

Novel carrier systems for targeted drug delivery in the treatment of Arthritis

PhD Synopsis

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By

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Abstract-

Arthritis is a major cause of disability and morbidity, particularly in older individuals. The symptoms and signs of arthritis and related conditions include pain, stiffness, swelling, muscle weakness, and limitation of movement of the joints. The objective of the present work was to develop a dosage form for localized action of drugs in the affected arthritis sites for prolonged period and to avoid side effects in non-target organs. To achieve this, the drug loaded vesicular systems of Nonsteroidal anti inflammatory drug Aceclofenac and Indomethacin were prepared and further incorporated in transdermal gel formulation. The formulations were prepared by experimental design using screened factors and their levels and by optimized process parameters.

The size of both drug carriers was found to be uniform and in the nanometric range with zeta potential values indicating stability of drug carrier suspension. The formulation of liposome was optimized based on drug entrapment efficiency and in-vitro skin permeation. The ex-vivo studies through rat skin showed that better permeation of Aceclofenac occurs from optimized liposomal and transferosomal gel as compared to plain drug gel. Both the gel formulation showed sustained release of drug for more than 6 hrs. The C_{max} for liposomal gel (7.002 $\mu\text{g/ml}$) and transferosomal gel (8.879 $\mu\text{g/ml}$) was found to be higher than that for the plain drug gel (5.998 $\mu\text{g/ml}$) however the T_{max} was 6 hrs for all the three. The bioavailability as indicated by AUC was found to be highest for transferosomal gel (67.14 $\mu\text{g.hr/ml}$) followed by liposomal gel (53.87 $\mu\text{g.hr/ml}$) as compared to plain gel of Aceclofenac (42.92 $\mu\text{g.hr/ml}$). The radio labeling experiment demonstrated that transdermal permeation of drug is rapid followed by depot formation in skin indicating sustained release of drug.

The drug Indomethacin, which is of the same therapeutic class, also shows better skin permeation, sustained release characteristics, Anti-inflammatory activity and Analgesic activity than its plain drug gel when formulated as transferosomal gel. The transferosomal dispersion and gel of both the drugs Aceclofenac and Indomethacin were also found to be stable for 6 months when kept at accelerated stability conditions.

The pH & the rheological properties of transdermal gel formulation of both the drugs indicated potential to withstand stress conditions of handling and packaging & compatibility with the skin.

The prepared formulation of drug carriers incorporated in gel can be a novel approach for treatment of Arthritis through topical route through which the drug can permeate through skin and also show sustained release characteristics. The localized action of drug through novel drug carriers containing gel

for prolonged period at the site of pain can provide more relief to patients as well as can reduce the side effects of drug associated with conventional oral route such as gastro-intestinal disorders in particular dyspepsia, abdominal pain, nausea and diarrhea.

Brief description on the state of the art of the research topic:

Nonsteroidal anti-inflammatory drugs (NSAIDs) that act by inhibiting cyclooxygenase and the formation of prostaglandins, are known to cause GI toxicity, leading to peptic ulcers, unwanted antiplatelet effects (nonselective inhibitors of cyclooxygenase), cardiotoxicity, renal toxicity and anaphylactic reactions in selected patients.^{2,3} The currently available oral dosage forms of NSAIDs like tablet and capsules etc do not circumvent above mentioned adverse effects of these drugs. The intravenous administration of these drugs leads to distribution throughout the whole body and rapid clearance, thus a high and frequent dosing is necessary to achieve an effective concentration of drug at inflamed target sites.⁴ Moreover, the activities of drug in many different tissues increase the risk of adverse effects in patients. Thus, we need to develop a drug delivery system in particular carriers, with enhanced localization to the target site and sustained drug release. Topical delivery of drugs can be a suitable option as it has many advantages such as avoidance of hepatic first-pass metabolism., improved patient compliance and ease of access, provide a means to quickly terminate dosing sustained therapeutic drug levels, possible self administration, non-invasive (no needles or injections needed), avoids food related Interaction, reduction of doses as compared to oral dosage forms and intravenous therapy. Anti-inflammatory and analgesics drugs used for the treatment of arthritis, are preferably administered by transdermal route.⁵

Topical route allows drug to diffuse out of its vehicle onto the surface tissues of skin. In fact, ease of applicability makes this route more comfortable for the patient which results in better patient compliance. There is no significant obstacle to penetration, once the drug permeates through stratum corneum of epidermis of skin.⁶ Topical NSAIDs have been reported to have reduced incidences of systemic side effects like gastric bleeding and peptic ulcer ⁷.The feasibility of topical route over parenteral route in treatment of RA has been evaluated in several studies. Topical Methotrexate gel prepared with Poloxamer 407 polymer have been observed to produce sustained and higher drug levels in muscle tissues beneath the site of administration⁸ . For the purpose to enhance the skin permeability of Ketoprofen, topical Oleo Hydrogel Ketoprofen is prepared with enhanced skin permeability.⁹ Most of the current therapies for arthritis do not achieve target specificity and to reach effective drug concentrations in affected joint tissues, high dose of drug must be administered, which may lead to

significant adverse systemic side effects.¹⁰ Currently anti-inflammatory drugs are mainly delivered by transdermal iontophoresis.^{11,12} New drug application (NDA) of Alza corporation for iontophoretic fentanyl containing transdermal analgesic have been approved by US FDA.¹² Iontophoresis is a special method of applying drug to and pushing it through the skin to reach the blood vessels and surrounding deeper tissues by electric transmission. A significant amount of Piroxicam was retained in the skin after transdermal iontophoresis from Piroxicam.¹³ In order to increase penetration and a prolonged release, lipid nano/submicron emulsion can be used as a vehicle for topical delivery of drugs.¹⁴

The most prominent advantage of drug carriers such as Liposomes, Transferosomes etc. over conventional drug delivery systems is the option to improve selective delivery of drugs to the site of action.

Liposomes:-This drug delivery system include easy encapsulation of hydrophilic drugs into their core compartment and hydrophobic drugs into their lipid bilayer, excellent biocompatibility, ability to penetrate effectively into cell membranes, delivery of drugs into the cell compartments and diversity in modifying the surface properties by altering or introducing new components into the lipid bilayer.^{14,15} For transdermal absorption of NSAIDs at the localized site of action, liposomes may be a useful tool.^{16,17} A single topical application of Diclofenac liposomal suspension has shown concentrations of Diclofenac in transudate within 6 hours and significantly attenuated carrageenan-induced local production of Prostaglandin. Results of this study suggest that DLS is readily absorbed transdermally and may be efficacious for reducing subcutaneous inflammation¹⁸ . In another study, Diclofenac sodium loaded liposomes were prepared by thin film hydration technique using soya lecithin, cholesterol followed by sonication and then incorporation into 1% carbopol gel. The particle size, polydispersity index and zeta potential of liposomes were found to be 230 nm, 0.247 and -41 respectively and the entrapment efficiency was found to be 62%. The cumulative amount of drug permeated in 24 hour from the liposomal gel formulation was found to be 1176.7 $\mu\text{g}/\text{cm}^2$ ¹⁹ .From the drug entrapment efficiency study of ketoprofen liposomal gel formulation, maximum drug encapsulation of 97.51% was observed in formulation, in which lipid and cholesterol were used in ratio of 1:2. Therefore, it can be interpreted that, in liposome preparation, cholesterol was found to act as fluidity buffer and provided stability and rigidity to liposome. The marketed gel of Ketoprofen released approximately 92% of drug within 24 hour, whereas the liposomal formulations showed 87% drug release in 24 hour. Liposomal formulations showed sustained drug release compared to normal gel, also an increase in release rate was observed after 12 hour²⁰ . The In-vivo findings revealed that the anti-inflammatory effect was more prolonged

when Indomethacin was delivered from a liposomal gel formulation rather than from a gel formulation without liposomes. In particular, the Indomethacin-loaded gel formulation LUV-A showed a sustained release, possibly related to an interaction between LUV lipids and stratum corneum lipid structure.²¹ The encapsulation of Piroxicam produced an increase of topical anti-inflammatory effect. In addition it was also observed that, anti-inflammatory effect can be achieved using lower drug concentrations when formulated as liposomal gel.^{22,23,24} Liposomal gel of Dex-Ibuprofen prepared by rotary evaporation followed by sonication using phosphatidylcholine and cholesterol with particle size of 5.40 µm and entrapment efficiency of 61% showed sustained drug delivery for 12 hours²⁵. In a study, liposome preparation consisted of a combination of a methyl prednisolone, phospholipid and cholesterol. In the work, methyl prednisolone derivative encapsulated in a liposome was essentially retained in said liposome for 6 months; liposomes were uniformly sized to a selected size range between 70-100 nm. (AU2005/281351A1). In one study, Charge-inducing lipids, such as Phosphatidylglycerol were incorporated into the liposome bilayer to decrease vesicle-vesicle fusion and to increase interaction with cells, while cholesterol and sphingomyelin were included in formulations in order to decrease permeability and leakage of encapsulated drugs. (US 2008/0003276A1).

Transferosomes: These are ultra deformable vesicle, elastic in nature which can squeeze itself through a pore which is many times smaller (1/10th) than its size owing to its elasticity. These are applied in a non-occluded method to the skin and have been shown to permeate through the stratum corneum lipid lamellar regions as a result of the hydration or osmotic force in the skin. Transferosomes are made up of a phospholipid component along with a surfactant mixture (Sodium Cholate, Spans and Tweens)^{26, 27}

The ratio and total amount of surfactants which acts as edge activator controls the flexibility of the vesicle. The unique property of this type of drug carrier system lies in the fact that it can accommodate hydrophilic, lipophilic as well as amphiphilic drugs. These ultra deformable drug carriers trespass the intact skin spontaneously, probably under the influence of the naturally occurring, transcutaneous hydration gradient. The 'moisture seeking' tendency (hydrotaxis) of transferosomes permits the carrier to bring more than 50% of the epicutaneously administered drug across the skin barrier.²⁷ In a study on Ibuprofen transferosomes the best formulations were observed with the use of Span 80 and Tween 80 where vesicle size was found to be 962 nm and 2250 nm respectively, and zeta potential (negatively charged) for Span 80 and Tween 80 was found to be -16.1 and -17.5 respectively. The %EE of Ibuprofen in the vesicles was 47.8 and the elasticity of both increases with increase in surfactant concentration and were found to be 34.4 and 26.5, in vitro skin permeation studies were carried by

human cadaver skin using Franz diffusion cell, and drug release and flux was found to be 2.5824 and 1.9672 $\mu\text{g}/\text{cm}^2/\text{hr}$ respectively after 24 hrs²⁶ .

It is evident from one of the studies, where transferosomes of Diclofenac sodium were prepared using soya Phosphatidylcholine by suspending lipids in aqueous phase containing drug and thereafter sonication, size achieved was in the range of 100-200 nm. Diclofenac association with ultra deformable carriers have a longer effect and reach 10-times higher concentrations in the tissues under the skin in comparison with the drug from a commercial hydrogel. In rats, a single epicutaneous application of 2 mg of Diclofenac per kg bodyweight in highly deformable carriers produced at least 4 times higher drug concentration in the treated muscles than a drug-loaded hydrogel²⁸ .

Ketoprofen transferosome formulation has been granted marketing approval by the Swiss regulatory agency (Swiss Medic) in 2007; the product is under the trademark Diractin of IDEA AG (Munich) U.S. Pat. No. 6,165,500 (Idea AG) describes an adaptable bilayer vesicle comprising a phospholipid combined with edge activators which include alcohols and surfactants such as cholates or polyoxyethylene ethers. These ultra deformable particles are termed Transferosomes and are suitable for delivering hydrophilic and lipophilic agents through the hydrophilic pores in the skin. Transferosomes ranging from 200 to 600 nm in size physically appear as milky emulsions. For dermal delivery applications, a particle sizes in the range of 100 to 200 nm is preferred. A pharmaceutical composition was prepared which comprises of components such as bilayer forming lipid, an amphipathic analgesic drug and a surfactant capable of self-aggregation in the suspension medium. Surfactants selected were preferably nonionic such as polyethylene glycol-sorbitan-long fatty chain ester, a polyethylene glycol-long fatty chain ester or ether and a polyhydroxyethylene-long fatty chain ester (EP 1551370 B1).

Definition of the problem:

Arthritis is a major cause of disability, particularly in older individuals. More than 30 percent of females have some degree of osteoarthritis by age 65. The symptoms and signs of arthritis and related conditions include pain, stiffness, swelling, muscle weakness, and limitation of movement of the joints. Nonsteroidal anti-inflammatory drugs and corticosteroids are most widely used and effective drugs for treatment of arthritis¹. Nonsteroidal anti-inflammatory drugs (NSAIDs) that act by inhibiting cyclooxygenase and the formation of prostaglandins, are known to cause GI toxicity, leading to peptic ulcers, unwanted antiplatelet effects (nonselective inhibitors of cyclooxygenase), cardiotoxicity, renal toxicity and anaphylactic reactions in selected patients. The currently available oral dosage forms of NSAIDs like tablet and capsules etc are more likely to produce above mentioned adverse effects of these

drugs. The intravenous administration of these drugs leads to distribution throughout the whole body and rapid clearance, thus a high and frequent dosing is necessary to achieve an effective concentration of drug at inflamed target sites. Moreover, the activities of drug in many different tissues increase the risk of adverse effects in patients. Topical delivery of drugs can be a suitable option and is associated with advantages such as avoidance of hepatic first-pass metabolism, improved patient compliance and ease of access, provides a means to quickly terminate dosing, sustained therapeutic drug levels, possible self administration, non-invasive (no needles or injections needed), avoids food related interaction, reduction of doses as compared to oral dosage forms and intravenous therapy and most important is avoidance of GI adverse effects.

The topical drug delivery also suffers from some shortcomings such as poor permeability through skin, unpredictable drug release and skin irritation. These shortcomings can be overcome if we develop a drug delivery system in particular carriers, with enhanced localization to the target site and sustained drug release.

Objective and scope of work:

- To achieve an effective treatment of arthritis by NSAIDs localization at inflamed target sites with a prolonged period of activity and minimization of side effects.
- To establish improvement in Bioavailability, Anti-inflammatory activity and Analgesic activity as compared to existing formulations through animal studies.
- To enhance the efficacy of transdermal formulations by maximum skin permeation and sustained release characteristics of drug through Nanoscaled drug carriers based transdermal drug delivery.

To achieve the objectives, Nanoscaled drug carriers such as liposomes and transferosomes were formulated and then incorporated in transdermal gel formulation to provide localized action of drugs to the affected arthritis tissues for prolonged period and to avoid side effects in non-target organs. The desired size, drug entrapment and permeation characteristics was achieved by optimization of carrier composition by experimental design.

The present study is a novel approach in order to achieve a targeted drug delivery with minimization of side effects in arthritis, a very prevalent disease among elderly which leads to disability. The treatment of arthritis by conventional methods produces many side effects in long run due to which treatment cannot be continued for a long period. The present study is a unique attempt of efficacious treatment in arthritis tissue in a specific manner so that other tissues remains unaffected, thus cardiotoxicity and renal toxicity can be avoided. The research work will be added among new innovations in the field of Novel

drug delivery system to expand the horizons of Pharmaceutical research and to cater the needs of effective and safe treatment.

Original contribution by the thesis:

The present study is a unique attempt of transdermal drug targeting into the arthritis affected joints using small biomolecules so that drug acts on diseased tissue in a specific manner and the other tissues remains unaffected, thus cardiotoxicity and renal toxicity can be avoided.

In the present study, drug carrier based transdermal drug delivery system has been developed based on experimental design using optimized process parameters and screened excipients. The developed formulations were capable of overcoming the shortcomings of poor skin permeability and unpredictable drug release as associated with conventional transdermal drug delivery systems.

The prepared Liposomal and Transferosomal gel formulations were found to be efficacious for treatment of arthritis through transdermal route, as it permeated through skin and also exhibited sustained release characteristics. The localized action for prolonged period at the site of pain can provide more relief to patients as well as can reduce the side effects of drug associated with conventional oral route such as gastro-intestinal disorders in particular dyspepsia, abdominal pain, nausea and diarrhea. The prepared formulation showed better efficacy as compared to marketed transdermal formulation.

Methodology of research, results/comparisons:

The NSAIDs selected for studies were Aceclofenac and Indomethacin for the preparation of drug carriers based transdermal gel.

Process Optimization: - The process of rotary vacuum evaporation and probe sonication was varied by varying the process variables to investigate their effect of characteristics of carrier systems.

Rotary Vacuum Evaporation: - The process variables, temperature, RPM and time of operation were varied based on 3 factor, 3 level general factorial design and based on the quality of film produced, the process was optimized.

Probe Sonication- The probe sonicator was operated using 13 mm probe at an amplitude of 60% .The effect of sonication cycles on the transparency of vesicular dispersion and average size of vesicles was studied. %Transmittance was measured using U.V spectrophotometer and size was measured using trinocular microscope.

The process parameters selected for further studies after optimization were-

For Rotary Vacuum Evaporation- 50°C temperature, rotation at 90 rpm and time 20 minutes.

For Probe Sonication- 5 sonication cycles each of 2 minutes at amplitude of 60% for Liposomes and 2 sonication cycles each of 2 minutes at amplitude of 60% for Transferosomes.

Screening of excipients by 2² full factorial designs:

The Phospholipids and surfactants were screened based 2 factors, 2 levels factorial design on the basis of outcomes of size and entrapment efficiency. The screened excipients were checked for compatibility with drug by DSC & FTIR studies.

Formulation of liposomes and transferosomes batches based on experimental design using statistical software Minitab 16:-

For Liposomes-

Factors-

Quantity of Phospholipids

Quantity of cholesterol

Responses-

Size

Entrapment efficiency

Permeation Flux

For Transferosomes-

Factors-

Quantity of Phospholipids

Quantity of cholesterol

Quantity of surfactant

Response-

Size

Entrapment efficiency

Permeation Flux

Process of synthesis of Liposomes as drug carriers by film hydration method:

1. Phospholipid 50-100 mg (0.067 mmol to 0.133 mmol), Cholesterol 25-50 mg (0.064 to 0.129 mmol) and Drug were solubilized in 10 ml chloroform-methanol (9:1) mixture. Drug: Lipid molar ratio=2.12 to 4.20 mmol). Hydration volume-10 ml.
2. Thin film formation was done by vacuum rotary evaporator at temperature of 50°C, 90 rpm for 20 minutes.
3. Thin film was evaporated under vacuum for removal of organic solvent and then kept in desiccator overnight.
4. Dried thin film was hydrated by 10 ml phosphate buffer saline pH 7.4 to prepare drug loaded multilamellar vesicles.

5. The vesicular dispersion was sonicated by probe sonicator (Vibra cell -Sonics) for 5 cycles of 2 minutes at pulse of 2 sec using standard 13 mm probe at amplitude of 60% to obtain small unilamellar vesicles from large multilamellar vesicles.

Process of Synthesis of trial batch of Transferosomes as Drug carriers by Film Hydration

Method:-

1. Phospholipid (0.067mmol-0.133mmol), surfactant (0.06-0.12 mmol) & Cholesterol (0.064-0.129 mmol) were solubilized in 10 ml chloroform-methanol (9:1) mixture. Drug: Lipid molar ratio=2.12 to 4.20 mmol. Hydration volume-10 ml
2. Thin film formation was done by vacuum rotary evaporator at temperature of 50°C, 90 rpm for 20 minutes.
3. Thin film was evaporated under vacuum for removal of even trace amount of organic solvent and then kept in desiccator overnight.
4. Dried thin film was hydrated by 10 ml phosphate buffer saline pH 7.4 containing 25-50 mg (0.06-0.12 mmol) surfactant to prepare drug loaded multilamellar vesicles.
5. The vesicular dispersion was sonicated by probe sonicator (Vibra cell -Sonics) for 2 cycles of 2 minutes at pulse of 2 sec using standard 13 mm probe at amplitude of 60% to obtain small unilamellar vesicles from large multilamellar vesicles.
6. The vesicles were observed under ZEISS trinocular Microscope at 100X magnification.

Formulation of drug carriers incorporated gel:

- A gel was prepared by dispersing Carbopol 934 in distilled water as a base for incorporation of drug loaded carriers for transdermal delivery
- 1 gm Carbopol 934 was dispersed in 88 ml water with continuous stirring.
- Then Propylene glycol-10 ml was added and the pH was checked with the help of pH meter.
- The pH was adjusted to 6.5 (near to pH of skin) with Triethanolamine and stirred till a transparent gel was obtained.
- Transferosomal and Liposomal suspension in PBS pH 7.4 free from untrapped drug was incorporated in gel base by slow gentle mixing. Gel formulation containing drug carriers was inspected for clarity, color, homogeneity, presence of particles and fibers.

Evaluation of carrier systems: Evaluation was carried out by following process-

a. Particle shape determination of drug carriers:

Shape was determined using trinocular microscope (Carl Zeiss) at BIP, Baroda

b. Particle size, Zeta potential and Polydispersity index determination of drug carriers:

Drug carriers particle size was determined using trinocular microscope (Carl Zeiss) and Malvern sizer (Malvern Instruments Ltd., UK, MAL100206)

c. Drug entrapment efficiency:

Drug loaded vesicles in phosphate buffer saline pH 7.4, were centrifuged at 15000 rpm for 15 minutes at 4° C on Remi Lab Centrifuge. The supernatant was collected and again centrifuged at 15000 rpm for 15 minutes and the drug content of supernatant was analyzed after 1/100 dilution to determine unentrapped drug content. The residue at bottom was soaked in methanol for 30 minutes and then agitated, and analyzed for the entrapped drug content.

Evaluation of drug loaded liposomes and transferosomes in transdermal gel:

1. Refractive index:

Refractive index was measured using Abbs refractrometer, dolphin at BIP, Baroda

2. Determination of pH:

The pH of gel was checked by using a digital pH meter at room temperature. Initially, the pH meter was calibrated using standard buffers of pH 7 and then 10 gm of gel was weighed and dispersed in 25 ml of distilled water and then electrode of pH meter was dipped in the dispersion and the pH was noted.

3. Spreadability:

Gel (0.5 g) was placed within a circle of 1 cm diameter (premarked glass slide) over which a second glass slide was placed. A weight of 2 g was allowed to rest on the upper glass slide for 1 min. The increase in the diameter due to spreading of the gel was noted. Spreadability was then calculated by using the formula-

$S = M.L / T$, Where, S = Spreadability, M = weight attached to upper slide, L = length of spread, T = time taken.

4. Gel strength:

The apparatus for measuring gel strength consist of plunger having pan to hold weights at one end and the other end was immersed into gel. Formulated gels were placed in glass bottle where

marking was done 1cm below the filling mark. The weight required for the plunger to sink to a depth of 1 cm through the prepared gel was measured for each formulation.

5. Extrudability: Prepared gel was filled in tube and sealed. Three markings were made at distance of 1.5 cm from bottom of tube. The tube was pressed at marking using Pfizer hardness tester with 1 kg/cm², the weight of gel in continuous ribbon expelled is measured for each formulation.

6. Rheological studies:

The viscosity of gels was determined by using Brookfield helipath (LVDV 2) viscometer. The gel was placed in the sample holder and no- 96 spindle was lowered perpendicularly into the sample. The readings of viscosity of the formulation were measured at different rpm.

7. Release studies:

In-Vitro and *Ex-Vivo* diffusion studies (IAEC Approval no. PhD/13-14/23 dated. 14th December 2013): *In-Vitro* diffusion studies was performed for prepared drug carrier based transdermal gel of Aceclofenac and Indomethacin using Franz diffusion cell and dialysis membrane (Himedia) having pore size of 2.4 nm followed by excised skin samples. Then, 0.2 g of gel was applied on the skin in donor chamber. The receptor chamber was filled with 20 ml of Phosphate buffer saline pH 7.4 as diffusion media in receptor compartment.

The cumulative amount of drug released across the Rat skin was determined as a function of time.

8. Permeation flux: The permeation flux for experimental batches of liposomal gel, transferosomal gel and plain drug gel were determined.

Permeation flux is the slope of percentage drug release v/s time. It is expressed as $\mu\text{gcm}^{-2}/\text{hr}^{-1}$

9. Plasma profile of drug administered through drug carrier incorporated transdermal gels:

Approval to carry out pharmacokinetic studies was obtained from the Institutional Animal Ethics Committee Approval no. AEP. PhD/13-14/23 .These studies were performed on optimized Liposomal gel (M3), Transferosomal gel (F3) and Plain gel. Male Wistar rats were housed under standard laboratory conditions (temperature $25 \pm 2^\circ\text{C}$ and relative humidity of $55 \pm 5\%$). The rats were kept in cages (6/cage) and fed with standard laboratory diet. About 15 cm² of skin was shaved on the abdominal side of rats in each group. They were fasted for the period of 12 h for observations of any unwanted effects. The rats were divided into 3 groups, each containing 3 rats. Group I received M3 transdermally, group II received F3 transdermally and group III received plain gel. The rats were anaesthetized using light ether anesthesia and blood samples

(0.5 ml) were withdrawn from the tail vein of rat at 0 (pre-dose), 30 minutes, 1, 2, 3, 4,6 and 8 h and kept in micro centrifuge tubes in which 6 mg of EDTA was added as an anticoagulant. The blood collected was mixed with the EDTA properly and centrifuged at 5000 rpm for 25 min for separation of plasma. The separated plasma was stored at -21°C until drug analysis was carried out using high performance liquid chromatographic (HPLC) method.

10. Radioactive tagging experiment for skin permeation studies:

- In vitro saline stability of radiolabelled compound was determined by instant thin layer chromatography. The radiolabeling of compound was performed as per standard protocol. Symbia 2T dual Head SPECT-CT machine (Siemens) was used for scintigraphy.
- 25 cm² round skin area of rabbit was shaved and cleaned with normal saline prior to application of the formulations.
- Application area of 2 cm² was marked by a marker pen and transferosomal gel was applied. Scintigraphic images of pre-wash area and post-wash area were taken at the end of 1, 2 and 3 hour after the gel application on individual rabbits.

11. Anti inflammatory activity:

The anti inflammatory activity was carried out by carrageenan induced paw oedema method to compare the activity of Aceclofenac Liposomal gel, transferosomal gel and Hifenac gel using Plethysmometer. The anti-inflammatory activity was also measured for Indomethacin transferosomal gel. After 30 minutes of topical application of formulations on the right hind paw of rats, 0.1 ml of 1% w/v carrageenan (in 0.9% saline solution) was injected in the subplantar region of right hind paw of rats. The initial paw volume just after injection and subsequent readings up to 6 hours and then at 24 hrs were measured.

12. Analgesic activity:

The time of latency was determined as the time period between the zero point, when the animal is placed on the hot plate surface, and the time when the animal jumps off to avoid thermal pain.

Analgesic activity was determined for liposomal gel, transferosomal gel and marketed gel of Aceclofenac. The analgesic activity was also performed for transferosomal gel of Indomethacin.

Results and discussion:

The drug loaded vesicular systems of Non steroidal anti inflammatory drug Aceclofenac and Indomethacin were prepared and further incorporated in transdermal gel formulation. The formulations

were prepared by experimental design using screened factors and their levels and by optimized process parameters.

The excipients were found to be compatible with the drug based on the results of drug excipient compatibility studies. The transferosomes and liposomes batches were prepared based on experimental design using Minitab software 16. The process of rotary vacuum evaporation at 50° C and 90 rpm, for 20 minutes followed by probe sonication for 5 cycles and 2 cycles each of 2 minutes at amplitude of 60% using 13 mm standard probe produce transparent vesicular dispersion with reproducibility and uniformity in vesicle size for Liposomes and Transferosomes respectively. For liposome preparation 1,2-distearoyl-sn-glycero-3-phospho-ethanolamine sodium salt was found to be better among the phospholipids, and for transferosome preparation, span 60 was screened as most suitable surfactant on the basis of size, drug entrapment efficiency and drug release.

The size of both drug carriers was found to be in the nanometric range with size uniformity and zeta potential values indicate stability of drug carrier suspension. The formulation of liposome was optimized based on drug entrapment efficiency and in vitro drug permeation, having Phospholipid 109.9 mg (0.14 mmol) and cholesterol 27.68 mg (0.071 mmol) with 100 mg (0.28 mmol) Aceclofenac, whereas the optimized formula of transferosomes contained phospholipid 91.41 mg (0.12 mmol), surfactant 25 mg (0.06 mmol) and cholesterol 35.60 mg (0.09 mmol) in formulation with 100 mg (0.28 mmol) Aceclofenac. The % drug entrapment of optimized formulation of liposomes was 51.02 % and that of transferosomes was found to be 57.44 % which were very close to the target responses fixed in response surface methodology. Also, permeation flux of optimized formulation of liposomes and transferosome formulations was very close to the target responses fixed in response surface methodology (26.88 and 28.69 $\mu\text{gcm}^{-2} \text{hr}^{-1}$ respectively) that shows that optimized formulation follow the prediction of possibility to meet the target responses.

The ex-vivo studies through rat skin showed that better permeation of Aceclofenac occurs from optimized liposomal and transferosomal gel as compared to plain drug gel. The transdermal drug permeation was found to be highest for transferosomal gel, whereas drug was found to be slightly retained in skin during permeation from liposomal gel formulation. The reason may be fusion of phospholipids during diffusion through skin. Both the gel formulation showed sustained release of drug for more than 6 hrs.

The C_{max} for liposomal gel (7.002 $\mu\text{g/ml}$) and transferosomal gel (8.879 $\mu\text{g/ml}$) was found to be significantly more than that for the plain drug gel (5.998 $\mu\text{g/ml}$) however the T_{max} was 6 hrs for all the

three. The bioavailability as indicated by AUC was found to be highest for transferosomal gel (67.14 $\mu\text{g}\cdot\text{hr}/\text{ml}$) followed by liposomal gel (53.87 $\mu\text{g}\cdot\text{hr}/\text{ml}$) as compared to plain gel of Aceclofenac (42.92 $\mu\text{g}\cdot\text{hr}/\text{ml}$). Both the transferosomal and liposomal gel showed better anti-inflammatory action than Hifenac (marketed) gel as can be correlated to the pharmacokinetic parameters as measured by rat paw edema method using Plethysmometer. The analgesic activity was measured by Eddy's hot plate method and it was found that transferosomal gel and liposomal has better analgesic action than marketed gel.

The radio labeling experiment was carried out to understand the kinetics and dynamics of drug permeation and release from transferosomal gel and it demonstrated that percutaneous permeation of drug is rapid followed by depot formation in skin, thus the drug can be released in a sustained manner.

As the results of transferosomal gel of Aceclofenac was found to be more promising as compared to liposomal gel, the transferosomal gel was prepared for another drug Indomethacin and tested. It was observed that Transferosomal gel of Aceclofenac and Indomethacin, both were found to have better permeation characteristics as compared to their plain drug gel for arthritis, whereas the permeation flux of Aceclofenac transferosomal gel was also found to be slightly higher than Indomethacin transferosomal gel.

The formulation of Indomethacin Transferosome was optimized based on Size, drug entrapment efficiency and in vitro drug permeation, having Phospholipid 99.03 mg (0.13 mmol), Surfactant-25 mg (0.05 mmol) and cholesterol 49.74 (0.12 mmol) mg in formulation with Drug to Lipid ratio of 0.27:0.13 mmol. The % drug entrapment of optimized formulation was 52.32 % which were very close to the target responses fixed in response surface methodology. Also, permeation flux of optimized transferosomal gel formulations was also very close to the target responses fixed in response surface methodology (26.11 $\mu\text{gcm}^{-2} \text{hr}^{-1}$ respectively) that shows that optimized formulation follow the prediction of possibility to meet the target responses. The ex-vivo studies through rat skin showed that better permeation of Indomethacin from optimized transferosomal gel as compared to plain drug gel.

The gel characteristics and the analgesic activity and anti inflammatory activity of transferosomal gel were also found to be better than plain drug gel for both the drugs Aceclofenac and Indomethacin.

The rheological properties of gel formulations indicated good spreadability, gel strength and extrudability for ease of application and a potential to withstand stress conditions of handling and packaging. The pH of the gel formulation was found to be compatible with the skin and the formulation was also found to be free of any toxic organic solvent.

Optimized transferosomal gel formulations of Aceclofenac and Indomethacin were stable as no statistically significant difference at 95% CI was observed among the size, zeta potential and drug permeation profile for the batches stored at accelerated stability testing conditions for 6 months.

The prepared formulation of drug carriers incorporated in gel can be a novel approach for treatment of Arthritis through topical route through which the drug can permeate through skin and also show sustained release characteristics. The localized action of drug through novel drug carriers containing gel for prolonged period at the site of pain can provide more relief to patients as well as can reduce the side effects of drug associated with conventional oral route such as gastro-intestinal disorders in particular dyspepsia, abdominal pain, nausea and diarrhea.

Achievements with respect to objectives:

- The transferosomal gel of NSAIDs Aceclofenac showed maximum skin permeation, better bioavailability, analgesic action and anti-inflammatory action followed by liposomal gel. Both the transferosomal and liposomal gel of Aceclofenac showed better efficacy than marketed gel.
- The Transferosomal gel of Indomethacin showed better skin permeation, analgesic action and anti-inflammatory action as compared to plain drug gel.
- The transferosomal gel of both NSAIDs Aceclofenac and Indomethacin were found to be stable and compatible with skin and having ease of handling and application.
- The work done has successfully produced Nanocarriers based novel drug delivery system for drugs utilized for long term treatment of chronic joint pain and disability due to Arthritis. The drug delivery system developed can target the inflamed tissue and avoid the accumulation of drugs in other tissues, thereby minimizing the side effects of drugs.

Conclusion:

The prepared formulation of drug carriers incorporated gel is be a novel approach for treatment of Arthritis through Transdermal route through which the drug can permeate through skin and also show sustained release characteristics. The NSAIDs Aceclofenac and Indomethacin in transferosomal gel formulation was found to have better bioavailability, Anti-inflammatory & Analgesic activity as compared to existing formulations of the mentioned drugs. The statistical analysis at 95% CI also proves stability and significant improvement in efficacy of formulation.

The localized action of drug through novel drug carriers based transdermal gel for prolonged period at the site of pain can provide more relief to Arthritis patients and can replace the oral therapy thereby avoiding the gastrointestinal side effects of drug.

Copies of papers published and a list of all publications arising from the thesis:

1. Review paper titled "Nanocarriers: a novel treatment approach for arthritis" *International Journal of Pharmaceutical Sciences & Research*, 2013; Vol. 4(11), pp. 4165-4174.
2. Research Paper titled "Effect of process parameters on design of vesicular systems carrying Aceclofenac" published in *European Journal of Biomedical and Pharmaceutical Sciences*, 2016, Vol. 3(6),pp. 529-532.
3. Research paper titled "Nanocarrier based transdermal formulation of NSAID: optimization of drug loading and analysis of permeation characteristics" accepted and to be published in *Journal of Innovations in Pharmaceuticals and Biological Sciences*, 2016, Vol. 3(3), pp.01-12.

Patent Filed:

Patent application has been filed in Indian Patent office. The application no is 1340/MUM/2014. Title of invention is "Novel topical composition of non steroidal anti-inflammatory drugs"

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