Formulation and Characterization of Flexible Phosphatydilcholine Vesicles for Systemic Delivery of Piroxicam

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Abstract

The purpose of this study was to formulate and optimise the piroxicam loaded transfersomes for the bioavailability enhancement. Transfersomes had been prepared by rotary evaporation method and characterized for various parameters including determination of vesicle size, shape and size distribution, drug entrapment studies and *in vitro* skin permeation and histopathological studies. On the best drug entrapment efficiency and permeation studies through wistar rat skin was found for the formulation of TF PIROX4 and found to be 91.4 and 73.16. Histopathological examination and skin irritation studies had been done which showed 0.26 value for transfersomal gel, which means all the excipients used in this formulation were safe for transdermal drug delivery system. Further pharmacodynamic studies had been performed in wistar rats and compared with conventional oral capsule, conventional piroxicam gel and transfersomal gel. The result showed much higher therapeutic effect of piroxicamtransfersomal formulation as compared to the conventional formlations available in market and other Novel drug delivery systems designed for Piroxicam. Finally the stability studies for piroxicamtransfersomal gel had been carried out.

Key words: Transfersomes,Piroxicam,Phosphotedylcholine, Sodium cholate, Osteoarthritis, transdermal delivery.

INTRODUCTION:

About 74% of drugs are administered orally but they are not giving the desired effect always.To overcome the disadvantages of oral drug administration the transdermal drug administration has been used (1). poor permeability characteristics of the skin is one of the very important points mentioned in researches which lead to aim of increasing dermal and systemic drug delivery in a reproducible and reliable way(2). There are various advantages in dermal drug delivery, as instant, convenience, patient compliance and elimination of the first-pass effect(3) and it also provides the controlled delivery of the drug for extended period of time(4).The greatest problem for transdermal delivery is the barrier property of the stratum corneum (SC)(5,6). Piroxicam is one of the most potent non-steroidal anti-inflammatory drugs which used in treatment of the symptoms of rheumatoid and osteoarthritis, primary dysmenorrhoea, postoperative pain; and act as an analgesic, especially where there is an inflammatory component. It was first developed by Pfizer and Co. Piroxicam entered into medical practise in 1970's.It is an effective analgesic and anti-inflammatory agent in treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondilitis and acute pain in musculoskeletal disorders and in acute gout(7). If piroxicam administer trough transdermal route it can overcome it`s adverse effects and more concentrations, this is the desirable factor for an anti-inflammatory agent(8,9,10). Osteoarthritis (OA) is one of the most common

and problematic chronic diseases which indicates it`s symptoms on elder stages. The concept of this disease is to involve the entire joint organ, including the subchondral bone, menisci, ligaments, periarticular muscle, capsule, and synovium(11).
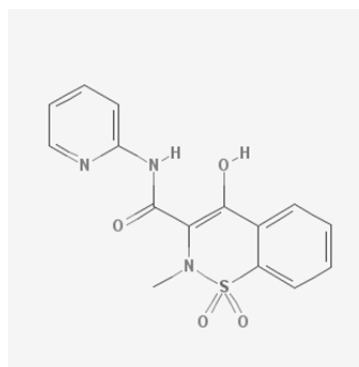
MATERIALS AND METHODS

Materials

Piroxicam was received as a gift sample from ZydusCadila Pharmaceuticals (P) Ltd. Phosphatidylcholine was received from Lipoid, Germany. Monosodium iodoacetate and dextran were purchased from Sigma Aldrich Co. Carbapol 940, methanol and ethanol (Analytical grade) from SD. Fine Chem. Ltd., was purchased from S.D Fine chemicals, New Delhi. All other chemicals used were of analytical grade and were used as received. Double-distilled water was used for all experiments.

METHOD
Formulation of Transfersome
Preparation of vesicular formulation

Transfersomes were prepared by conventional rotary evaporation sonication method(12,13). The mixture of 15ml methanol- chloroform (1:1), phospholipids and surfactant were taken in a clean, dry round bottom flask and the drug was added to the above mixture. The organic solvent was removed by vacuum rotary evaporation above the lipid transition temperature. Last traces of solvent were
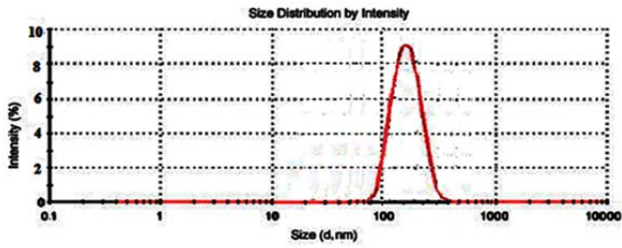
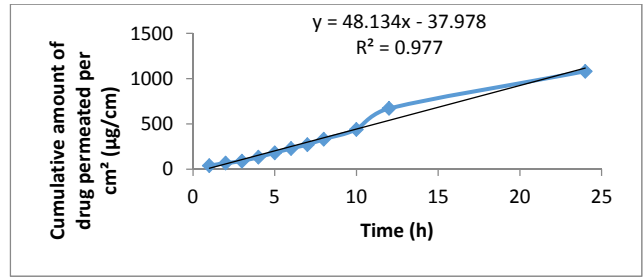**Fig.1.(m) Vesicle size and it`s distribution of formulation TF PIROX13 = 175.81 nm.**

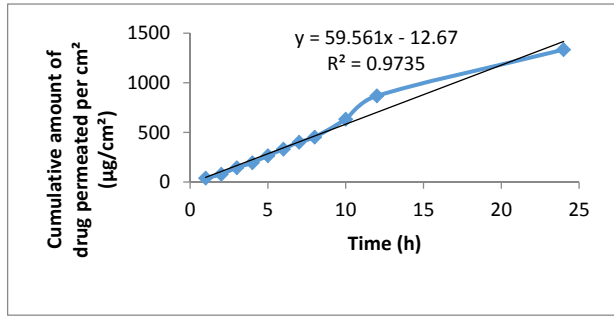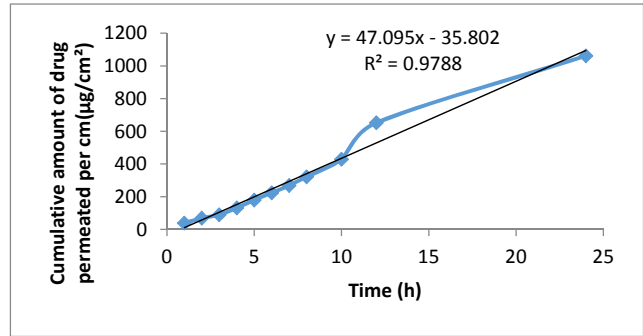**Fig.2.** *In vitro* **skin permeation of Piroxicam from transfersome TF PIROX1**

Fig.3. *In vitro* **skin permeation of Piroxicam from transfersome TF PIROX2**
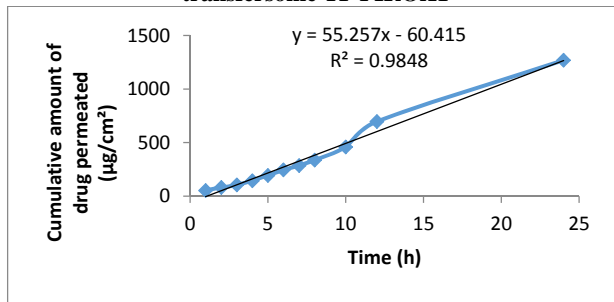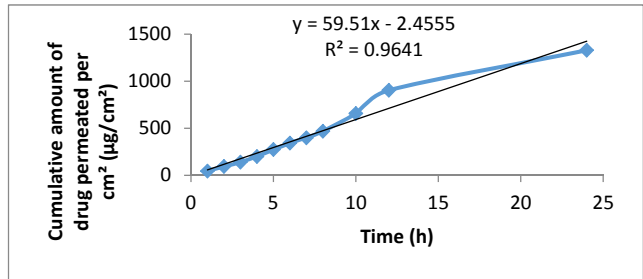

Fig.4. *In vitro* **permeation of Piroxicam from transfersome TF PIROX3**

Fig.5. *In vitro* **skin permeation of Piroxicam from transfersome (TF PIROX4)**

Fig.6. *In vitro* **skin permeation of Piroxicam from transfersome TF PIROX5**

Fig.7.*In vitro* **skin permeation of Piroxicam from transfersome TF PIROX6**

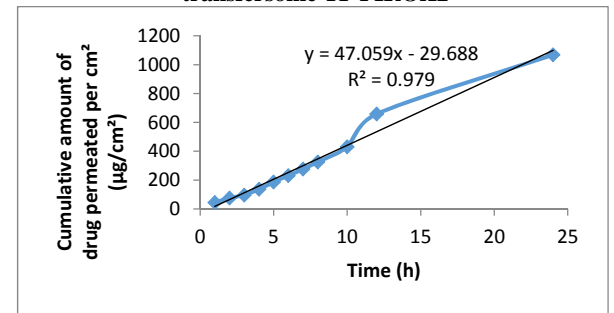**Fig.8.** *In vitro* **skin permeation of Piroxicam from transfersome TF PIROX7**

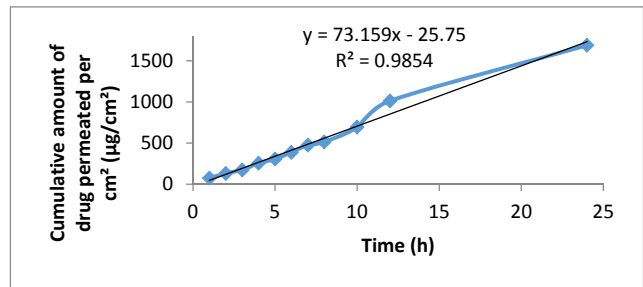**Fig.9.** *In vitro* **permeation of Piroxicam from transfersome TF PIROX8**

Fig.10. *In vitro* **permeation of Piroxicam from transfersome TF PIROX9**

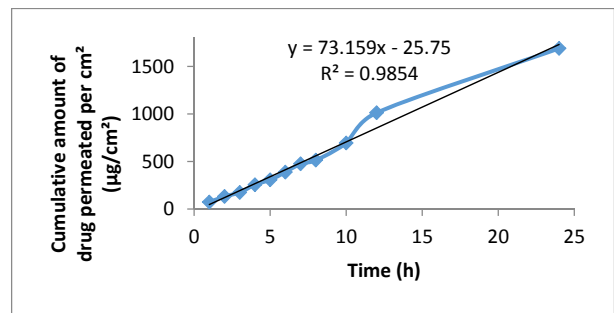**Fig.11.** *In vitro* **permeation of Piroxicam from transfersome TF PIROX10**

Fig.12. *In vitro* **skin permeation of Piroxicam from transfersome TF PIROX11**

Fig.13. *In vitro* **permeation of Piroxicam from transfersome TF PIROX12**

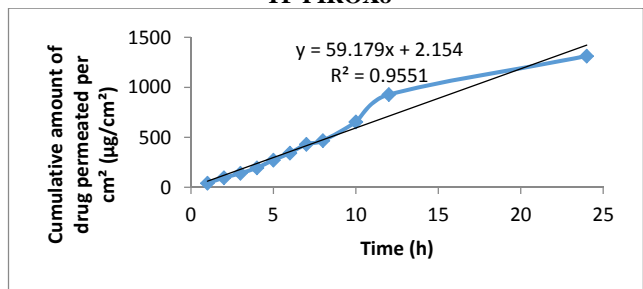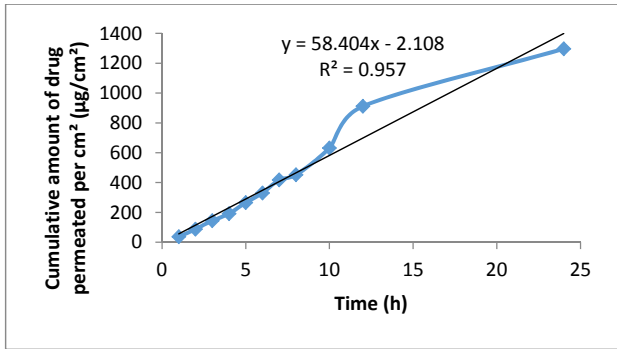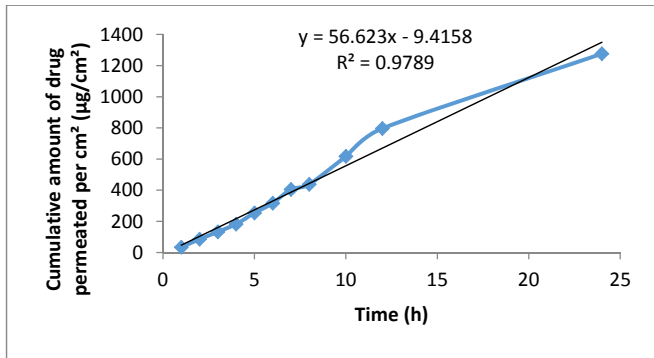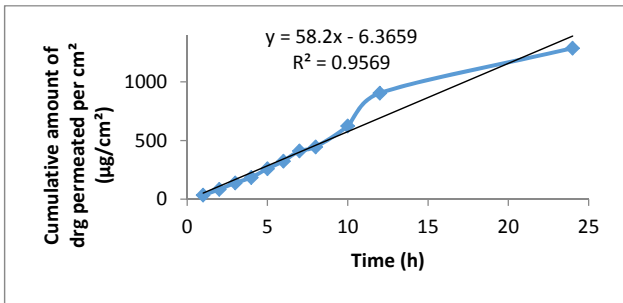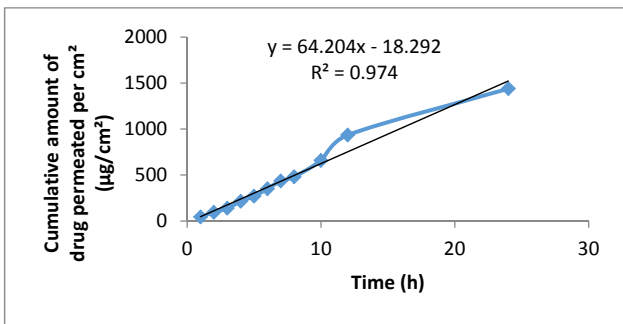**Fig.14.** *In vitro* **skin permeation of Piroxicam from transfersome TF PIROX13**
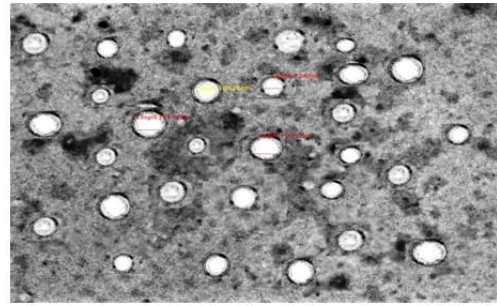
Fig.15. TEM image of optimized formulation and Vesicle size and it`s distribution of formulation TF PIROX4 = 100.18 nm.
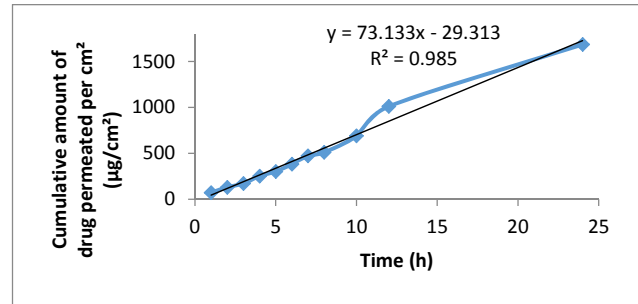
Fig.16. *In vitro* **drug permeation of Piroxicam from transfersomal gel.**
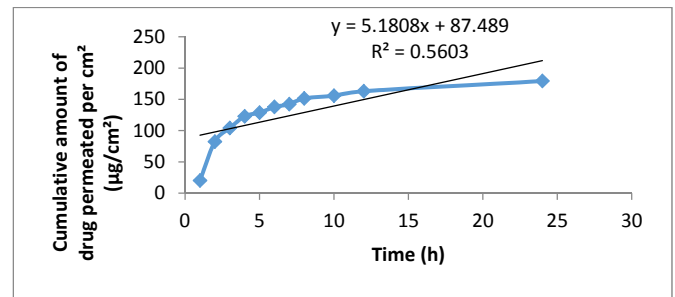
Fig.17. *Ex vivo* **permeation of Piroxicam from marketed formulation gel 0.5% w/w.**
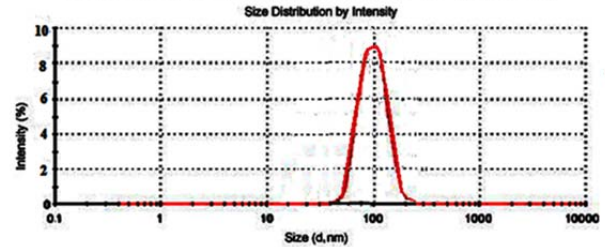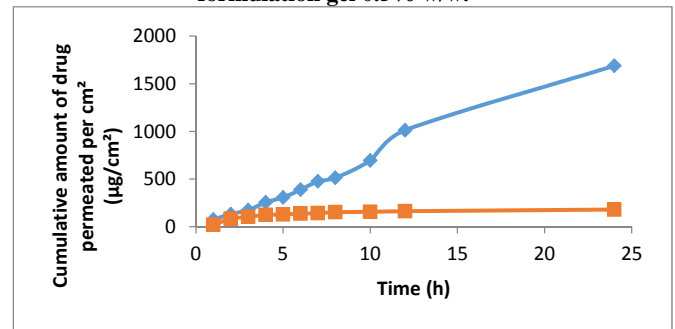

Fig.18. Comparative permeation profile of transfersomal gel and Marketed gel formulation(0.5% w/w)
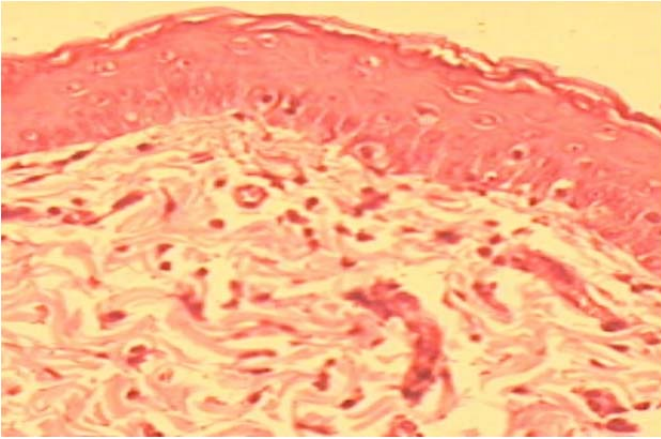
Fig.19. Photomicrograph of control skin
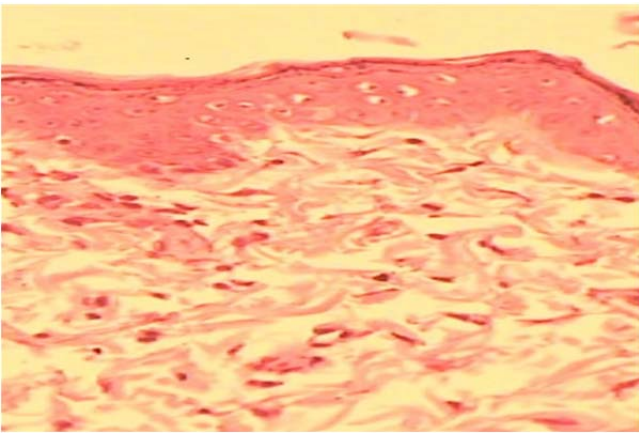
Fig.20. Photomicrograph of treated skin
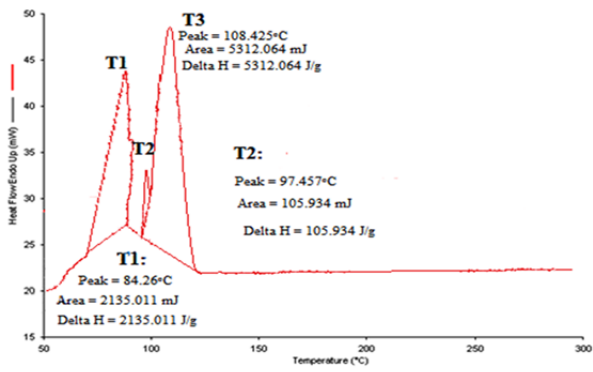
Fig.21. DSC thermogram of control treated rat skin
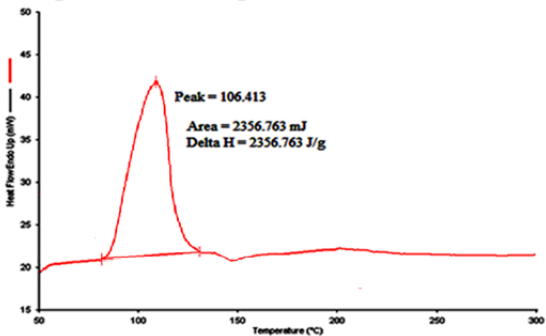
Fig.22. DSC thermogram of transfersomal gel treated skin
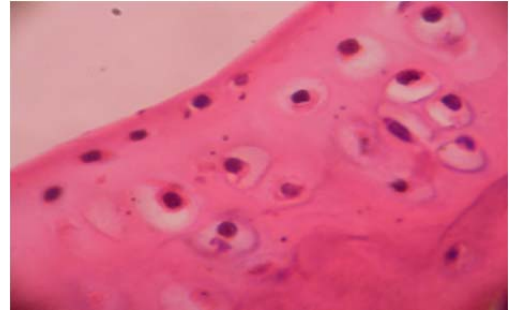
Fig.23. Control
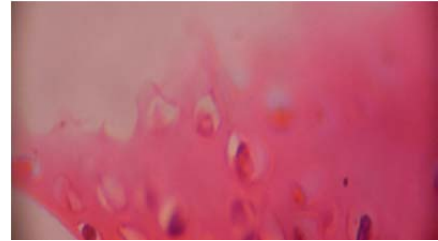
Fig.24. Toxic control

Fig.25. Treatment (oral piroxicam suspension)
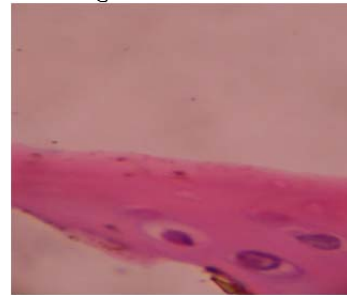
Fig.26.Treatment (marketed piroxicam gel formulation)
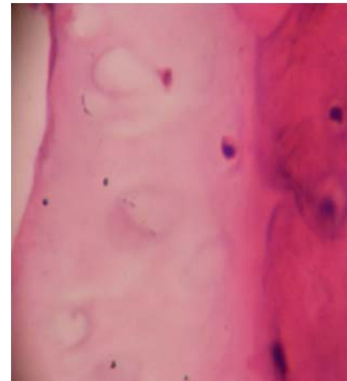
Fig.27. Treatment (Piroxicam transfersomal gel)
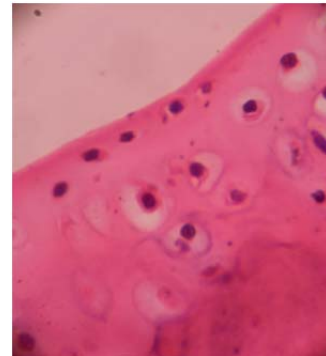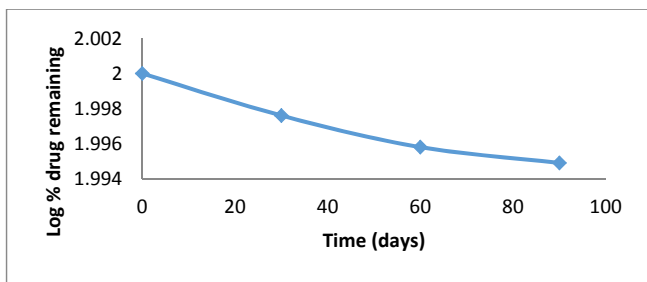
Fig.28. Degradation kinetics of Piroxicam in transfersomal gel(40±2°C &75±5% RH)

Fig.29. Log percentage remaining vs time plot for transfersomal gel

Fig.30. Arrhenius plot for the calculation of shelf life

The data of the skin permeation studies of the transfersomal gel was evaluated in order to ascertain permeation kinetics. From the permeation studies the rate constant for zero and first order rate kinetics were calculated for each time interval and their coefficient of variation (C_v) and standard deviation (SD) were calculated. It was seen in the case of transfersomal gel that small value of C_v was observed for zero order kinetics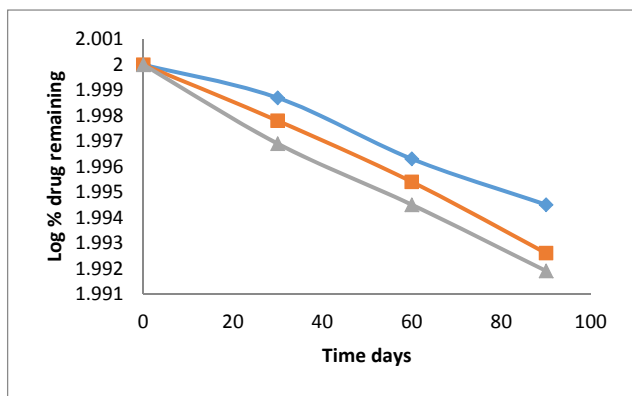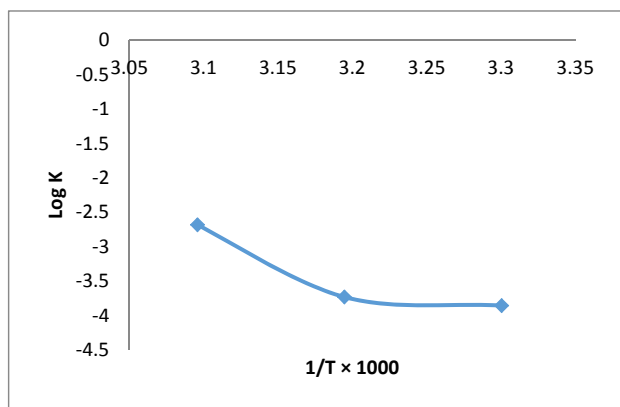 and more of C_v was observed for first order kinetics. Therefore, it was found that permeation from transfersome followed zero order permeation kinetics. *In vitro* permeation data of conventional gel was also evaluated for permeation kinetics. The studies conducted on conventional gel revealed that less C_v was observed for first order rate kinetics while more C_v was found for zero order kinetics.

Thus it was concluded that the permeation of the drug from transfersomal gel through the skin follows zero order kinetics.

The optical photomicrographs of control (untreated skin) showed normal skin with well defined epidermis and dermal layers. Keratin layer was well formed and lied just adjacent to the top most layer of the epidermis. Dermis was devoid of any inflammatory cells. Skin appendages were within normal limits (Fig.19).When the skin was treated with transfersomal gel formulation for 24 h, significant changes were observed in the skin morphology. Low power photomicrograph of skin sample showed epidermis with a prominent keratin layer, a normal dermis and subcutaneous tissues. Dermis does not show any edema or inflammatory cell infiltration. The disruption of lipid bilayers was clearly evident as distinct voids and empty spaces were visible in the epidermal region.

There were no apparent sign of skin irritation (erythma and edema etc.) observed on visual examination of skin specimens treated with transfersomal formulation.

Van Abbe *et al* (20) mentioned that a value of skin irritancy score between 0 and 9 indicates that the applied formulation is non irritant and safe for human skin. The mean value of skin irritancy score for formulation transfersomal gel was found to be 0.26. This value indicates that all excipients used in formulation were safe for transdermal drug delivery.

DSC thermogram of untreated rat epidermis revealed three endotherms (Fig.21). The three endotherms were recorded at 84,26°C (T1), 97.457°C (t2), 108.425°C (T3) respectively. T3 produced a sharp and prominent peak at 108.425°C which is attributed to SC proteins and the second endotherm (having the lowest enthalpy) was attributed to sebaceous section and to minor structural rearrangement of lipid bilayer. The first endotherm (T1) appeared due to the melting of SC lipids (24).

It was observed that both T1 and T2 endotherm were shifted to lower melting points in thermograms of SC treated with transfersomal gel formulation.

According to the previous studies, formulation TF PIROX4, which was showing the best results among all other Transfersomal formulations of piroxicam was selected for Transfersomal gel formulation for the anti inflammatory effects. The percent inhibition value after 24h administration was found to be high for Transfersomal gel i.e. 59.8% as compared to prepared conventional gel sample (22.8%).The difference was extremely significant at 1% level of significance (p<0.01) when compared with Transfersomal gel.

It was observed that Piroxicam when applied transdermally in the form of transfersomal gel reduced the cartilage necrobiosis of the tibial plateau and decreased proteoglycan destruction due to its matrix metalloproteinase inhibition ref. The transfersomal formulation of Piroxicam showed much higher achievement in treatment of osteoarthritis as compared to its conventional formulations which are already available in the market. In the control image Fig.23. the cartilage remains intact featuring prominent cell density and proteoglycan. In the toxic control image Fig.24, it was seen that there was an extension of the area of central chondrocyte necrobiosis with cartilage thinning in the surrounding area, marked loss of proteoglycans and a decreased cell density. This is due to the increase in activity

of matrix metalloproteinase and a decreased cell density. This is due to the increase in activity of matrix metalloproteinase enzymes which cause the loss of proteoglycans and thinning of cartilage was observed. The treatment images 25, 26 and 27 showed slight loss of proteoglycan with a decreased cartilage thinning and slight decrease in cell density.

The value of K at 25°C (K$_{25}$) was extrapolated from the Arrhenius plot (Fig.28) and shelf life was calculated by substituting k$_{25}$ in the equation for calculating shelf life. Shelf life was found to be 1.286 years. The result showed that formed transfersomal gel is stable. Thus the prepared transfersomal gel, if stored properly, can be effective for a period of 1.286 years from the date of its manufacturing.

DISCUSSION

The observation that shows increase in the amount of surfactant to SPC ratio resulted in increased entrapment efficiency of the formulation to a certain ratio can be probably explained by enhancing emulsification effect of sodium cholate (surfactant) over the phospholipid to a certain degree.

According to the results appeared the entrapment efficiency and vesicle size of different formulations were dependant on the ratio of lipid:surfactant and sonication time.

The content of lipid and surfactant mixture in transfersome affected the skin permeation rate of Piroxicam significantly.

As reduction in the drug permeation, which could be because of the fact that, at higher content of lipid and surfactant mixture the affinity of Piroxicam to the transfersome is increased while its affinity to stratum corneum is decreased.

The spreadability plays a significant role in patient compliance and helps in uniform application of gel to the skin. A good gel takes less time to spread and will have high spreadability.

. In order to ascertain that whether the zero order kinetics achieved in the case of transfersomal gel above was because of the formulation or due to the nature of the skin.

This shows that the permeation of drug from the conventional gel through the skin followed first order kinetics.

Observations of histopathological studies support the in vitro skin permeation data of Piroxicam.

The skin irritation test was performed to confirm the safety of the transfersomal gel formulation.

DSC of rat skin indicated that the components (lipid and surfactant) of the transfersome enhanced skin permeation of piroxicam through disruption of lipid bilayers. Change in peak area and transition temperature (peak shift) indicated increase in fluidity of skin bilayer which could be the mechanism of permeation enhancement.

The enhanced anti-inflammatory effects of ultraflexibleTransfersomal gel formulation could be due to enhanced permeation of Piroxicam through the skin.

It is reported that iodoacetate injection into the knee of rats results in histopathological features of OA such as a loss of proteoglycan, cartilage degeneration, osteophyte formation, a dose dependent reduction in spontaneous locomotion(25)

and altered gait (26). In this study we have demonstrated the important role of Piroxicam in the chondroprotection against iodoacetate induced knee osteoarthritis in albino wistar rat model.

The results of pharmacodynamic studies can be due to the antiinflammatory effect of Piroxicam which result from the reversible inhibition of cyclooxygenase, causing the peripheral inhibition of prostaglandin synthesis. The prostaglandins are produced by an enzyme called Cox-1. Piroxicam blocks the Cox-1 enzyme, resulting into the disruption of production of prostaglandins. Piroxicam also inhibits the migration of leukocytes into sites of inflammation and prevents the formation of thromboxane A2, an aggregating agent, by the platelets.

CONCLUSION:

The present study is my M.Pharm thesis carried out on July 2011 till August 2012 which developed and evaluated the transdermal transfersomal gel of piroxicam, since the oral administration piroxicam causes severe hypoglycaemic reaction. The transfersomal formulation of Piroxicam showed the much higher achievement in treatment of osteoarthritis as compare to its conventional formulations which are already available in the market. Further research is recommended for validating the efficacy and safety of the above formulation by long term pharmacokinetic and pharmacodynamic studies in healthy volunteers and patients.

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