a drug (tamoxifen), polymer (poly (SA:RA), and cryoprotectant (glucose and mannitol) at multiple concentrations. The detail of multiple formulations is summarized in Table 1.

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Table 1: Composition of formulations

Formulation	Drug	Polymer	Cryoprotectant	
	Tamoxifen citrate (mg)	Poly (SA:RA) 50:50 (mg)	Glucose (mg)	Mannitol (mg)
F1	10	190	14	14
F2	20	180	14	14
F3	40	160	14	14
F4	60	140	14	14

Materials and Methods

Evaluation of *in vivo* antitumor property

The study was performed after performing acute toxicity as OECD 423[7] A mixture containing 25 mg of DMBA was dissolved in 1 ml of a vehicle and injected by subcutaneous route. Tumor yield was evaluated after the end of the study. Seven different which composed 12 female albino rats in each group were used. Each group comprised of 12 female albino rats.

i. Group 1: The normal control
ii. Group 2: The cancer group negative control
iii. Group 3: The tamoxifen group positive control (PC)
iv. Group 4: F-1
v. Group 5: F-2
vi. Group 6: F-3
vii. Group 7: F-4.

Test sample treatment

Tamoxifen was injected i. p. for PC. Normal group was injected with vehicle. Similarly, Groups 4, 5, 6, and 7 were treated with multiple formulations, and the study was carried for 30 days. At the end of the study, multiple physical and biochemical parameters were evaluated.

Parameters evaluated

Body weight was measured at the end of the study, and each group was compared to evaluate the role of formulation in tumor treatment. Similarly, hematological parameters such as hemoglobin, white blood cell (WBC), and red blood cell (RBC) counts were also determined in peripheral blood. Further, serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) were also quantified using commercially available kits. Institutional ethics approval was obtained from the institutional ethics committee Ref . No KLECOP/CPCSEA-Reg No 221/Res-13-2010.

Results

Effect on final body weight

In the disease group, there was a considerable increase in body weight compared to a normal group. Similarly, in the PC group, there was a considerable decrease in body weight compared to the cancer group. Similarly, formulation F1 showed a considerable decrease in body weight compared to the cancer group. Likewise, formulation F2 showed a considerable decrease in body weight compared to the cancer group. Further, the F3 group showed a considerable decrease compared to the cancer group. Similarly, the F4 group showed a considerable decrease compared to the cancer group [Figure 1].

Effect on hemoglobin content

The hemoglobin content in the disease group was found to be considerably decreased in the disease group compared to normal control. Similarly, there was a considerable increase in hemoglobin content in the PC compared to the disease group. Similarly, formulations F1, F3, and F4 also showed a considerable increase in hemoglobin content compared to the normal disease group. However, formulation F2 did not affect hemoglobin content compared to the disease group [Figure 2].

Effect on red blood cell count

RBC count was considerably decreased in the disease group compared to a normal group and was increased in the PC compared to the disease group. There was an increase in RBC count in formulation treatments. However, the results were not considerable [Figure 3].

Effect on white blood cell count

There was a considerable decrease in WBC count in the disease group compared to the normal group. Similarly, there was a considerable increase in WBC count in PC compared to the disease group. There was an increase in WBC count in formulation treated groups; however, the results were not considerably different compared to the disease control group [Figure 4].

Effect on tumor weight

The PC group showed a considerable decrease in tumor weight in the PC group compared to the disease group. Likewise, treating with formulations F1 and F2 showed an equal level of a considerable decrease in tumor weight compared to disease control. Likewise, formulation F3 showed a considerable decrease in tumor weight compared to the disease group. However, formulation F4 had no effect in decreasing tumor weight compared to the disease group [Figure 5].

Effect on SGPT

A considerable increase in the SGPT level was found in the disease group compared to normal. However, there was a considerable decrease in the SGPT level in tamoxifen and formulation (F1–F4)-treated groups. Furthermore, there was a decreased level in the SGPT level in formulations compared to the tamoxifen group [Figure 6].

Effect on SGOT level

SGOT level in the disease group was found to be

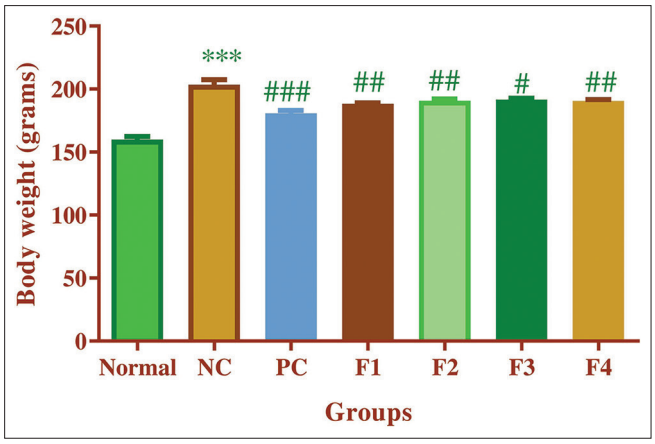

Figure 1: Effect of multiple formulations in body weight ***$P < 0.001$ compared to normal control, #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ compared to cancer group

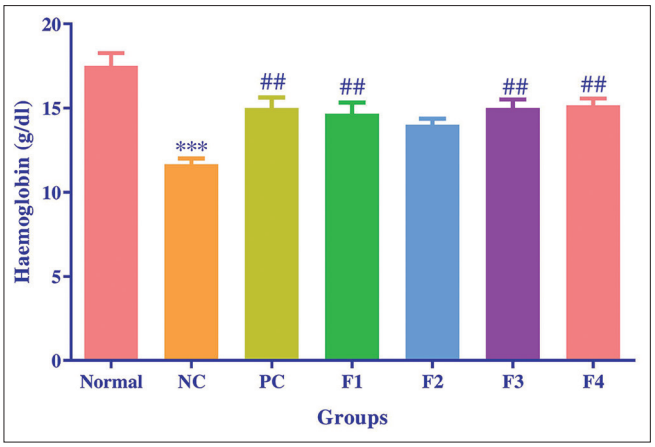

Figure 2: Effect of formulations on hemoglobin content (gm/dl) ***$P < 0.001$ compared to normal group, ##$P < 0.01$ compared to disease control group

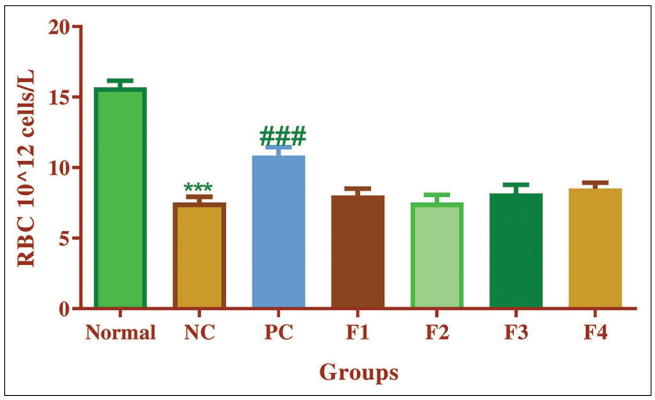

Figure 3: Effect of formulations in red blood cell ***$P < 0.001$ compared to normal, ###$P < 0.001$ compared to disease group

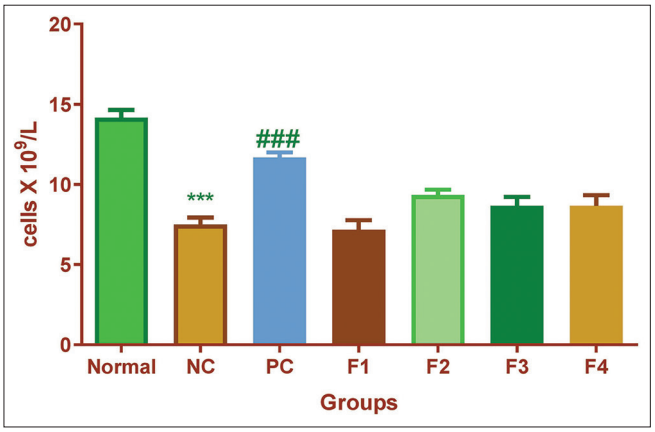

Figure 4: Effect of formulations in white blood cell ***$P < 0.001$ compared to normal, ###$P < 0.001$ compared to disease group

considerably higher compared to normal. However, there was a considerable decrease in the SGOT level in tamoxifen and formulation (F1–F4)-treated groups. Furthermore, there was a decreased level in the SGOT level in formulations compared to the tamoxifen group [Figure 7].

Discussion

In the present study, we investigated four different formulations and were assessed for multiple parameters including body weight, tumor weight, RBC, WBC, and hemoglobin content. All the results were also compared with the gold standard treatment procedure for tamoxifen.

Nanoformulations are well-accepted formulations in the pharmacotherapy of multiple diseases including breast cancer.[8] Further, breast cancer includes a polygenic risk for the development of its pathogenesis.[9] It is more convenient in the pharmacotherapy of such polygenic conditions since the treating is quite easy and the drug release is fast through this approach. The present study also showed a decrease in tumor weight by formulations F1, F2, and F3. Likewise, there was a decrease in body weight in the formulation-treated group. This represents that the formations have the capacity to release the tamoxifen in the targeted site.

Furthermore, cancer is a polygenic condition.[10] The pharmacotherapy of this condition could be complicated, though we target a specific molecule or protein. On the other hand, a single compound can modulate multiple proteins and regulate multiple pathways.[11] Although the tamoxifen-loaded formulation has been studied in the present study, there is always a probability that it could modulate multiple proteins and pathways as previously predicted,[12-15] which needs to be still applied for tamoxifen-loaded formulations.

Previous literature suggests that there is a decrease in hemoglobin in cancer pathogenesis,[16] which has been revealed by the formulation treated in the present study. Further, there was an increase in RBC and WBC, but the results were not considerable compared to the disease group. This reflects that the sympathomimetic relief has not been achieved within the lowered time which could be achieved after long-term treatment and is the scope of future study.

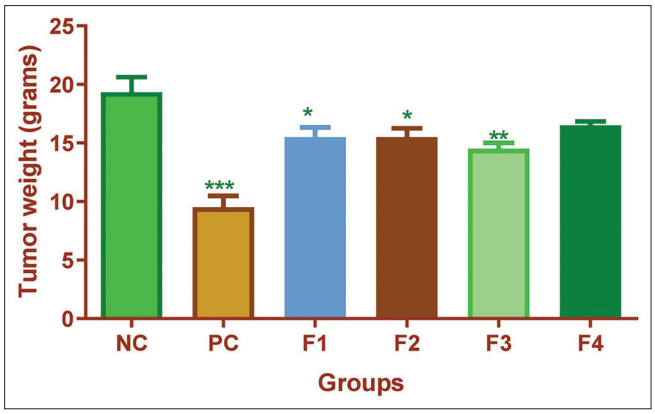

Figure 5: Effect of formulations in tumor weight *P < 0.05, **P < 0.01, ***P < 0.001 compared to disease group

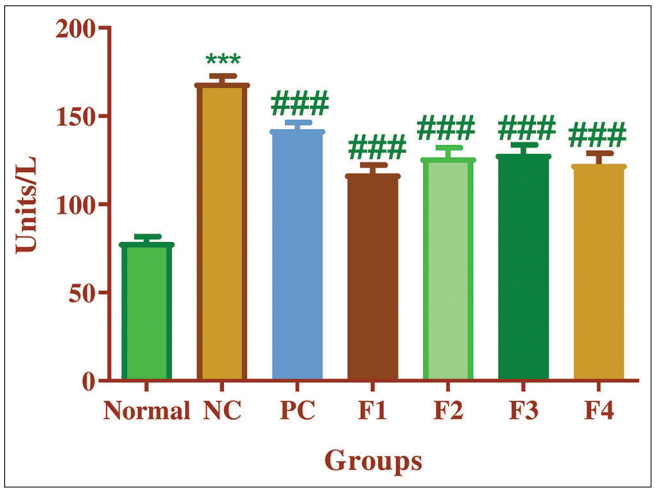

Figure 6: Effect on SGPT levels ***P < 0.001 compared to normal group, ###P < 0.001 compared to disease group

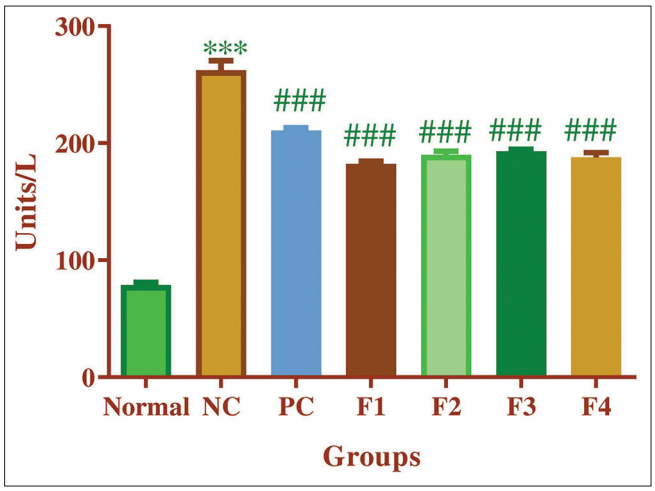

Figure 7: Effect on SGOT levels ***P < 0.001 compared to normal group, ###P < 0.001 compared to disease group

One of the major limitations in the pharmacotherapy of cancer is increased SGOT and SGPT levels reflecting the hepatotoxicity,[17] which needs to be minimized. In our study, there was a decrease in the SGOT and SGPT levels after tamoxifen treatment. Further, our formulation showed a decreased level of SGOT and SGPT compared to the tamoxifen group, which could be the outcome of targeted drug delivery.

Conclusion

The present study demonstrated the anticancer activity of multiple formulations and their effectiveness in multiple biochemical and hematological parameters reflecting the importance of nanoparticles in cancer pharmacotherapy and targeted drug delivery system.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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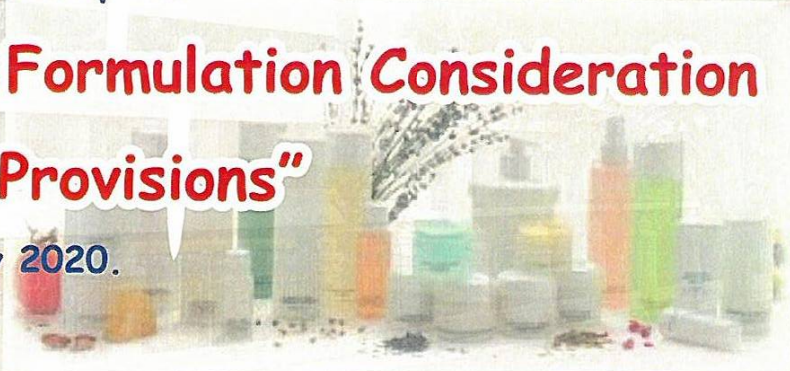

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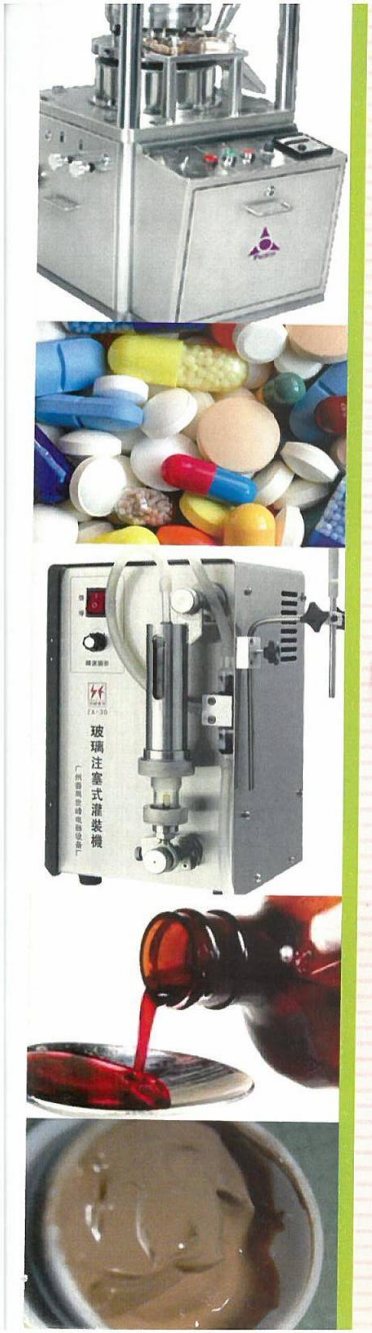

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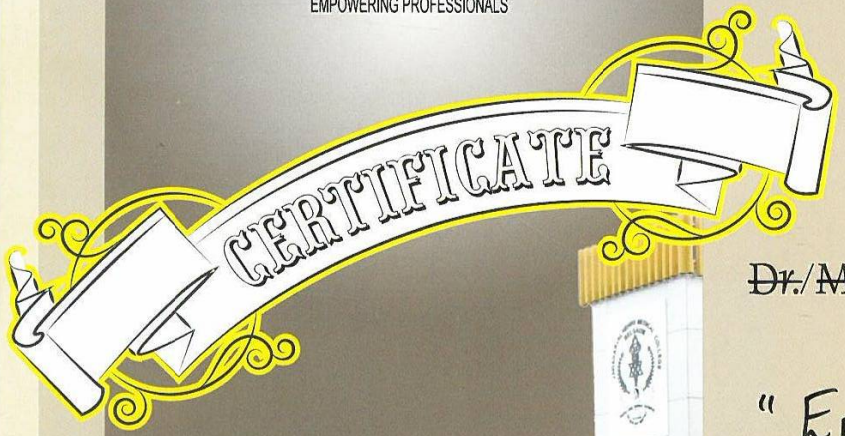

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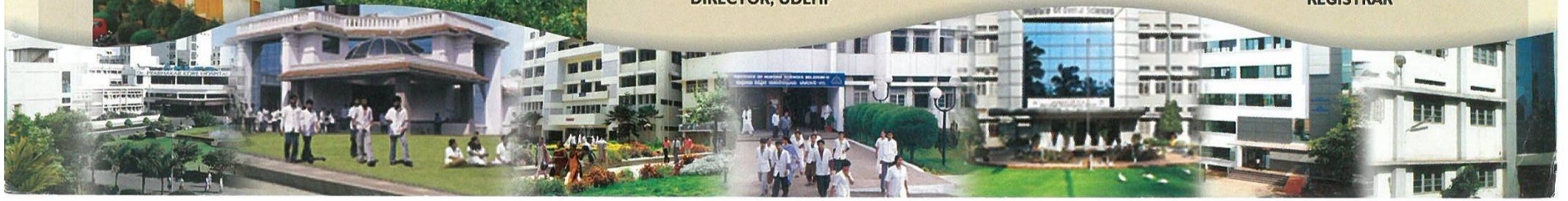

MANIPAL UNIVERSITY

8th NATIONAL CONFERENCE

On

P4
Pharmacovigilance
Pharmacoeconomics
Pharmaceutical Care
Patient Related Outcomes

KLE UNIVERSITY
EMPOWERING PROFESSIONALS

Organised by ACPI-KSPOR Belagavi Local Chapter and Manipal University
in association with KLEU's College of Pharmacy, Belagavi

CERTIFICATE

This is to certify that

Mrs. ANJANA ADHYAPAK

has participated as a Delegate / Presented a paper (oral/ poster) in the 8th National Conference on P4 at KLEU's College of Pharmacy, Balagavi between 24 and 25 March, 2017

Prof N Udupa	Prof A N Nagappa	Prof B M Patil	Prof M S Ganachari
President KSPOR	President ACPI	Co-ordinator	LOC, Convener

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Placed in Category 'A' by MHRD, Govt. of India Accredited 'A' Grade by NAAC

Nehru Nagar, Belgaum - 590 010, Karnataka State, India

Ph. : 0831-2444444 FAX : 0831-2493777 **Web**: http://www.kleuniversity.edu.in **E-mail**: info@kleuniversity.edu.in

UNIVERSITY DEPARTMENT OF EDUCATION FOR HEALTH PROFESSIONALS

CERTIFICATE

This is to certify that

~~Dr.~~/~~Mr.~~/Mrs. Anjana Adhyapak

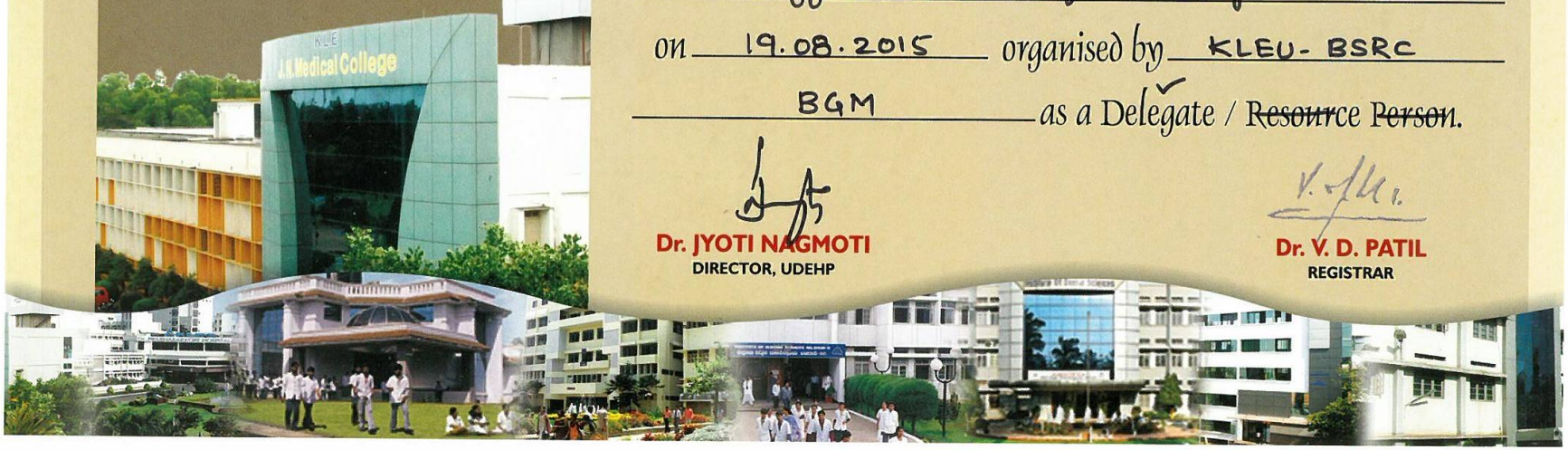
has participated in the Workshop entitled

Effective Scientific writing skills

on 19.08.2015 organised by KLEU-BSRC BGM as a Delegate / ~~Resource Person~~.

Dr. JYOTI NAGMOTI
DIRECTOR, UDEHP

Dr. V. D. PATIL
REGISTRAR



UGC Networking Resource Centre for Training in Pharmaceutical Sciences

University Institute of Pharmaceutical Sciences

(UGC Centre for Advanced Studies)

Panjab University, Chandigarh 160 014

UGC Autumn Training Programme

October 8-13, 2012

Certificate

This certificate is awarded to MRS ANJANA ADHYAPAK

for active participation and successful completion of a ***1-week*** *UGC Networking Autumn School under* ***Module B-2*** *on* ***"Nanotechnology in Drug Delivery : Promises and Concerns".***

Indu P. Kaur
Professor Indu Pal Kaur
Course Coordinator

Kanwaljit Chopra
Professor Kanwaljit Chopra
Course Coordinator

Professor V. R. Sinha
Chairperson, UIPS & Programme Coordinator
UGC Networking Resource Centre

CERTIFICATE OF ATTENDANCE

This is to certify

Dr/Prof/Mr/Mrs ANJANA ADHYAPAK

attended the International Workshop on Bioavailability and Bioequivalence

8-9 August 2012, conducted by

KLE University , India

in association with

Louvain Drug Research Institute,

Catholic University of Louvain, Belgium

UCL Université catholique de Louvain

KLE UNIVERSITY EMPOWERING PROFESSIONALS

Chair: ***Prof. Dr Roger K. Verbeeck***
Louvain Drug Research Institute

Coordinator: ***Prof. Dr Srinivas Patnala***
Basic Sciences Research labs - KLE University

Prof. Dr P. F. Kotur
Registrar - KLE University

AICTE

AICTE Sponsored

Two Weeks Staff Development Programme

On

"Recent Advances in Drug Delivery & Technology"

(1st to 14th July, 2011)

GANPAT UNIVERSITY

ग विद्यया समाजोत्कर्षः ग

Organized By

S. K. Patel College of Pharmaceutical Education and Research

Ganpat University, Kherva, Mehsana (N. Gujarat), 382711.

Certificate

This is to certify that Prof./Dr./Mr./Ms. Anjana Adhyapak

has participated / ~~delivered a lecture on~~ _____

_____ as a Delegate / ~~Resource person~~ /

~~Organizing committee member~~ in the AICTE sponsored two weeks staff development programme, entitled "**Recent Advances in Drug Delivery & Technology**", held from 1st to 14th July, 2011.

Dr. Rakesh P. Patel
Programme Co-ordinator

Dr. N. J. Patel
Principal

Dr. B. G. Prajapati
Asst. co-ordinator

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Belgaum - 590 010, Karnataka State, India, Website : http://www.kleuniversity.edu.in

UNIVERSITY DEPARTMENT OF EDUCATION FOR HEALTH PROFESSIONALS

This is to certify that

~~Dr./Mr.~~/Mrs. Anjana. Adhyapak

has participated in the Workshop / ~~CME~~ entitled

"Significance of particle size & their characterization in formulation development"

on 27th FEBRUARY, 2010 organised by Dept. of pharmaceutics

~~K.L.E. College of pharmacy, Hubli~~ as a Delegate / ~~Resource~~ person.

Dr. JYOTI NAGMOTI
DIRECTOR, UDEHP

Dr. P. F. KOTUR
REGISTRAR

KLE UNIVERSITY
EMPOWERING PROFESSIONALS

Certificate

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Belgaum - 590 010, Karnataka State, India, Website : http://www.kleuniversity.edu.in

UNIVERSITY DEPARTMENT OF EDUCATION FOR HEALTH PROFESSIONALS

This is to certify that

Dr./~~Mr.~~/Mrs. Anjana Adhyapak

has participated in the Workshop / CME entitled

Qualitative Research and Bio-Ethics

on 10-11/5/2010 organised by UDEHP

as a Delegate / ~~Resource person.~~

Dr. JYOTI NAGMOTI
DIRECTOR, UDEHP

Dr. P. F. KOTUR
REGISTRAR