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FORMULATION AND DEVELOPMENT OF KETOROLAC TROMETHAMINE PROTRANSFEROSOMAL GEL

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ABSTRACT

In present study proultraflexible lipid vesicles "protransfersome" were proposed that will be converted into the ultraflexible vesicles, transfersomes in situ by absorbing water from the skin. The proposed system is more stable and having higher entrapment efficiency.Protransferosomal gel of Ketorolac tromethamine used for nociceptive somatic pain . Protransfersomal gel (PTG) of Ketorolac tromethamine was prepared by the method in which lecithin was used as vesicle forming component, surfactant for providing flexibility, alcohol as a solvent and aqueous phase (buffering agent) as a hydrating medium. Optimization of the formulations was done by selecting three process variables: effect of lecithin: surfactant ratio, effect of various solvents and effect of surfactants. The effect of these three factors was studied on the entrapment efficiency of drug, vesicle size and optical microscopy of drug. Among all the formulations, LCI-1 was selected as a optimized formulation. LCI-1 (94.17 ± 0.95) exhibited very high entrapment efficiency. Protransfersomes may be a promising carrier for Ketorolac and other drugs, especially due to their simple production and facile scale up.

INTRODUCTION

There has been keen interest in the development of a novel drug delivery system. Novel drug delivery system aims to deliver the drug at a rate directed by the needs of the body during the period of treatment, and channel the active entity to the site of action. A number of novel drug delivery systems have emerged encompassing various routes of administration, to achieve controlled and targeted drug delivery. Encapsulation of the drug in vesicular structures is one such sy3stem, which can be predicted to prolong the existence of the drug in systemic circulation, and reduce the toxicity, if selective uptake can be achieved. Consequently a number of vesicular drug delivery systems such as liposomes, niosomes, transfersomes, and pharmacosomes were developed [8,16]

More recent approach to drug delivery is to deliver the drug in to systemic circulation at a predetermined rate which is known as controlled release drug delivery system. Such systems helped to overcome the side effects associated with conventional system of medication, which require multidose therapy. In recent years, the development of transdermal dosage forms has been attracting increasing attention, owing to the several advantages that this administration route offers. Transdermal delivery system, when compared with conventional formulations, generally show a better control of blood levels, a reduced incidence of systemic toxicity, no hepatic first pass metabolism, and a higher compliance**.** Transdermal therapeutic systems are defined as self contained discrete dosage forms which when applied to the intact skin deliver the drug through the skin at a controlled rate to the systemic circulation. Thus it is anticipated that transdermal drug delivery system (TDDS) can be designed to input drugs at appropriate rates to maintain suitable plasma drug levels for therapeutic efficacy by using skin as the port of entry of drugs **.**Nevertheless, drug delivery via the skin is not an easy task because of the formidable barrier properties of the stratum corneum (SC). The majority of drugs do not appear to penetrate the skin at a sufficiently high rate to have therapeutic effectiveness [12]

One of the possibilities for increasing the penetration of drugs through the skin is the use of vesicular systems such as liposomes. Due to their biocompatibility and capability of incorporating both hydrophilic and lipophilic drugs, liposomes have recently been investigated as transdermal drug delivery systems**. T**ransdermal delivery is important because it is a noninvasive procedure for drug delivery. Further, problem of drug degradation by digestive enzymes after oral administration and discomfort associated with parenteral drug administration can be avoided. It is the most preferred route for systemic delivery of drugs to

pediatric, geriatric and patients having dysphasia. Hence, transdermal dosage forms enjoy being the most patient compliant mode of drug delivery.

1.1 Transdermal Drug Delivery System:

Transdermal therapeutic systems are defined as self-contained, discrete dosage forms which, when applied to the intact skin, deliver the drug(s), through the skin at a controlled rate to the systemic circulation[13]

1.1.1 Rationale for selecting the transdermal drug delivery system:

- Convenient and safe
- Increased patient acceptability (Non-invasiveness)
- Avoid GIT degradation and first pass effect
- Avoid GIT disturbances due to drugs
- Minimize side effects
- Avoiding fluctuation

1.3 Transfersomes (Vesicular System):

Transfersomes are vesicles, which are self optimized aggregates with ultra flexible membrane. These vesicular transfersomes are more elastic than the standard liposomes and thus well suited for the skin penetration.[14]

1.3.1 Rationale for selecting the lipid vesicles (Transfersomes) as a TDDS:

There are several instances where the most convenient drug intake methods, like oral route, were not feasible and alternative routes had to be sought. Although, intravenous administration of the medicament avoids many of these shortfalls (such as gastrointestinal and hepatic metabolism), its invasive and apprehensive nature (particularly for chronic administration) has encouraged the search for alternative strategies. Transdermal/Topical drug delivery offers several distinct advantages including relatively large and readily accessible surface area for absorption, ease of application and termination of therapy.

- Transfersomes are amphiphilic in nature so able to accommodate both hydrophilic as well as lipophilic drugs.
- Transfersomes release the drug in a sustained manner for a prolonged period of time at a predetermined rate.

- Transfersomes can deform and pass through narrow constriction (from 5-10 times less than their own diameter) without measurable loss.
- Transfersomes can act as a carrier for low and high molecular weight drugs
- Transfersomes have high entrapment efficiency.
- Transfersomes are used for both, topical and systemic delivery of drugs
- They protect the encapsulated drug from metabolic degradation.[16]

1.3.2 Limitations of transfersomes:

- Chemically unstable
- Expensive
- Less purity of phospholipids

TABLE 1.3: DIFFERENT DRUGS USED AND RESULTS OBTAINED OF DIFFERENT STUDIES OF ELASTIC LIPOSOMES FOR TRANSDERMAL APPLICATION

S.No	System	Drug	Results	References
1	Transfersomes	Insulin	High entrapment efficiency, Improved transdermal flux.	Cevc et al., 2003
2	Transfersomes	Interferon-α	Efficient delivery means (because delivery by other routes is difficult).	Hofer et al., 2000
3	Transfersomes	Interleukin-2	Controlled release, reduce stability problem.	Hofer et al., 2004
4	Transfersomes	Soluble proteins	Permits non-invasive immunization.	Paul et al, 1998
5	Transfersomes	Hydrocortisone Dexamethasone	Increased biological potency, Prolonged effect, Reduced dosage.	Cevc et al., 2004
6	Transfersomes	Triamcinolone acetonide	Both for local and systemic delivery.	Cevc et al., 2003
7	Transfersomes	Diclofenac Tetracaine Lidocaine	Non-invasive treatment of local pain on direct topical application.	Cevc et al., 2001
8	Transfersomes	Oestradiol	Improved transdermal flux.	Maghraby et al., 1999
9	Transfersomes	Tamoxifen	Improved transdermal flux.	Gamal et al., 1999
10	Elastic liposome	Zidovudine	Sustained drug delivery.	Jain et al., 2006
11	Transfersomes	Vaccine	Both for Local and Systemic delivery	Gupta et al., 2005

1.3.3 Vesicular approaches for transdermal drug delivery:

Lipids present in the skin contribute to the barrier properties of skin and prevent systemic absorption of drugs. Due to the ampiphilic nature, lipid vesicle may serve as non-toxic penetration enhancer for drugs. In addition, vesicles can be used for encapsulating hydrophilic and lipophilic as well as low and high molecular weight drugs. Therefore these lipid rich vesicles are hypothesized to carry significant quantity of drugs across the skin thus, enhancing the systemic absorption of drugs.[16]

1.3.4 Provesicular approach:

The vesicular approach i.e. Liposome in transdermal drug delivery system has been studied for many purposes but the unstable nature limits their use at clinical and industrial levels. In order to increase the stability of liposomes concept of proliposomes has been proposed. This approach has been extended to niosomes, which exhibit superior stability compared to traditional liposomes and overcome their limitations by proniosomal approach. But all approaches, because of poor skin permeability, breaking of vesicles, leakage of drug, aggregation and fusion of vesicles are not much successful for effective transdermal drug delivery.

To overcome problems of poor skin permeability recently introduced two new vesicular carrier system, transfersomes for non-invasive delivery of drugs into or across the skin. Transfersomes incorporated edge activators (surfactants) to influence the properties of vesicles and stratum corneum. Provesicular approach has been extended to the transfersome. Protransfersome developed for transdermal delivery which possess superior skin penetration ability and better stability. Each transfersomes consists of at least one inner aqueous compartment; this is surrounded by a lipid bilayer, but the problem in this carrier is self-stability. Transfersomes are also known as elastic liposomes, which pass through nanometer pores of the stratum corneum.So in present study liquid crystalline proultraflexible lipid vesicles (protransfersome) were proposed that will be converted into the ultraflexible lipid vesicles (transfersome) in situ by absorbing the water from the skin.[8]

1.3.5 Protransfersome:

Protransfersome developed for transdermal delivery possessed superior skin penetration ability and better stability. Protransfersome are reported to have lamellar liquid crystalline structure which will be converted into the ultraflexible vesicles, transfersomes (also known as elastic liposomes), in situ by absorbing water. [8]

1.3.6 Proposed mechanism for transfersomes skin penetration:

The presence of surface active agent in the transfersomes enhance the rheological properties and sensitivity to the driving force (Osmotic gradient), which results from water concentration gradient across the skin. This enhances the propensity of sufficiently large but deformable penetrants, transfersomes to move across the skin barrier (fig. 1.5). Such capacity combined with inclination to deform into elongated shapes, although maintaining the vesicle integrity can explain the usually high efficiency of transfersomes across the skin.[16]

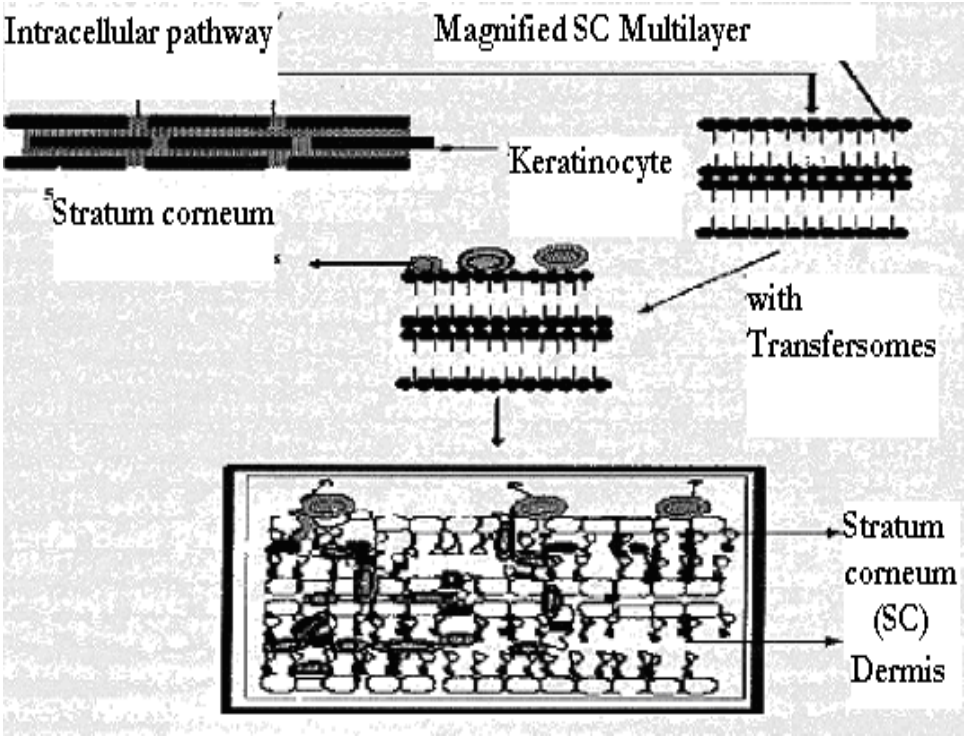


Fig. 1.5: Mechanism of penetration of transfersomes

Transfersomes when applied under suitable conditions (Non occlusive) can transfer 0.1 to 0.5 mg of lipid per hour and cm^2 area across the intact skin. This value is substantially higher than that which is typically driven by the transdermal concentration gradient. Epidermis (75% water content) and completely dry stratum corneum, near to skin surface (15% water content). All polar lipids attract some water; this is due to the interaction between the hydrophilic lipid residue and their proximal water. Lipid bilayers resist dehydration, consequently all polar lipid vesicles move to the site with a sufficiently high water concentration.

1.4.3.1 NonSteroidal Anti-Inflammatory Drugs (NSAIDs):

The NSAIDs achieve their effects by blocking the activity of cyclooxygenase. In addition to reducing the fever and pain of inflammation, NSAIDs also inhibit clotting.

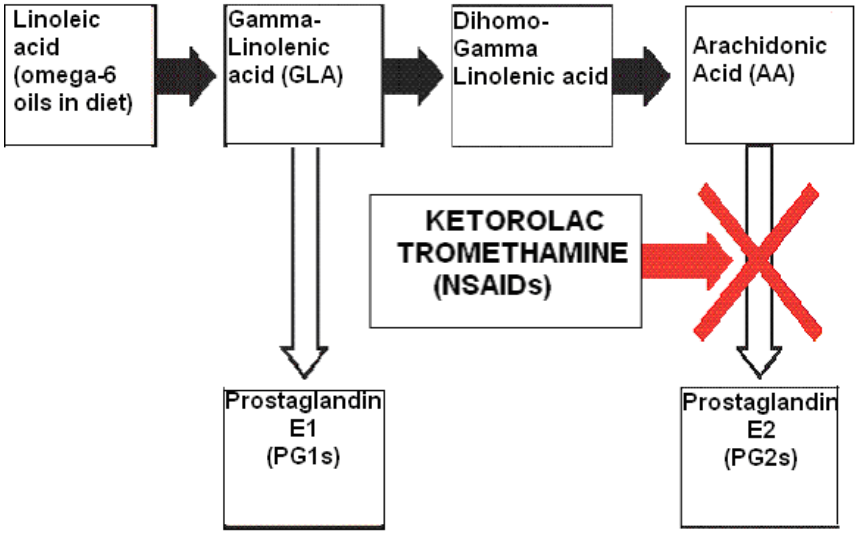


Fig. 1.9: Inhibition of inflammation by NSAIDs

Material:

Soya lecithin

Sodium cholate

sodium deoxycholate

Span 80

Isopropyl alcohol

Ethanol

Butanol

Carbopol 940

Method:

The present investigation aimed at formulation and performance evaluation, of new vesicular drug carrier system protransfersomes for transdermal delivery of the anti-inflammatory agent, Ketorolac tromethamine. Protransfersome gel (PTG) formulations of Ketorolac tromethamine were prepared and characterized for vesicle shape, size, entrapment efficiency, and drug permeation across human cadaver skin. The stability studies of the optimized formulation were also carried out[14]

4.1 Preparation of Protransfersomal gel (PTG):

Protransfersomal gel (PTG) was prepared by using Soya lecithin (Phospholipid), sodium cholate/sodium deoxycholate/span 80 (Surfactant) and isopropyl alcohol/ethanol/butanol (Alcohol). All the ingredients were taken in a small, clean, dry and wide mouth round bottom flask and the open end was covered to prevent the loss of solvent. The flask was warmed in a water bath at 60-70^{0}C, until all the ingredients were dissolved. The Ketorolac tromethamine in PBS (pH 7.4) was added (approx 2 ml) to the round bottom flask and warmed on a water bath until clear solution was formed. This mixture was converted into protransfersomal gel on cooling. The proliposomal gel (PC + Ch: alcohol:aqueous phase, 5:4:5 w/w and drug concentration 1% w/w) that was used for comparison was also prepared by using the above method. Plain drug gel was prepared by carbopol 940 (2 gm.) by means of 10 ml solution of drug in PBS pH 7.4.

- Optimization of variables:

The preparation of PTG containing Ketorolac tromethamine involves various formulation variables, which could affect the preparation and properties of the PTG, but optimization was done by selecting three of them.[14]

- Effect of Lecithin: Surfactant ratio
- Effect of various solvents
- Effect of various surfactants

TABLE 4.1: COMPOSITION OF PTG WITH SODIUM CHOLATE

S.No.	Formulation code	PC:S (w/w)	Alcohol
A	LCI-1	1.71:0.3	Isopropanol
B	LCI-2	2.1:0.3	Isopropanol
C	LCI-3	0.9:0.3	Isopropanol
D	LCE-1	1.71:0.3	Ethanol
E	LCE-2	2.1:0.3	Ethanol
F	LCE-3	0.9:0.3	Ethanol
G	LCB-1	1.71:0.3	Butanol
H	LCB-2	2.1:0.3	Butanol
I	LCB-3	0.9:0.3	Butanol

TABLE 4.2:COMPOSITION OF PTG WITH SODIUM DEOXYCHOLATE

S.No.	Formulation code	PC:S (w/w)	Alcohol
A	LDCI-1	1.71:0.3	Isopropanol
B	LDCI-2	2.1:0.3	Isopropanol
C	LDCI-3	0.9:0.3	Isopropanol
D	LDCE-1	1.71:0.3	Ethanol
E	LDCE-2	2.1:0.3	Ethanol
F	LDCE-3	0.9:0.3	Ethanol
G	LDCB-1	1.71:0.3	Butanol
H	LDCB-2	2.1:0.3	Butanol
I	LDCB-3	0.9:0.3	Butanol

TABLE 4.3: COMPOSITION OF PTG WITH SPAN-80

S.No.	Formulation code	PC:S (w/w)	Alcohol
A	LSI-1	1.71:0.3	Isopropanol
B	LSI-2	2.1:0.3	Isopropanol
C	LSI-3	0.9:0.3	Isopropanol
D	LSE-1	1.71:0.3	Ethanol
E	LSE-2	2.1:0.3	Ethanol
F	LSE-3	0.9:0.3	Ethanol
G	LSB-1	1.71:0.3	Butanol
H	LSB-2	2.1:0.3	Butanol
I	LSB-3	0.9:0.3	Butanol

PC indicates phosphatidyl choline (Soya); S,surfactant; L,Lecithin; C, NaCholate;

DC, Na Deoxycholate; S**,** Span 80; I, Isopropyl alcohol; E, Ethanol; B, Butanol;

TABLE 4.4: FORMULA FOR PREPARATION OF OPTIMIZED PROTRANSFERSOMAL GEL:

S.No.	Ingredients of protransfersomal gel (PTG)	Quantity
1	Drug	0.01 g.
2	Soya lecithin	1.7 g.
3	Sodium cholate / Sodium deoxycholate	0.3 g.
4	Alcohol (Isopropyl alcohol)	2.0 ml
5	PBS (pH 7.4)	2.0 ml

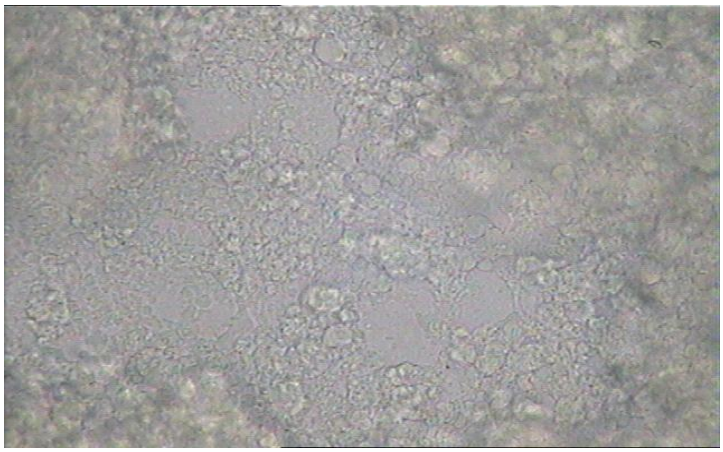
RESULT:

4.3 In vitro characterization:

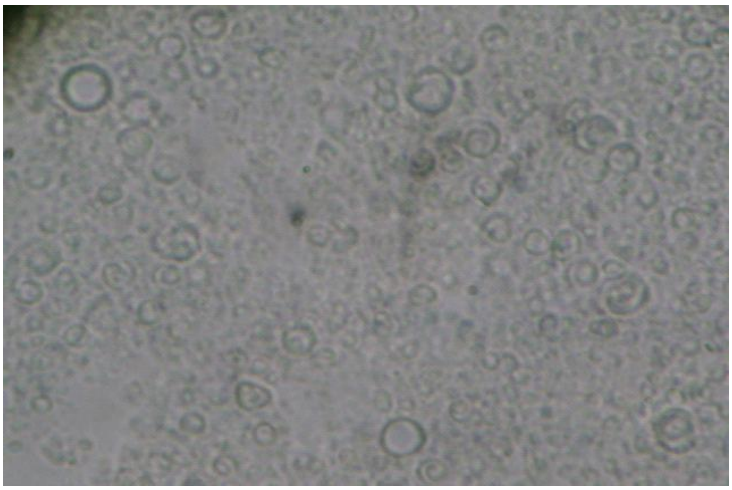
4.3.1 Visualization of vesicles:

4.3.1.1 Visualization of vesicles by optical microscopy:

A thin layer of PTG was spread on a slide and a drop of phosphate buffer was added through the side of the cover slip into the cavity slide and observed by optical microscope (Photomicrograph 4.1a and 4.1b). Photomicrographs were taken at 400**x**, before and after addition of water (Labomed). [14]



(a)

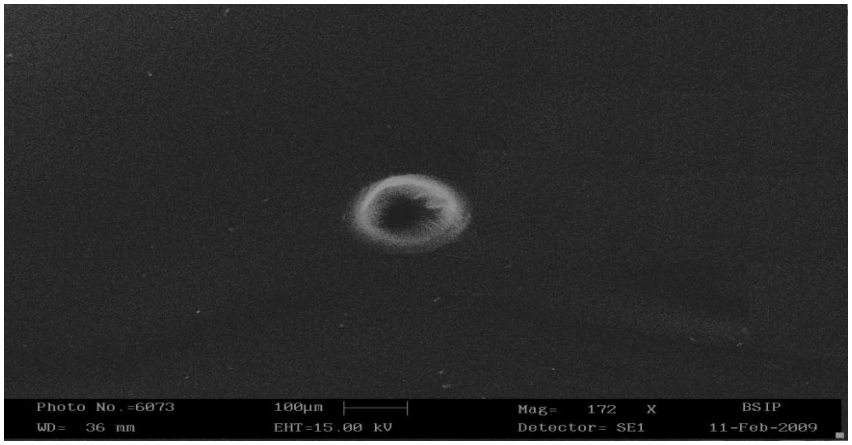


(b)

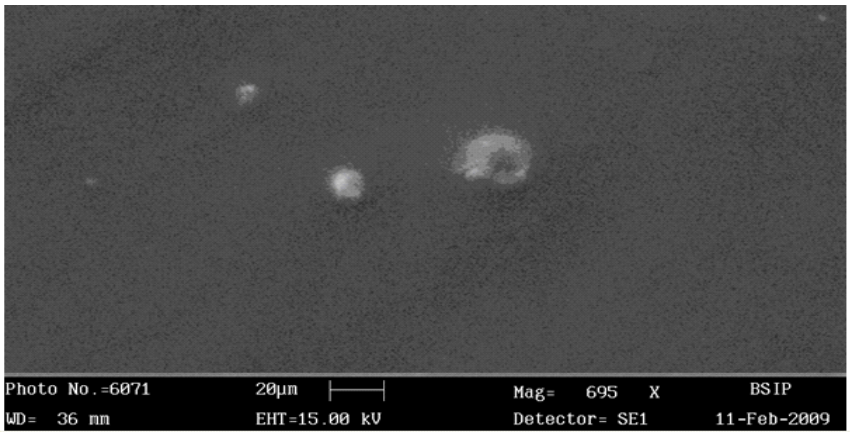
Photomicrograph *4.*1 Visualization of lamellar structure of protransfersomal gel (a) and vesicular structure of transfersomes formed upon hydration of protransfersomal gel (b) at X400 magnification.

4.3.1.2 Visualization of vesicles by scanning electron microscope (SEM):

The shape, surface characteristics, and size of the transfersomes were observed by scanning electron microscopy (SEM) (Photomicrograph. 4.2a and 4.2b). 0.2 g of the protransfersomal gel in a glass tube was diluted with 10 ml of phosphate buffer saline (pH 7.4). The lyophilized transfersomes were mounted on an aluminum stub using double-sided adhesive carbon tape. Then the vesicles were sputter-coated with gold palladium (Au/Pd) using a vacuum evaporator (Edwards) and examined using a scanning electron microscope JSM-5510 (Jeol Ltd, Tokyo, Japan) equipped with a digital camera, at 20 kV accelerating voltage. [(11)]



(a)

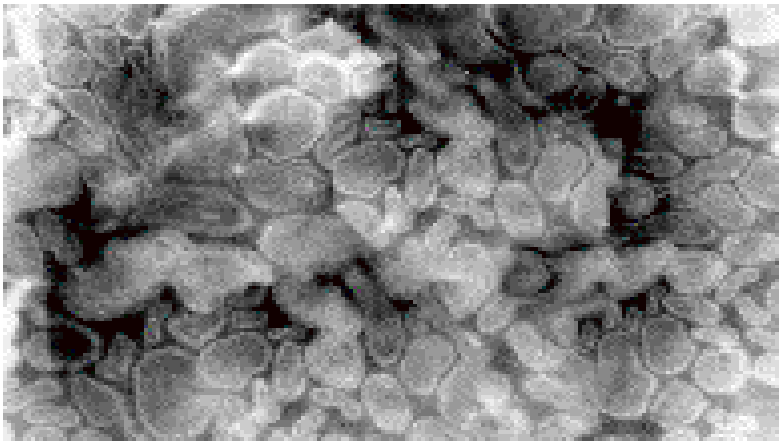


(b)

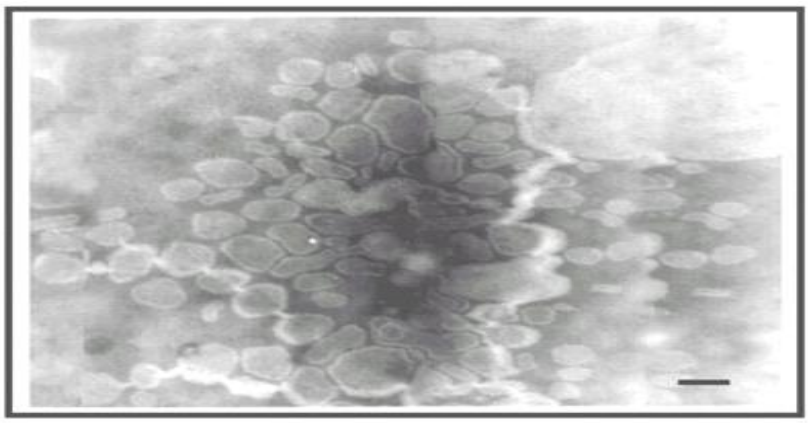
Photmicroograph 4.2 vesicle with out drug (a) and drug-loaded vesicle (b), observed by SEM.

4.3.1.3 Visualization of vesicles by transmission electron microscope (TEM):

Reconstitution of transfersomes from PTG formulation after hydration was confirmed by Transmission electron microscopy (TEM) (Photomicrograph 4.3). Samples were prepared by adding phosphate buffer (pH 7.4) to PTG and shaking the mixture manually for 1 minute. A drop of the sample was placed on a carbon-coated copper grid after 15 minutes and negatively stained with 1% aqueous solution of phosphotungstic acid. The grid was allowed to air dry thoroughly and samples were viewed on a TEM (Philips, TEM, New Brunswick, Canada).



(a)



(b)

Photomicrograph 4.3 TEM photomicrograph of optimized PTG formulation (LCI-1) (a) and LDCI-1(b)

4.3.2 Vesicle size analysis:

Optimized PTG (LCI-1) 100 mg was hydrated with 10 mL of PBS (pH 7.4) using manual shaking for 5 minutes. The vesicle size (Table 4.6, 4.7 and 4.8) of protransfersomal gel after hydration was determined by dynamic light scattering (DLS) method using Zeta Sizer (Nano ZS, Malvern Instruments, UK) [14]

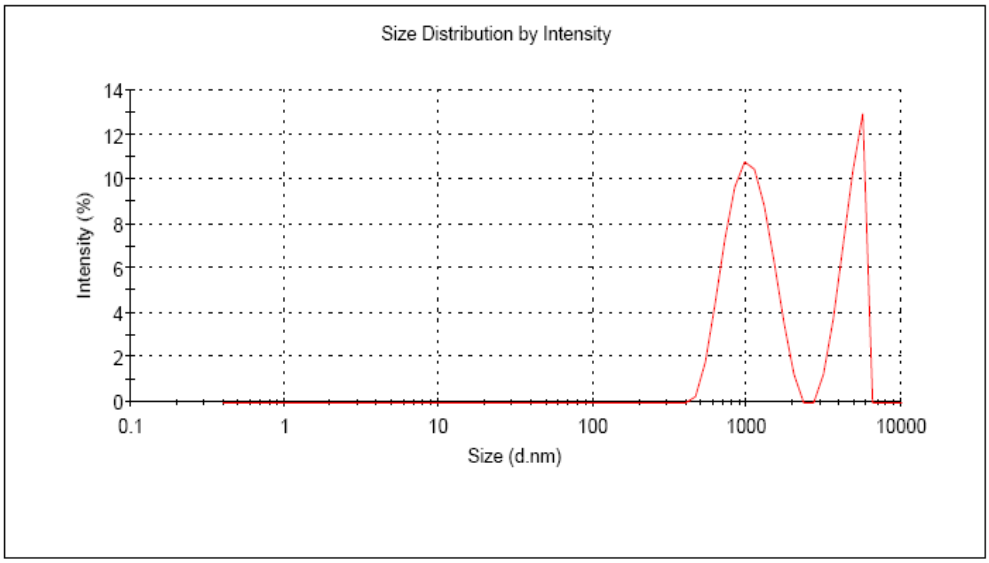


Fig. 4.1 size distribution curve of optimized PTG formulation (LCI-1)

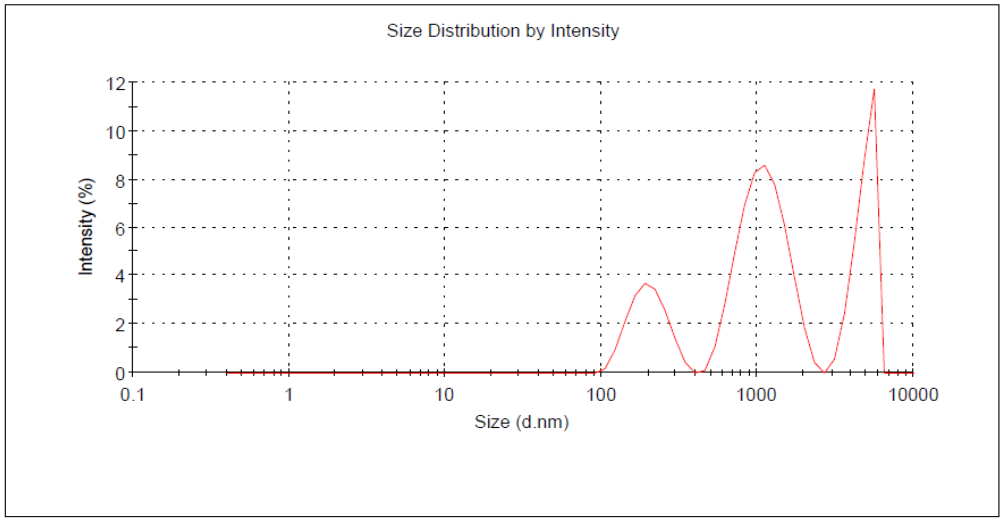


Fig. 4.2 size distribution curve of optimized PTG formulation (LDCI-1)

4.3.3 Determination of Zeta-Potential:

The method involves the preparation of dispersion of Protransfersomal gel in PBS (pH 7.4). Then this dispersion was filled in zeta cell and placed in the Zeta Sizer (Nano ZS, Malvern Instruments, UK) to determine the zeta-potential. The values of zeta potential are as follows:

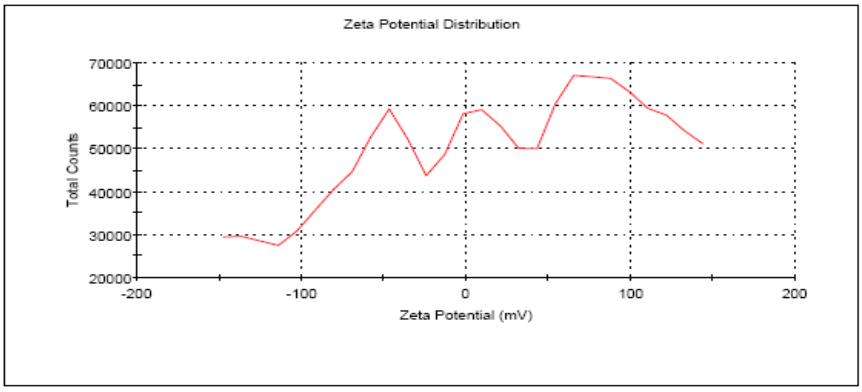


Fig. 4.3 zeta potential analysis of optimized PTG formulation (LCI-1)

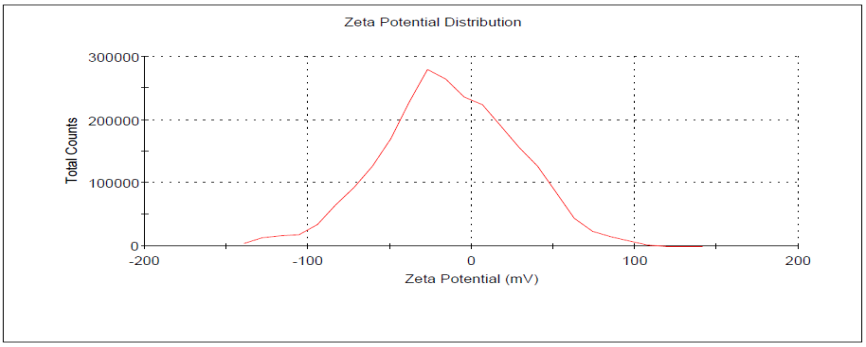


Fig. 4.4 zeta potential analysis of optimized PTG formulation (LDCI-1)

TABLE 4.5: VESICLE SIZE AND ZETA POTENTIAL ANALYSIS OF OPTIMIZED PTG FORMULATIONS

S.No.	Formulation	Average vesicle size (nm)	Zeta potential (mV)
1.	LCI-1	0984 ± 32	-2.84 ± 0.1
2.	LDCI-1	1834 ± 47	-13.9 ± 0.8

Values represented as mean ± SD, n=3

4.3.4 Determination of entrapment efficiency:

The entrapment efficiency was determined after separating the unentrapped drug. Protransfersomes gel (100mg) was hydrated with 10 ml of phosphate buffer saline (pH

7.4) using manual shaking for 5 minutes, to form transfersomal dispersion. For the separation of unentrapped drug the transfersomal dispersion was centrifuged at 15000 rpm for 30 minutes at 4^0C. The clear supernatant was siphoned off carefully to separate the unentrapped drug. The sediment (1ml) was resuspended in 1ml of Triton X-100 (0.1% v/v) was used to lyse the transfersomes after appropriate dilution of the sample with PBS (pH 7.4), absorbance was recorded at λ_{max} 323 nm.[14,15]

$$\text{Percentage entrapment} = \frac{\text{Amount of drug in sediment x100}}{\text{Total amount of drug added}}$$

TABLE 4.6: ENTRAPMENT EFFICIENCY AND VESICLE SIZE OF DIFFERENT COMPOSITION OF PROTRANSFERSOMAL GEL (PTG) FORMULATION (LECITHIN WITH SODIUM CHOLATE)

S.No.	Formulation code	% Entrapment efficiency	Vesicle size (nm)
a	LCI-1	94.17 ± 0.95	984 ± 32
b	LCI-2	78.60 ± 0.75	1072 ± 26
c	LCI-3	69.90 ± 0.96	1012 ± 22
d	LCE-1	89.64 ± 0.96	1243 ± 28
e	LCE-2	77.50 ± 0.80	1174 ± 42
f	LCE-3	68.80 ± 2.15	1120 ± 36
g	LCB-1	81.70 ± 0.45	1072 ± 27
h	LCB-2	75.27 ± 1.20	1202 ± 40
i	LCB-3	65.50 ± 1.40	1448 ± 37

Values represented as mean ± SD, n=3

TABLE 4.7: ENTRAPMENT EFFICIENCY AND VESICLE SIZE OF DIFFERENT COMPOSITION OF PROTRANSFERSOMAL GEL (PTG) FORMULATION (LECITHIN WITH SODIUMDEOXY CHOLATE)

S.No.	Formulation code	% Entrapment efficiency	Vesicle size (nm)
A	LDCI-1	90.77 ± 0.83	1834 ± 47
B	LDCI-2	82.00 ± 0.56	1889 ± 32
C	LDCI-3	69.22 ± 1.81	1842 ± 28
D	LDCE-1	83.20 ± 1.10	1677 ± 24
E	LDCE-2	76.00 ± 1.05	1942 ± 18
F	LDCE-3	67.30 ± 1.60	1900 ± 19
G	LDCB-1	80.10 ± 0.9	1800 ± 31
H	LDCB-2	77.10 ± 1.65	2000 ± 33
I	LDCB-3	67.70 ± 2.01	1934 ± 27

Values represented as mean ± SD, n=3

TABLE 4.8: ENTRAPMENT EFFICIENCY AND VESICLE SIZE OF DIFFERENT COMPOSITION OF PROTRANSFERSOMAL GEL (PTG) FORMULATION (LECITHIN WITH SPAN 80)

S.No.	Formulation code	% Entrapment efficiency	Vesicle size (nm)
A	LSI-1	68.0 ± 2.04	2248 ± 21
B	LSI-2	65.0 ± 1.28	1754 ± 16
C	LSI-3	59.33 ± 2.05	2108 ± 36
D	LSE-1	66.90 ± 1.41	1600 ± 32
E	LSE-2	64.60 ± 1.13	1178 ± 20
F	LSE-3	59.00 ± 1.62	1300 ± 16
G	LSB-1	65.80 ± 1.62	1154 ± 19
H	LSB-2	66.20 ± 2.19	2470 ± 39
I	LSB-3	58.20 ± 1.66	1734 ± 30

Values represented as mean ± SD, n=3

4.3.5 *Invitro* drug permeation study:

Permeation of Ketorolac tromethamine from different PTG formulations was studied using a Franz glass diffusion cell. The effective permeation area of the diffusion cell and receptor cell volume was 1 cm^2 and 10 ml, respectively. The receptor compartment contained PBS (pH 7.4) and maintained at 37^0C ± 1°C by magnetic stirrer. Egg membrane was mounted between the donor and receptor compartment. PTG (200mg) containing 1 mg of drug was applied to the surface of the egg membrane. Samples were withdrawn through the sampling port of the diffusion cell at predetermined intervals over 48 hours and analyzed by UV-Visible Spectrophotometer (SHIMADZU 1700 JAPAN) at 323nm. An equal volume of fresh PBS, pH 7.4 was replaced into the receptor compartment after each sampling. (Fig. 4.5)

TABLE 4.9: INVITRO DRUG PERMEATION OF PTG, PLG AND PDG FORMULATION

Time (hours)	Cumulative drug permeated (µg)		
	PTG (LCI-1)	PLG	PDG
1	69.44 ± 3.83	34.10 ± 4.95	0.407 ± 0.005
2	98.21 ± 3.08	54.86 ± 4.50	0.862 ± 0.02
3	170.31 ± 6.04	77.24 ± 5.23	01.85 ± 0.20
4	241.94 ± 5.26	103.51 ± 5.19	03.01 ± 0.08
5	289.30 ± 5.76	145.32 ± 6.44	05.18 ± 2.78
6	350.38 ± 6.62	170.48 ± 4.57	06.30 ± 3.24
7	414.14 ± 8.63	190.36 ± 4.95	09.56 ± 5.57
8	473.00 ± 11.74	200.19 ± 3.80	14.45 ± 5.78
9	519.56 ± 11.96	220.22 ± 3.71	17.77 ± 4.06
10	574.75 ± 8.90	242.66 ± 3.31	22.27 ± 4.64
11	608.19 ± 12.86	258.75 ± 4.95	29.18 ± 3.83
12	642.25 ± 10.26	288.21 ± 3.75	34.52 ± 2.90
24	737.64 ± 13.17	348.08 ± 3.06	37.56 ± 4.89
48	830.52 ± 5.06	488.50 ± 4.67	42.20 ± 4.80

Values represented as mean ± SD, n=3

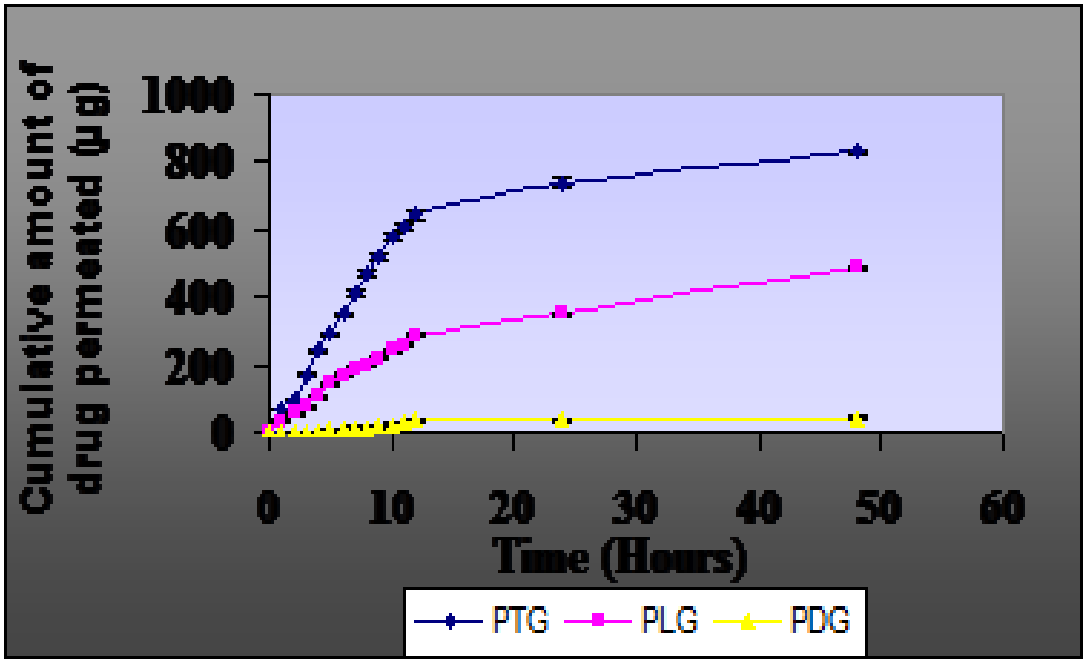


Fig. 4.5 invitro drug permeation of protransfersomal gel (PTG), proliposomal gel (PLG) and plain drug gel (PDG)

- Compatibility studies using FTIR:

  To investigate any possible interactions between the drug and the used excipients fourier transform infrared spectroscopy (ABB, FTLA-2000, India) was adopted. Physical mixture of pure drug (Ketorolac tromethamine) and the excipients (lecithin, Na cholate and Na deoxycholate) in ratio of 1:1 were subjected to the FTIR compatibility studies. The FT-IR spectra were carried out by placing the drug directly on the cavity and was determined in the wave number region of 4000-400 cm^{-1} (1)

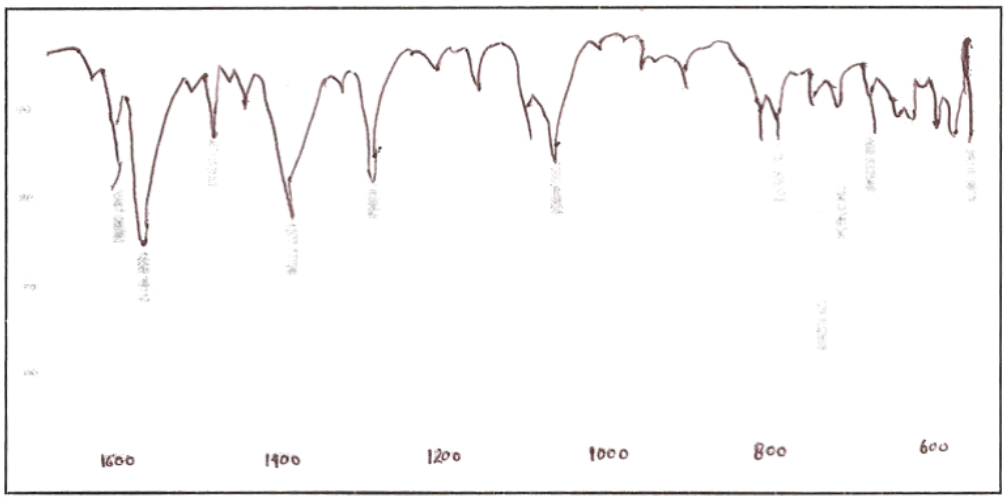


Fig. 4.6 FTIR spectrum of pure drug (Ketorolac tromethamine)

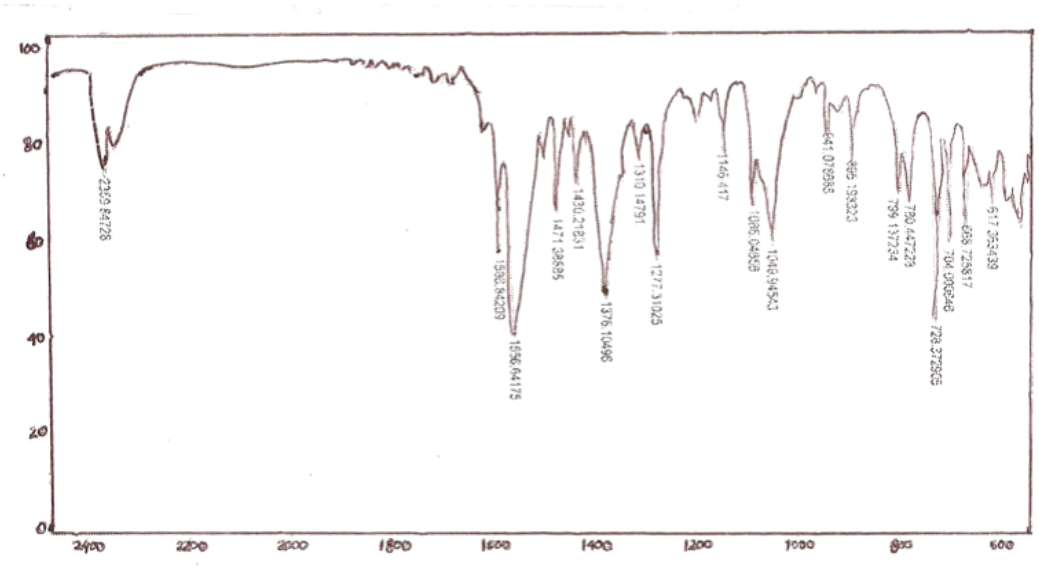


Fig. 4.7 FTIR spectrum of Protransfersomal Gel (PTG) containing drug

4.3 DISCUSSION:

The method of preparation of Ketorolac tromethamine loaded protransfersomal gel is based on the principle of coaecervation phase separation, in which concentrated protransfersomal gel was converted in to stable protransfersomal dispersion by dilution with excess aqueous phase**.** The basic composition of the Protransfersomal gel comprises phospholipid (stabilizing agent) and surfactant (destabilizing agent) in optimum ratio to impart elasticity to vesicle membrane of transfersomes. Protransfersomal gel was initially characterized by entrapment efficiency and electron microscopy [(17)] Optimization of protransfersomal gel formulations with regard to various attributes has been a subject of importance in the area of formulation research. Different formulations using different surfactants, composition and alcohols were prepared. Compositions of different protransfersomal gel formulation for optimization were given in table 4.1, 4.2 and 4.3. Formulation LCI-1 and LDCI-1 were found to be optimum. Optimized formulation, composed of Soya lecithin and sodium cholate/ sodium deoxycholate (85:15 wt/wt). it was found that as the concentration of lecithin increases further at constant sodium cholate/ sodium deoxycholate concentration, it form a rigid wall that retards the release of drug from vesicles, while on decreasing the concentration of lecithin from the optimized formula, showed vesicles were not formed.

Optical microscopy showed lamellar structures of protransfersomal gel (PTG) in liquid crystalline form (photomicrograph 4.1a) and Hydration of this gel formed spherical vesicular structure (photomicrograph 4.1b). The transformation of lamellar liquid crystalline PTG to transfersomes can be ascribed to different degree of hydration of surfactant and phospholipid molecules. Initially, due to the presence of limited solvent, the PTG formed was a mixture of lamellar liquid crystals resembling palisades and vesiculating lamellas linked together. Further addition of water resulted in swelling of the lipid bilayer due to interaction of water with polar groups of surfactants and above a limiting concentration of solvent, the bilayers formed spherical structures randomly giving rise to vesicular structures . The Scanning electron microscopy **(**SEM) images of the transfersomes were observed to be mostly spherical, with a few being slightly elongated (photomicrograph 4.2a and 4.2b). Morphological characterization of the protransfersomes as well as existence of their

vesicular structure after hydration was confirmed by Transmission electron microscopy (TEM) (photomicrograph 4.3a and 4.3b).

The vesicle size, size distribution and zeta potential were determined by dynamic light scattering method Malvern Zetasizer (DTS version 4.10, Malvern, UK) following hydration of the Ketorolac tromethamine loaded PTG formulations. The mean vesicle diameter of different PTG formulations were given in table 4.6, 4.7 and 4.8. Vesicle size of optimized PTG formulation, (LCI-1) and (LDCI-1) was found to be 984.55 ± 32 nm nm and 1896 ± 39 nm (Table 4.6). The highest and lowest vesicle size, LCI-1 (85:15) and LSB-2 (105:15) was found to be 984 ± 32 and 2470 ± 39 respectively. Reducing the lecithin content contributed an increase in the hydrophobicity, due to decrease in surface energy which is responsible for reduction in vesicle size [18]

Zeta potential is an important and useful indicator of vesicle surface charge, which can be used to predict and control the stability. The zeta potential of optimized formulation (LCI-1 and LDCI-1) was found to be -2.36 and -13.6 mV (Table 4.5). In this experiment, lecithin (steric stabilizer) is used that can easily compensate for the missing electrostatic repulsion.

As shown in table 4.6 and 4.7, formulation LCI-1 (94.17 ± 0.95) and LDCI-1 (90.77 ± 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 housed almost completely with in the lipid bilayer of the transfersomes [2]

As shown in table 4.9, Protransfersomal gel, LCI-1 (830.52 ± 5.06) showed better Invitro permeation than Proliposomal gel (488.50 ± 4.67) and plain drug gel (42.20 ± 4.80). High entrapment efficiency of protransfersomal gel is probably the reason for its better skin permeation[14]**.** Na cholate and Na deoxy cholate used as a surfactant in the preparation of protransfersomal gel formulation, which provide the flexibility to transfersomes. Due to elastic nature, when transfersomes pass through the skin pores, it will deform their original size and retain their original size after crossing the skin pores. Thus the large amount of transfersomes come in to the systemic circulation and release the drug for a longer period of time. Proliposomal gel comprise of cholesterol as a surfactant, which is not elastic in nature. In the influence of skin hydrostatic pressure liposomes will be break on the skin surface, so the amount of the liposomes which come in to the systemic circulation will be less. So it can be concluded that

cumulative amount of drug is proportional to the amount of drug which comes in to the systemic circulation.

The FTIR spectra of pure drug with excipients show that there is no evidence of chemical interaction between them. Characteristic peaks of pure drug appeared with no shift in the physical mixture with excipients (Fig. 4.7).

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