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FORMULATION, CHARACTERIZATION AND IN VITRO EVALUATION OF PIROXICAM TRANSFEROSOMAL GELS

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ABSTRACT

Transferosome formulations were prepared by thin film hydration technique and were incorporated into 2% carbapol gel. The Formulation PT3 containing Lecithin: Tween-80 in ratio 85:15(%w/w) has higher entrapment efficiency and maximum drug release. In-vitro skin permeation study studies showed that, transferosome gels were found to increase the skin permeation and deposition showing a sustain effect. Stability studies performed for optimized transferosome gel formulations indicates that prepared transfersomes have more stability at freezing temperature than that of room temperature. Based on the above data, it was confirmed that prepared Piroxicam transferosome gel (PT3) can be considered as one of the promising approach to reduce the dosing frequency and to maintain drug concentration at the desired site for longer time. Finally, it can be concluded from the results of present study that Transferosomes gel improve the transdermal delivery, prolong the release, and improve the site specificity of the drug Piroxicam. Transferosomes creates a new opportunity for the well-controlled transdermal delivery of a number of drugs that have a problem of administration by other routes.

KEYWORDS: Transferosome, Drug - Piroxicam, Phosphate Buffer, Transferosome gel, Lecithin, Phospholipid – Soya Lecithin, Edge Activator – Span 80, Tween 80, Volatile Solvents – Methanol, Chloroform, Gelling Agent - Carbopol 934.

INTRODUCTION

Piroxicam is a non-steroidal anti-inflammatory agent with analgesic and antipyretic properties. The mode of action of piroxicam has not been fully established; however, independent studies, both in vitro and in vivo, have demonstrated that piroxicam interacts at several steps in the immune and inflammation responses.[1-3] Transdermal delivery of drugs through the skin to the systemic circulation provides a convenient route of administration for a variety of clinical indications.[4] Transdermal delivery is gaining importance recently because of certain advantages over the conventional oral one. The application of transdermal delivery to a wider range of drugs is limited due to the significant barrier to penetration across the skin which is associated primarily with outermost stratum corneum layer of epidermis.[5-7] The skin structure looks as if stratum corneum cells are embedded in a pool of intercellular lipid lamellae. These lamellae have a crucial role in imparting barrier properties to the stratum corneum. As a result, only milligram quantities of drug can be delivered by this route. This limits the application of this route to only potent drugs. Extensivework has been done in order to overcome the barrier properties of intact human skin. These include augmentation of skin permeability using penetration enhancers, use of forces which are not dependent on concentration gradient (iontophoresis, electroporation, phonophoresis, microneedles, jet injectors, etc.,) and many more. Transferosomes or other drug carrier systems like vesicles belong to the latter category.[8-11]

The main objective of the study is to formulate and evaluate Piroxicam transferosome gel formulation for effective topical delivery of drug. The aim and objective of the study is to develop analytical method for the estimation of drug in the formulations, to carry out pre-formulation studies for possible drug-excipient interactions by FTIR. To prepare transferosomes containing Piroxicamusing different ratios of Phospholipid and Edge activator using thin film hydration technique. Characterization of transferosomes with respect to vesicle shape, formulation of transferosome gel and studying its in-vitro drug release using diffusion cell Entrapment efficiency and Drug content.[11-15]

MATERIALS AND EQUIPMENTS

All the materials and equipments used in the formulation, evaluation and other experiments

are given below.

Table No. 1: List of Materials.

Category	Chemical name	Supplier
Drug	Piroxicam	Chandra Labs Hyderabad, India
Phospholipid	Soya Lecithin	Bright Laboratories
Edge activator	Span 80,Tween 80	Merck specialties pvt. limited (Mumbai)
Volatile solvents	Methanol, Chloroform	S.D.Fine Chemicals, Mumbai
Gelling agent	Carbopol-934	Research lab fine chem. Industries(Mumbai)

INSTRUMENTS USED

Table No. 2: List of Equipments.

S.No.	INSTRUMENTS	SUPPLIER	MODEL
1	FT-IR spectrophotometer	BRUKER	ALPHA-T-1020
2	UV-Visible spectrophotometer	Lab India	UV 3200
3	Hot air oven	Universal	Q-5247
4	Electronic balance	Shimadzu	AX-200
5	Centrifuge	Remi	TROI
6	Probe sonicator	Heldolph	VCX750
7	PH meter	Labindia	SAB 5000
8	Magnetic stirrer	Remi	5MLH
9	Weighing balance	Shimadzu	ATX224
10	Homogenizer	Remi	RQT-124A

METHODOLOGY

PREFORMULATION STUDIES

Preformulation may be described as a phase of the research & development process where the that enter the development process during this evaluation possible interaction with various ingredients intended for use final dosage form are also considereding the present study formulation scientist characterizes the physical, chemical and mechanical properties of new drug substances, in order to develop stable, safe and effective dosage forms. Ideally the Preformulation phase begins early in the discovery process such the appropriate physical, chemical data is available to aid the selection of new chemical entities.

Organoleptic properties

The color, odor and taste of the drug were recorded using descriptive terminology.

Solubility

The solubility of the drug sample was carried out in different solvents (aqueous and organic) according to the Indian Pharmacopoeia. The results are then compared with those

given in the official books and Indian Pharmacopoeia.

Melting point

The melting point of Piroxicam was found out by capillary method using programmable melting point apparatus.

Determination of λ max for piroxicam

On the basis of preliminary identification test, it was concluded that the the drug complied the preliminary identification. From the scanning of drug, it was concluded that the drug had λ max of 254 nm.

Preparation of standard calibration curve of piroxicam

The standard calibration curve for Piroxicam was prepared using DMSO and pH 6.8 buffer solution.

Standard solution: 1 mg/ ml(1000µg/ml).

100 mg of Piroxicam was dissolved in DMSO (drop by drop) first and made upto a volume of 100 ml with pH 6.8 buffer solution to give a concentration of 1 mg/ ml(1000µg/ml).

Stock solution

From standard solution take 1 ml of solution in 100 ml of pH 6.8 buffer solution to produce the 10 µg/ml concentration. different aliquots of solutions were taken to produce 2,4, 6, 8 and 10 µg/ml concentrations. The absorbance of prepared solution of piroxicam was measured at 254 nm in Shimadzu UV/visible 1700 spectrophotometer against pH 6.8 buffer solution as blank. The absorbance data for standard calibration curve are given in Table and plotted graphically. The standard calibration curve yields a straight line, which shows that drug obeys Beer's law in the concentration range of 2 to 8 mcg/ml.

Table No. 3: Parameters of Piroxicam.

Parameters		**Piroxicam**
Wavelength(nm)		254
Beer's limit(ppm)	**Law**	0-10
R2 value		0.9976
Regression equation(6.8 buffer)	**pH**	Y=0.0823x+0.0069

COMPATIBILITY STUDIES

IR spectroscopy can be used to investigate and predictany physicochemical interactions between different components in a formulation and therefore it can be applied to the selection of suitable chemically compatible excipients. The aim of the present study was to test, whether there are any interactions between the carriers and drug. One part of the sample and three parts of potassium bromide were taken in a mortar and triturated a small amount of triturated sample was taken into a pellet. Maker and was compressed at 10kg/cm2 using z hydraulic press. The pellet was kept on to the sample holder and scanned from 4000cm-1 to 400cm-1 in Bruker IR spectrophotometer. Then it was compared with original spectra.

IR spectra was compared and checked for any shifting in functional peaks and non – involvement of functional group. From the spectra it is clear that there is no interaction between the selected carriers, drug and mixtures. Hence, the selected carrier was found to be compatible in entrapping the selected Piroxicam with carriers without any mutual interactions.

FORMULATION OF TRANSFERSOME GEL

1) Preparation of transfersomes containing Piroxicam
2) Preparation of topical Transfersome gel

Preparation of Transfersomes by Modified Hand shaking lipid film hydration technique

Six Transfersome formulations were prepared by thin film hydration method using Piroxicam, Soya Lecithin, and different concentrations of surfactants (Span- 80, Tween80). The amount of drug is kept constant (8mg) in all the formulations. Different formulations were prepared by using different ratios of phospholipid and surfactants in different ratios. The details about the surfactants used and amount of lecithin and surfactant used in each formulation are given in the table. Lecithin, surfactants and the drug are dissolved in 5ml of organic solvent (Chloroform: Methanol 3:1). The organic solvent is then removed by evaporation while hand shaking above lipid transition temperature (43^0c). Final traces of solvent are removed under vacuum. The deposited lipid film is hydrated with the phosphate buffer (pH 6.8) by rotation at 60 rpm for 1 hour at room temperature. The resulting vesicles are swollen for 2 hours at room temperature. The multilamellar lipid vesicles (MLV) are then sonicated using sonicator for 30 minutes.

Table No. 4: Quantity of substances taken for preparation of transfersomes.

FORMULATION	Piroxicam (mg)	Lecithin(mg)	Tween 80 (mg)	Span 80 (mg)
PT1	120	95	5	--
PT2	120	90	10	--
PT3	120	85	15	--
PT4	120	95	--	5
PT5	120	90	--	10
PT6	120	85	--	15

In each of the formulation, 5ml of chloroform and methanol ratio were added separately.

Preparation of topical transfersome gel

As a vehicle for incorporation of transfersomes for topical delivery, carbopol gels were prepared. Transfersomes aqueous dispersion was utilized for the formulation of topical gel. Gel polymer such as carbopol 934 was utilized to prepare transfersome gel. 2g of carbopol- 934 powder was dispersed into vigorously stirred (stirred by magnetic stirrerRemi 5MLH) distilled water (taking care to avoid the formation of in dispersible lumps) and allowed to hydrate for 24 hrs. The dispersion was neutralized with tri ethanolamine to adjust the pH [6.8] by using pH meter (Lab India Sab 5000).

OPTIMIZATION OF FORMULATION

There are various process variables which could affect the preparation and properties of the transfersome. The preparation procedure was accordingly optimized and validated. The preparation of transfersome containing Piroxicam involves various process variables such as effect of Lecithin: Surfactant ratio and effect of surfactant, optimization was done by selecting in vitro release of drug as optimizing parameter. During the preparation of a particular system, the other variables were kept constant.

CHARACTERIZATION OF TRANSFERSOMES

Transfersome suspension

Vesicle shape and type

Transfersomes vesicles can be visualized by SEM and optical microscope. The Morphological characterization of transfer some vesicle such as shape and surface feature were projected by using optical microscope and SEM.

Optical microscope method

A drop of transfersome suspension was placed over the slide and Photo micrograph was

taken at 10x resolution.

Transfersome gel Determination of PH

The value of pH of topical transfersome gels was measured by using digital pH meter(Lab India Sab 5000 pH meter) at the room temperature.

Determination of entrapment efficiency percentage

The amount of Piroxicam entrapped in transfersome gel was estimated by centrifugation method. 1gm of Transfersome gel was taken and diluted with 10ml phosphate buffer (pH 6.8). This suspension was sonicated using bath sonicator for 20 minutes. Later this solution was placed in centrifugation tube and centrifuged at 14000 rpm for 30 minutes. 0.5ml of supernatant was withdrawn and diluted before going for absorbance measurement using UV spectrophotometer (UV-3200 Lab India) at 254nm. This gives us the total amount of unentrapped drug. Entrapment efficiency is expressed as the percent of drug trapped.

$$\% \text{ Entrapment} = \frac{\text{Total drug-Diffused drug}}{\text{Total drug}} \times 100$$

% Drug content

1g of transfersome gel formulation was taken and the vesicles were lysed with 25 ml of methanol by sonication [citizen, India] for 15 min. Later this solution was placed in centrifugation tube and centrifuged at 14000 rpm for 30 minute. Then 10 ml of solution was diluted to 100 ml with phosphate buffer pH 6.8. Aliquots were Withdrawn and drug contentwas calculated for Piroxicam by using UV spectrophotometer at 254 nm.

$$\% \text{ Drug Content} = \frac{\text{Amount of Drug obtained after centrifugation}}{\text{Amount of Drug Taken}} \times 100$$

In-vitro drug release studies

Modified Franz diffusion cell with a receiver compartment volume of 30ml and effective diffusion area of $2cm^2$ was used for this study. *In-vitro* drug study was performed by using egg membrane in phosphate buffer solution (pH 6.8).

To perform *in-vitro* drug release study, egg membrane was mounted horizontally on the receptor compartment of Franz diffusion cell. The effective permeation area of donor compartment exposed to receptor compartment was $2cm^2$ and capacity of receptor

compartment was 30ml. The receptor compartment was filled with 30ml of phosphate buffer (pH 6.8) maintained at 37± 0.5^0C and stirred by a magnetic bar at 100rpm. Transfersome gel formulation equivalent to 8mg drug was placed on the skin and the top of the diffusion cell was covered. At appropriate time intervals 5 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh phosphate buffer (pH 6.8) to maintain sink conditions. The samples were analyzed spectrophotometrically at λ max 254 nm.

RESULTS

Description

These tests were performed and the results were illustrated in the following table.

Table No. 5: Table showing the description of Piroxicam (API)

Test	Description
Colour	A pale yellow powder

Result

The results were found as per specifications.

Solubility.

These tests were performed and the results are illustrated in the table.

Table No. 6: Table showing the Solubility of Piroxicam (API) in various solvents.

Solvents	Solubility
Water	Sparingly soluble
pH6.8 Phosphate buffer	Soluble
DMSO	Freely Soluble
Ethanol	Freely Soluble

Melting Point

This test is performed and the result was illustrated in the following table.

Table No. 7: Table showing the melting point of API.

Material	Melting Point	Melting Point Range
piroxicam	1990c	198-2000c

Result: The Result was found to be within limit.

PREFORMULATION STUDIES

Standard calibration curve

In the pre-formulation study, it was found that the λmax of Piroxicam by spectrophotometric method in phosphate buffer pH 6.8 was found to be 254 nm.

Table No. 8: Calibration Curve of Piroxicamin Phosphate Buffer pH 6.8

S.no	Concentration (µg/ml)	Absorbance
1	0	0
2	2	0.173
3	4	0.333
4	6	0.508
5	8	0.689
6	10	0.808

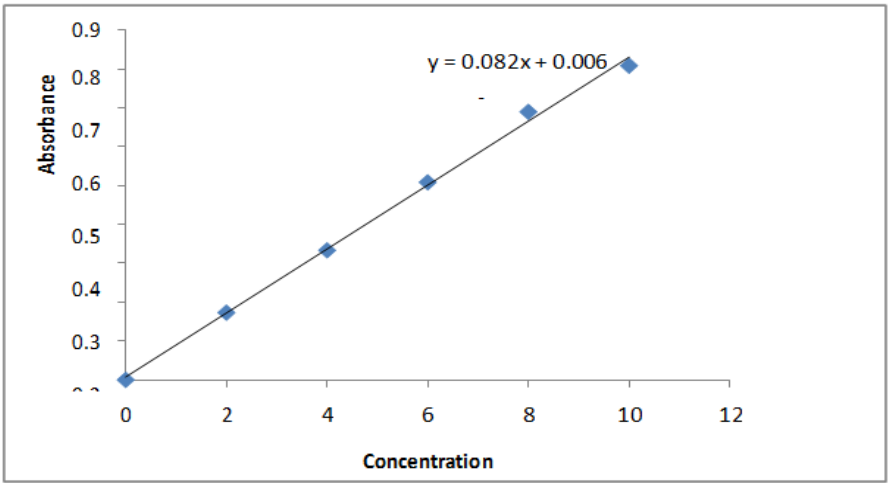

Fig: 1: Standard Graph of Piroxicam in Phosphate Buffer pH 6.8.

Drug excipient compatibility study

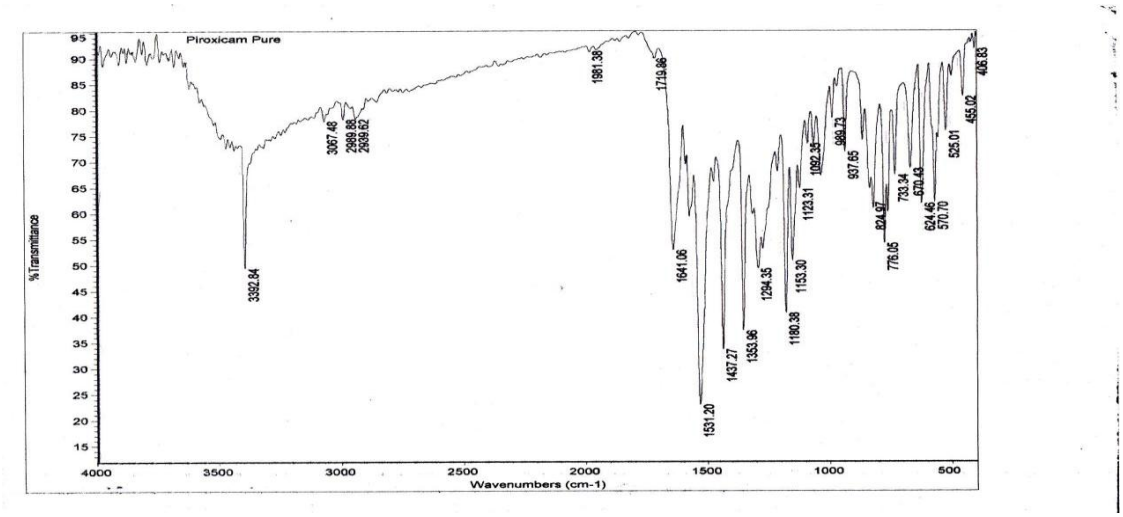

Fig: 2: FTIR spectra of Piroxicam

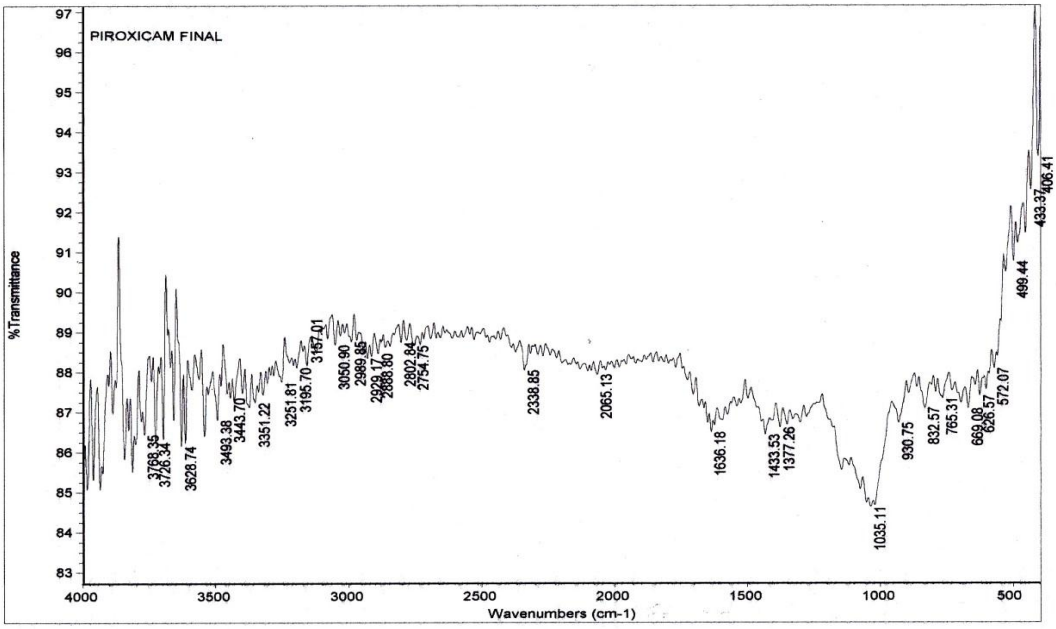

Fig 3: FTIR spectra of Piroxicam optimized.

CHARACTERIZATION OF TRANSFEROSOMES EVALUATION OF TRANSFEROSOME GEL

Entrapment efficiency

The % entrapment efficiency of deformable vesicles formulations were found to be in the range of 84.92to 94.11 (Table). Entrapment efficiency of the PT3 formulation was high (maximum 94.11).

% Drug content

% drug content of transferosome formulations (PT1to PT6) were determined according to procedure described in chapter 4. The results obtained shows 92.0- 95.07% drug content in the formulations. The results obtained are shown in table.

Table No. 9: % Drug entrapped and % Drug content in transferosomes

Formulation	% Entrapment Efficiency	% Drug content
PT1	86.5	94.14
PT2	84.92	92.0
PT3	94.11	94.81
PT4	73.40	94.6
PT5	74.25	95.0
PT6	79.32	95.07

pH value of topical transferosome gel

The value of pH of topical transferosome gels was measured by using digital pH meter (Lab India Sab 5000 pH meter) at the room temperature. The pH of all topical transfersomal gels were found to be in the range of 6.6±0.82 to 6.9±0.71.

In-vitro drug release study

The *in-vitro* diffusion study in phosphate buffer pH 6.8 were carried out using Franz diffusion cell according to procedure explained in section 4.7.6 of chapter 4. The results are shown in tables.

Table No. 10: *In-Vitro* drug release of transferosome gel (PT1 to PT6).

Time(hr)	PT1	PT2	PT3	PT4	PT5	PT6
0	0	0	0	0	0	0
1	20.51	21.5	23.1	22.71	24.81	24.39
2	24.28	28.04	27.81	28.54	29.88	26.51
3	29.41	31.41	30.44	33.14	34.36	30.06
4	34.51	38.82	37.91	39.51	40.80	37.87
5	40.28	43.61	42.84	44.80	44.72	39.87
6	48.17	45.81	44.90	48.71	47.81	43.10
8	56.78	48.31	53.28	53.20	50.20	49.29
12	60.43	59.80	66.94	58.18	66.43	68.18
24	82	79.0	85.30	63.83	74.04	77.50

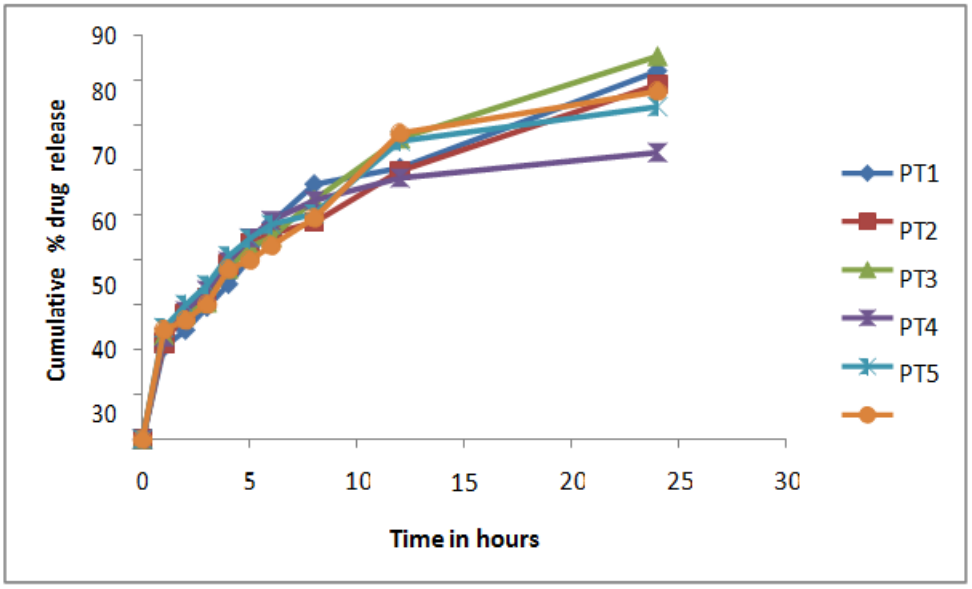

Fig: 4: *In-Vitro* drug release study for Tansferosome Gel formulation PT1-PT6.

SUMMARY AND CONCLUSION

The work was carried out to prepare Piroxicam transferosome gel to achieve sustain release effect at site of administration. The pre-formulation studies like UV analysis of Piroxicam, FTIR were complied with BP standards. The FTIR spectra revealed that there was no interaction between the drug and excipients. Transferosome formulations were prepared by thin film hydration technique and were incorporated into 2% carbapol gel. The Formulation PT3 containing Lecithin: Tween-80 in ratio 85:15(%w/w) has higher entrapment efficiency and maximum drug release. In-vitro skin permeation study studies showed that,transferosome gels were found to increase the skin permeation and deposition showing a sustain effect. Stability studies performed for optimized transferosome gel formulations indicates that prepared Transferosomes have more stability at freezing temperature than that of room temperature. Based on the above data, it was confirmed that prepared Piroxicam transferosome gel (PT3) can be considered as one of the promising approach to reduce the dosing frequency and to maintain drug concentration at the desired site for longer time.

CONCLUSION

Finally, it can be concluded from the results of present study that Transferosomes gel improve the transdermal delivery, prolong the release, and improve the site specificity of the drug Piroxicam. Transferosomes creates a new opportunity for the well-controlled transdermal delivery of a number of drugs that have a problem of administration by other routes.

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