ORIGINAL ARTICLE

Formulation and Development of Proniosomal Gel for Transdermal Delivery of Ketorolac Tromethamine

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Abstract

Aim: The aim of this study was to formulate and perform characterization, permeability enhancement studies, *in-vitro*, *in-vivo* evaluation of prepared formulation and stability studies of transdermal proniosomal (PN) gel for the anti-inflammatory and analgesic activity containing ketorolac tromethamine (KT). **Materials and Methods:** KT is heterocyclic acetic acid derivative with a molecular weight of approximately 255.27 g/mol, water-soluble and poorly absorbable, and act by inhibition of cyclooxygenase enzyme. PN gel formulations of KT were prepared from optimized PN suspension and effects of formulation variables such as solvents and surfactants on transdermal permeability profile were assessed. Carbopol 940 and dimethyl sulfoxide were added as gelling and penetration enhancing agents, respectively. **Results and Discussion:** PN gel formulations LCI-1-a showed better transdermal flux *ex-vivo* permeation (3.172 ± 0.041 µg/cm^2/h) than LCI-2-b (2.651 ± 0.069 µg/cm^2/h) and plain gel (1.941 ± 0.031 µg/cm^2/h). PN gel LCI-1-a showed the highest drug release 94.048% in 17 h; marketed preparation showed drug release 90.987 in 17 h; plain gel showed drug release 41.154% in 10 h. The percentage inhibition of carrageenan-induced paw edema after 5 h of applying the LCI-1-a gel was found to be 63% while marketed preparation and plain gel showed 46.86% and 29%, respectively. **Conclusion:** The PN gel formulations showed good stability at refrigerated than room temperature after 3 months of storage. Among distinctive PN gel formulations, LCI-1-a (containing sodium cholate and isopropanol) showed promising results with respect to drug entrapment and percentage drug release. The formulations were subjected to pharmacokinetic and pharmacodynamic analysis by *ex-vivo* permeability studies; *in-vivo* anti-inflammatory, skin irritation studies, etc., and the results obtained were satisfactory.

Key words: Ketorolac tromethamine, proniosomes, solvents, surfactants, transdermal delivery

INTRODUCTION

Drug targeting can be defined as the ability to express a therapeutic agent exclusively to desired site of action in such way that little or no interaction with non-target tissue.[1] Several novel approaches used for delivering these drugs comprise liposomes, microspheres, nanotechnology, microemulsions, antibody-loaded drug delivery, magnetic microcapsules, implantable pumps, and niosomes.[2] Niosomes and liposomes are formations of vesicles through mixture of cholesterol, nonionic surfactants or lipid but offer certain disadvantages[3] restrictions of niosomes can be avoided by proniosomes as degradation by hydrolysis, because they are dispersed aqueous systems, require special storage and handling, difficulty in sterilization, transportation, distribution, storage uniformity of dose and scale up, use of unacceptable solvents during preparation, incomplete hydration of lipid/surfactant film on walls during hydration.[4] Proniosomes minimize the problems like sedimentation, aggregation or fusion on storage along with better stability during storage, sterilization, ease of transfer, distribution, measuring, and storage. Transdermal delivery is of great significance for drugs that may cause systemic side effects such as non-steroidal anti-inflammatory drugs (NSAIDs).[6] Ketorolac tromethamine (KT) (NSAIDs), a prostaglandin synthesis inhibitor is a NSAID with potent analgesic and moderate anti-inflammatory activities.[7] Administered through oral and injectable formulations, it has revealed a high analgesic potency nearly equivalent to that of morphine.[8] It is presently administered orally, intramuscularly

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or intravenously for short-term management of moderate to severe pain including postpartum pain. Oral bioavailability of KT is reported to be 90% through a very low first pass metabolism. Although, the drug is reported to cause severe gastrointestinal side effects such as gastrointestinal bleeding, perforation, and peptic ulceration. Consequently, topical administration of KT is the preferred route of administration for moderate to severe pain management. The biological half-life of ketorolac ranges from 3.8 to 6 h, due to such a short life, recurrent dosing is required to alleviate pain in post-operative patients. Conversely, to avoid an invasive drug delivery technique and to reduce frequent dosing regimens, there is a necessitated for an alternative; non-invasive mode of delivery of KT.[9]

The objective of this research work was to develop proniosomal (PN) formulation by handshaking using an appropriate surfactant (sodium cholate) and permeability enhancer (dimethyl sulfoxide). The prepared proniosomes were characterized and *in-vitro, in-vivo* evaluation of prepared formulation and stability studies of proniosomes preformed. In the work reported here, we illustrate the preparation of dry niosomes, "proniosomes." As a dry, free-flowing, granular product which, on addition of water, disperses or dissolves to form a multilamellar niosome suspension suitable for administration by oral or topical routes.[5]

MATERIALS AND METHODS

Materials

Soya PC, cholesterol (Ch), sodium cholate, sodium deoxycholate, and Span-80 were purchased from Himedia Chemicals. Disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride, and isopropyl alcohol were purchased from Loba Chemie (Mumbai, India); ethanol was purchased from Changshu Yangyuan Chemical (China), and a gift sample of KT was procured from Piramal Health Care Pvt., Ltd. (Pithampur, India).

Preparation and optimization of formula

Preparation of PN suspensions

The proniosomes were prepared by handshaking method by dissolving cholesterol and surfactant in alcohol, and the thin film was formed along the sides of the flask by continuous vortexing. Drug was dissolved in 10 ml of phosphate buffer saline (PBS) pH 7.4 and added to the thin film and sonicated for 5 min. The PN suspension was kept refrigerated at 4°C.[10,11]

Evaluation of PN suspension

i. By scanning electron microscope (SEM): The sizes of the vesicles are measured by SEM. The small amount of PN suspension was placed on the specimen stub, coated with carbon and gold vapor using Hitachi vacuum evaporator and examined under SEM, and then photographed.[12]
ii. By optical microscope: PN suspension was diluted with phosphate buffer pH 7.4. A drop was placed on glass slide and shape of vesicles measured using optical microscope at ×6 (Besto Model 10 A).[13]
iii. Determination of zeta potential: Zeta potential of selected batch of PN formulation was determined at 25°C using Zetasizer (Malvern Instruments). PN suspension was diluted 100 times with double-distilled water and voltage was set at 50 or 100 V. Electrodes were placed in dispersion for the measurement of zeta potential.[14]
iv. Entrapment efficiency: The entrapment efficiency was determined after separating the unentrapped drug. Proniosomes (100 mg) were hydrated with 10 ml of PBS (pH 7.4), shaken for 5 min, to form a dispersion. To separate un-trapped drug, the dispersion was centrifuged at 15,000 rpm for 30 min at 20°C and then analyzed by ultraviolet (UV) spectroscopy at 322 nm.[15] The entrapment efficiency was calculated using the formula:

$$\% \text{ Entrapment efficiency} = \frac{\text{Total drug} - \text{Drug in supernatant liquid}}{\text{Total drug}} \times 100$$

v. *In-vitro* drug release: The release of KT from PN formulations was determined using membrane diffusion technique. The PN formulation equivalent to 5 mg of KT was placed inside the donor chamber and the receptor media consisted of 100 ml of phosphate buffer 7.4, maintained at 37 ± 0.5°C, and agitated at 75 rpm. Aliquots of 3 ml were withdrawn at regular intervals and replaced with fresh pre-warmed media. The collected samples were analyzed spectrophotometrically (Shimadzu-1800, Japan).[16]

Preparation of PN gel

About 2 ml of PN suspension was added to carbopol 940 (1% w/v) containing glycerin, methylparaben and dimethyl sulfoxide, mixed and the pH was adjusted using triethanolamine. The gel was sonicated for 15 min and kept overnight to remove air bubbles.[17,18]

Evaluation of PN gel

i. Physical appearance and homogeneity: The physical appearance and homogeneity were tested by visual observation
ii. Clarity: The clarity of formulations was determined by visual inspection under black and white background and graded as; turbid: +, clear: ++, very clear (glassy): +++[19,20]
iii. Viscosity: Viscosity was measured using Brookfield Viscometer (DV-E). 10 g of gel was taken into beaker, spindle was dipped and the viscosity was measured by rotating the spindle 06 at 100 rpm[21,22]
iv. Measurement of pH: The pH measurements were performed in triplicate by digital pH meter. Before measurements, pH meter was calibrated

v. *Ex-vivo* permeability study: Skin permeation study was performed using Franz diffusion cell with an effective diffusion area of 2.669 cm^2. The experiment was carried out using freshly killed goat dorsal skin obtained from a local slaughterhouse and stored at −18°C in Ringer solution. Skin was first flushed with physiological solution at room temperature for 2 h to remove any skin content and shaved using a hand razor to remove subcutaneous tissue. Dermis side was wiped with isopropyl alcohol to remove adhering fat, and then washed with distilled water. A circular piece of skin about 3 cm diameters was sandwiched between donor and receptor compartments. In the donor compartment, the formulation was placed and the receptor compartment was filled with phosphate buffer pH 7.4, kept at constant temperature of 37 ± 0.5°C and stirred. At appropriate intervals (1, 2, 3, 4, 5, 6, 7, 8 h), 3 ml aliquots were withdrawn and replaced with an equal volume of fresh receptor solution. Samples were analyzed by UV Spectrophotometer (Shimadzu-1800, Japan) at 322 nm using phosphate buffer (pH 7.4) as blank. Permeation study of plain gel and marketed gel was also conducted similarly.[23]

Calculation of permeation parameters:

The permeation studies were investigated using flow-through diffusion cell system and calculated by the following equation

$$J_{SS} = (dq/dt)ss.\ 1/A \quad (1)$$

A = The effective diffusion area,

$(dq/dt)_{ss}$ = The steady-state slope.

The permeability coefficient (Kp) was calculated using Fick's first law of diffusion as follow:

$$Kp = J_{SS}/C_0 \quad (2)$$

Where, C_0 is the initial drug concentration in the donor compartment.[24]

vi. *In-vitro* drug release: The release of KT from PN gel was determined using membrane diffusion technique. The PN gel equivalent to 1 mg of KT was placed in a dialysis bag tied with glass tube and placed in beaker containing 50 ml of phosphate buffer (pH 7.4) as receptor compartment. The temperature of receptor medium was maintained at 37 ± 0.5°C and agitated at 75 rpm. Aliquots of 3 ml sample were withdrawn periodically and analyzed by UV spectrophotometer.[25,26]

vii. *In-vivo* anti-inflammatory study

Carrageenan-induced rat paw edema: Effect of KT on acute inflammation was evaluated in rat carrageenan-induced edema model as described previously. Animals were divided into four groups, each group contains six animals. Group I served as control whiles the Groups II, III, and IV received formulations of the PN gel (200 mg). The animals were fasted overnight before experiment and deprived of water during the experiment. 0.1 ml of 1% carrageenan (wt/vol) in normal saline was intradermally injected into the left hind paw of rats. PN gel formulations (LCI-1-a, marketed preparation, and plain gel) were topically applied to the dorsal surface of rats (1.0 cm^2), 30 min before carrageenan injection.[27] The increase in paw thickness was measured with the help of plethysmometer before (time 0) and 30 min, 1, 2, 3, 4 and 5 h after carrageenan administration.[27] The percent inhibition was calculated as follows:

$$\text{Percent inhibition} = (Pc - PT/Pc) \times 100$$

Where, Pc is the increase in paw thickness of the control group and PT is the increase in paw thickness of the treatment groups.

viii. Stability study: Sufficient quantity of formulations were sealed in 5 g collapsible aluminum tube in triplicate and stored at refrigerated temperature (2-8°C) and room temperature (25 ± 2°C). Samples were withdrawn each month and analyzed for drug content, physical appearance, homogeneity, and viscosity.[28]

RESULTS AND DISCUSSION

Preparation and optimization of formulations of PN suspension

Evaluation of PN suspension

i. By SEM: SEM for the selected formulation LCI-1 was carried out and results are shown in Figure 1.

ii. By optical microscope: Microscopy for the selected formulation LCI-1 was carried out and result is shown in Figure 2.

iii. Determination of zeta potential: The formulation LCI-1 which was subjected to zeta potential analysis had a zeta value of −20.0 mV, which is a measure of net charge of proniosomes. High surface charge provides sufficient electrostatic repulsion between the vesicles which made them stable, by preventing aggregation and result is shown in Figure 3.

iv. Entrapment efficiency: Entrapment efficiency is the percentage fraction of the total drug incorporated into the proniosomes. Formulation LCI-1 (94.17 ± 0.95) and LDCI-1 (89.77 ± 0.83) exhibited very high entrapment efficiency. This could be explained on the basis that the highly lipophilic portion of the drug is expected to be

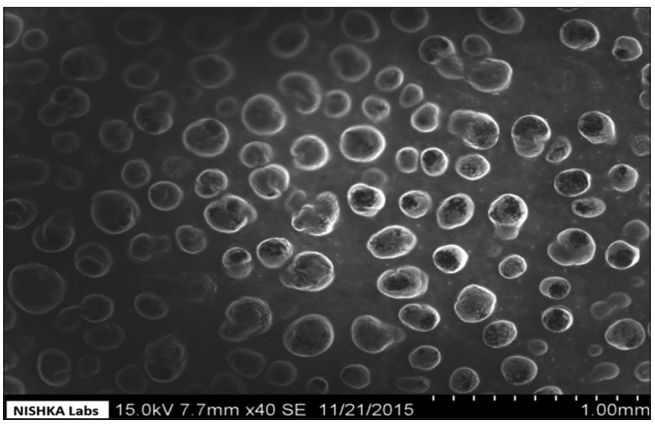

Figure 1: Proniosomes observed under scanning electron microscope

housed almost completely within the lipid bilayer of the proniosomes and result is shown in Figure 4.

v. *In-vitro* drug release: The release of KT from PN formulations containing isopropanol as solvent and sodium cholate as surfactant showed higher drug release, as compared to other surfactants (sodium deoxycholate and span-60) and solvents (butanol and ethanol) which were added to formulations. In the case of PN formulation LCI-1 which was prepared by addition of sodium cholate as surfactant showed 94.021% drug release and in the case of different solvents PN formulation LCI-1 which was prepared by addition of isopropanol as solvent showed 97.01% drug release and result is shown in Figure 5.

Preparation of PN gel

Evaluations of PN gel

i. Physical appearance and homogeneity: All the PN gel formulations had colorless to pale yellow appearance [Table 1]
Homogeneity: All the PN gel formulations showed good homogeneity with the absence of lumps [Table 1].

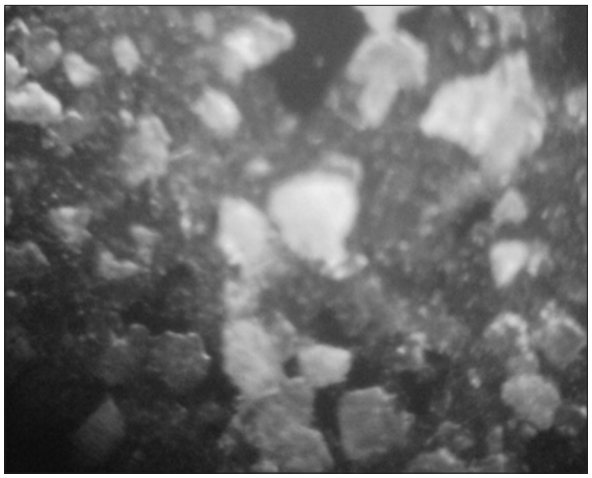

Figure 2: Microphotograph of proniosomes observed under optical microscope

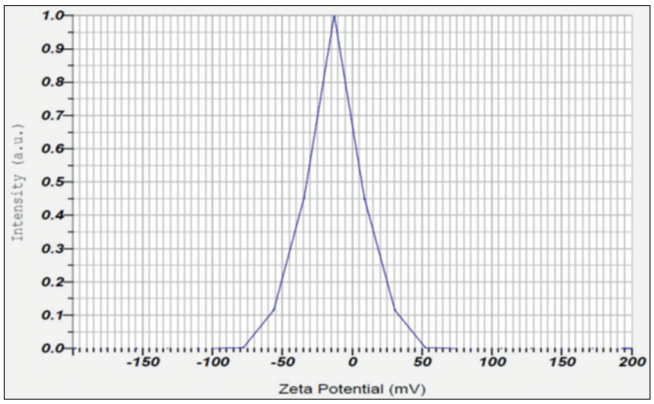

Figure 3: Zeta potential analysis

ii. Clarity: All the PN gel formulations were found to be transparent and free from presence of particles [Table 1]

iii. Viscosity: Viscosity was found in the range of 8529-10,511 cps [Table 1]

iv. Measurement of pH: The pH of all the PN gel formulations was in the range of 6.2-7.2, which lies in the normal pH range of the skin and would not produce any skin irritation [Table 1]

v. *Ex-vivo* permeability study: The results of *ex-vivo* permeation are shown in Figure 6. The flux values of, PN gel (LCI-1-a), LCI-2-b, and plain gel were found to be 7.518, 4.651, and 2.941 µg/cm² h, respectively. The permeability coefficient of PN gel (LCI-1-a), LCI-2-b and plain gel was found to be 3.759, 2.991 and 2.01 $cm.h^{-1}.10^{-3}$ [Table 2].

vi. *In-vitro* drug release of PN gel: The formulation LCI-1-a

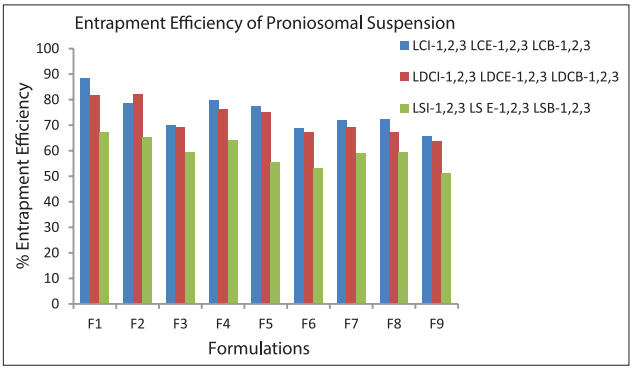

Figure 4: Entrapment efficiency of proniosomal suspension

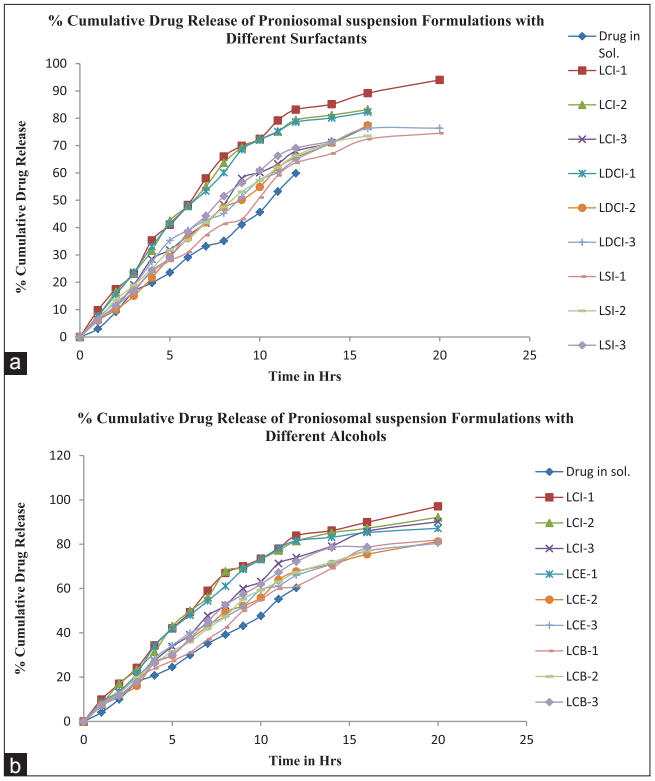

Figure 5: (a and b) % Cumulative drug release of proniosomal suspension

Table 1: Physical appearance, homogeneity, clarity, pH and viscosity of PN and plain gel

S. No.	Formulation code	Physical appearance	Homogeneity	Clarity	pH	Viscosity (cps)
F1	LCI-1-a	Colorless	Good	++	6.7±0.081	9840±1.632
F2	LCI-2-b	Colorless	Good	++	6.9±0.245	10,410±0.816
F3	LCI-3-c	Colorless	Good	++	6.5±0.164	10224±1.247
F4	LCE-1-d	Pale yellow	Good	++	6.4±0.286	9190±1.247
F5	LCE-2-e	Pale yellow	Good	++	7.2±0.087	9380±3.26
F6	LCE-3-f	Pale yellow	Good	++	7.0±0.091	10,340±1.47
F7	LCB-1-g	Colorless	Good	++	6.6±0.168	8529±2.054
F8	LCB-2-h	Colorless	Good	++	6.3±0.294	10,091±0.816
F9	LCB-3-i	Colorless	Good	++	6.2±0.085	10,511±0.816
Plain gel	-	Colorless	Good	++	6.5±0.113	10,220±1.632

B: Butanol, C: Sodium cholate, E: Ethanol, I: Isopropanol, L: Soya lecithin, + Turbid, + + Clear, + + + Very clear

Table 2: Permeation parameters of LCI-1-a, LCI-2-b and plain gel formulation

Formulation	Flux (J) μg/cm².h	Permeability coefficient (Kp) (cm.h⁻¹.10⁻³)
LCI-1-a	7.518±0.041	3.759±0.00005
LCI-2-b	4.651±0.069	2.991±0.00038
Plain gel	2.941±0.031	2.01±0.00001

C: Sodium cholate, I: Isopropanol, L: Soya lecithin

showed highest drug release of 94.05% in 17 h. After incorporation of PN vesicles into gel base, it showed a significant reduction in *in-vitro* drug release in 17 h as compared to PN suspension and result is shown in Figure 7.

vii. Pharmacodynamic study: Carrageenan-induced rat paw edema: The percentage inhibition of carrageenan-induced paw edema 5 h after applying the positive control was found to be 60.00%, the LCI-1-a gel was found to be 63.44% while LCI-1-b and LCE-d gel showed 57.44% and 49.08%, respectively [Table 3].

viii. Stability study: The stability studies of optimized PN gel (LCI-1, LCE-1 and LCB-1) formulations were performed as per ICH guideline at 5 ± 3°C and 25 ± 2°C/60 ± 5% RH. There were no aggregation, fusion or disruption of the vesicles during the study period and the prepared formulations could retain their multilamellar nature. At refrigerated condition (5 ± 2°C), the PN gel formulation (LCI-I-a) showed drug content 96.84% at the end of study durations, but the formulations (LCE-1-d and LCB-1-g) showed drug contents 95.78% and 80.68% [Table 4]. Thus, storage under refrigeration increases stability.

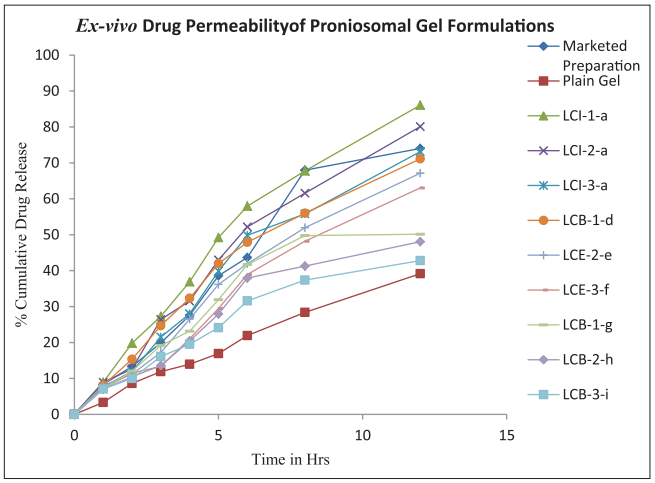

Figure 6: Cumulative amount of ketorolac tromethamine permeated through goat skin

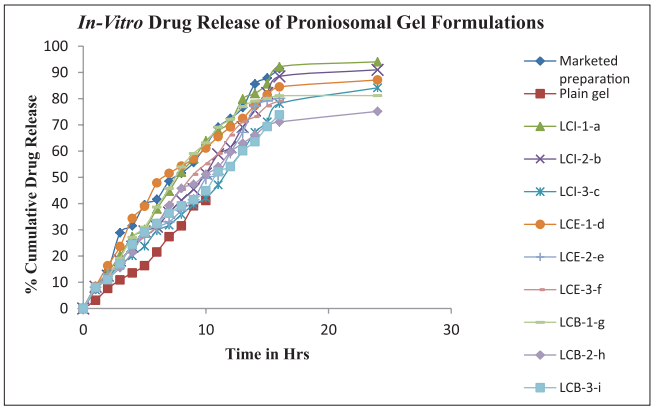

Figure 7: % Cumulative drug release of gel formulations

CONCLUSION

The results of the present investigation showed that the problems associated with the transdermal delivery of KT could be overcome by incorporating it into the new PN drug carrier, proniosomes. Among the three PN formulations developed for transdermal delivery of KT, LCI-1-a showed promising skin permeation potential, better stability, and higher entrapment efficiency than LCI-1-b and LCB-1-g formulation. LCI-1-a showed high drug release (94.048%) over 17 h after topical application of optimized PN formulation, as compared to LCI-1-b, LCB-1-g and marketed

Table 3: Anti-inflammatory activity of KT PN gel LCI-1-a, LCI-1-b and LCE-1-d

Group *N*=4	% Inhibition at different time intervals (h)							
	Time in h	0	30 min	1 h	2 h	3 h	4 h	5 h
Group I	Normal control	1.07±0.07	1.07±0.07	1.07±0.07	1.07±0.07	1.07±0.07	1.07±0.07	1.07±0.07
Group II	Negative control	1.09±0.07#	2.59±0.07#	3.06±0.04#	3.66±0.08#	3.76±0.05#	3.82±0.04#	3.83±0.05#
Group III	Positive control	1.12±0.06	2.47±0.07	2.72±0.07	1.07±0.07	1.65±0.04	1.58±0.05	1.53±0.06
	% inhibition	0.00	4.63	11.11	39.89	56.11	58.63	60.00
Group IV	LCI-1-a	1.04±0.04	2.49±0.05	2.59±0.07*	2.00±0.09*	1.58±0.04*	1.42±0.04*	1.40±0.04*
	% inhibition	0.00	3.86	15.35	45.35	57.97	62.82	63.44
Group V	LCI-1-b	1.12±0.02	2.48±0.05	2.70±0.07*	2.15±0.14*	1.73±0.06*	1.65±0.05*	1.63±0.05*
	% inhibition	0.00	4.24	11.76	41.25	53.98	56.80	57.44
Group VI	LCE-1-d	1.10±0.07	2.51±0.04	2.69±0.05*	2.35±0.07*	2.05±0.09*	1.97±0.08*	1.95±0.08*
	% inhibition	0.00	3.08	12.09	35.79	44.94	48.42	49.08

Value expressed as mean±SD. N=Number of animals per group. Statistical significance; #*P*<0.001 versus control; **P*<0.001 versus negative control: One-way ANOVA followed by Turkey's multiple comparison test. SD: Standard deviation, KT: Ketorolac tromethamine, PN: Proniosomal

Table 4: Stability studies performed as per ICH Guideline for optimized PN gel formulations

Formulation code	Characteristics	25±2°C/60±5% RH				5±3°C		
LCI-1-a	Color	White	NC	NC	NC	NC	NC	NC
LCE-1-d		Yellow	NC	NC	C	NC	NC	NC
LCB-1-g		Yellow	NC	NC	C	NC	NC	NC
LCI-1-a	Appearance of drug crystal	NA	NA	NA	NA	NA	NA	NA
LCE-1-d		NA	NA	NA	NA	NA	NA	NA
LCB-1-g		NA	NA	NA	A	NA	NA	NA
LCI-1-a	Morphology (optical microscopy)	Spherical vesicles	NC	NC	NC	NC	NC	NC
LCE-1-d		Spherical vesicles	NC	NC	C	NC	NC	NC
LCB-1-g		Spherical vesicles	NC	NC	C	NC	NC	NC

B: Butanol, C: Sodium cholate, E: Ethanol, I: Isopropanol, L: Soya lecithin, NC: No change, NA: Not appear, C: Change, PN: Proniosomal

formulation which suggests that PN formulation (LCI-1-a) is far way better mode of administration of KT through the systemic circulation.

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REFERENCES

1. Brahmankar DM, Jaiswal SB. A Text Book Biopharmaceutics and Pharmacokinetics a Treatise. 2nd ed. New Delhi: Vallabh Prakashan; 2009. p. 398.
2. Rooks WH 2nd, Maloney PJ, Shott LD, Schuler ME, Sevelius H, Strosberg AM, *et al.* The analgesic and anti-inflammatory profile of ketorolac and its tromethamine salt. Drugs Exp Clin Res 1985;11:479-92.
3. Alsarra IA, Bosela AA, Ahmed SM, Mahrous GM. Proniosomes as a drug carrier for transdermal delivery of ketorolac. Eur J Pharm Biopharm 2005;59:485-90.
4. Chen XH, Bai JY, Shen F, Bai AP, Guo ZR, Cheng GF. Imrecoxib: A novel and selective cyclooxygenase 2 inhibitor with anti-inflammatory effect. Acta Pharmacol Sin 2004;25:927-31.
5. Draize JH, Woodard G, Calvery HO. Method for the study of irritation and toxicity of substances applied topically to the skin and mucus membranes. J Pharmacol Exp Ther 1994;82:377-89.
6. Fry DW, White JC, Goldman ID. Rapid separation of low molecular weight solutes from liposomes without dilution. Anal Biochem 1978;90:809-15.
7. Gopinath D, Patel B, Deo M, Parakh SR. Proliposomes: A review. Indian Drugs 1997;34:181-3.
8. Hen G, Kim D, Chien YW. Dual controlled transdermal delivery of levonorgestrel and estradiol: Enhanced permeation and modulated delivery. J Control Release 1995;34:129-43.
9. Honeywell-Nguyen PL, Bouwstra JA. The *in vitro* transport of pergolide from surfactant-based elastic

vesicles through human skin: A suggested mechanism of action. J Control Release 2003;86:145-56.
10. Jain S, Umamaheshwari RB, Tripathi P, Jain NK. Ultra deformable liposomes: A recent tool for effective transdermal drug delivery. Indian J Pharm Sci 2003;65:223-31.
11. Kokki H. Nonsteroidal anti-inflammatory drugs for postoperative pain: A focus on children. Paediatr Drugs 2003;5:103-23.
12. El Maghraby GM, Williams AC, Barry BW. Oestradiol skin delivery from ultradeformable liposomes: Refinement of surfactant concentration. Int J Pharm 2000;196:63-74.
13. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
14. Ozgüney IS, Karasulu HY, Kantarc G, Sözer S, Güneri T, Ertan G. Transdermal delivery of diclofenac sodium through rat skin from various formulations. AAPS PharmSciTech 2006;7:E1-7.
15. Puglia C, Filosa R, Peduto A, de Caprariis P, Rizza L, Bonina F, *et al.* Evaluation of alternative strategies to optimize ketorolac transdermal delivery. AAPS PharmSciTech 2006;7:64.
16. Schreier R, Bouwstra J. Liposomes and niosomes as topical drug carriers: Dermal and transdermal drug delivery. J Control Release 1994;30:1-15.
17. Subramanian N, Yajnik A, Murthy RS. Artificial neural network as an alternative to multiple regression analysis in optimizing formulation parameters of cytarabine liposomes. AAPS PharmSciTech 2004;5:E4.
18. Vora B, Khopade AJ, Jain NK. Proniosome based transdermal delivery of levonorgestrel for effective contraception. J Control Release 1998;54:149-65.
19. Fathalla D, Abdel-Mageed A, Abdel-Hamid F, Ahmed M. *In-vitro* and *in-vivo* evaluation of niosomal gel containing aceclofenac for sustained drug delivery. Int J Pharm Sci Res 2014;1:1-11.
20. Nasseria AA, Aboofazelib R, Zia H, Needhama TE. Lecithin–stabilized microemulsion–based organogels for topical application of ketorolac tromethamine. II. *In vitro* release study. Iran J Pharm Res 2003;2:117-23.
21. Gupta A, Prajapati SK, Balamurugan M, Singh M, Bhatia D. Design and development of a proniosomal transdermal drug delivery system for captopril. Trop J Pharm Res 2007;6:687-93.
22. Mayuren C, Ilavarasan R. Anti-infl ammatory activity of ethanolic leaf extracts from Adenanthera pavonina (L) in Rats. Journal of Young Pharmacists 2009;1(2):125-8.
23. Aggarwal A, Saroha K, Nanda S. Formulation, evaluation and comparison of ketorolac tromethamine transdermal gel containing natural and synthetic permeation enhancers. Der Pharm Sin 2014;5:41-5.
24. Tavano L, Gentile L, Oliviero Rossi C, Muzzalupo R. Novel gel-niosomes formulations as multicomponent systems for transdermal drug delivery. Colloids Surf B Biointerfaces 2013;110:281-8.
25. Jain S, Garg VK, Sharma PK. Anti-inflammatory activity of aqueous extract of *Beta vulgaris* L. J Basic Clin Pharm 2011;2:83-6.
26. Habiba FS, Hassana MA, Sayeh F, Ela AE, Raheem RE. Different topical formulations of ketorolac tromethamine for anti-inflammatory application and clinical efficacy. Dig J Nanomater Biostruct 2014;9:705-19.
27. Jain PS, Bari SB. Anti-inflammatory activity of Abelmoschus manihot extracts. Int J Pharmacol 2010;6(4):505-9.
28. Sato K, Sgibayashi K, Morimoto Y, Species difference in percutaneous absorption of nicorandil. J Pharm Sci. 1991;80:104-7.

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