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Research Article

Formulation and Evaluation of Ketoprofen Loaded Protransfersome by Using Sodium Deoxycholate and Brij 35

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ABSTRACT

The study was aimed to characterize protransfersome gel as TDDS of ketoprofen for effective and sustain deliver of drug. The various formulation parameters were optimized by preparing 18 formulations using Sodium deoxycholate and brij 35 as edge activator. All protransfersome formulations were characterized and found that, in physical appearance protransfersome gel was Yellowish semisolid compact mass and the mean particle size of transfersome varied from 938-1374 nm. TEM micrographs transfersome suspension showed that the vesicles have a uniform spherical shape with a smooth surface. The formulation PTdC3-I have higher rate to produce transfersome than all formulations (12.331±0.246) X 10^3. Formulations with cholesterol have good entrapment capacity and formulations with isopropanol have a little advantage of higher entrapment in comparison with others either with butanol or ethanol. Release rate study through cellophane membrane indicate that the % cumulative release in 48 hr was found ranging in 34.9 to 64.35%, PTdC3-I formulation has shown highest percentage release.

Key words: protransfersome, ketoprofen, sodium deoxycholate and brij 35.

INTRODUCTION

Transfersomes are squeeze themselves even through pores much smaller than their own diameter. This is due to high flexibility of the transfersomes membrane and is achieved by judiciously combining at least two lipophilic/amphiphilic components (phospholipids plus biosurfactant) with sufficiently different packing characteristics into a single bilayer[1,2]. The high resulting aggregate deformability permits transfersomes to penetrate the skin spontaneously. Transfersomes are ultra deformable hydrophilic lipid vesicles that cross the skin under the influence of a transepidermal water activity gradient[3]. They are able to transport both high and low molecular weight into the body noninvasively. These vesicles are 105 time more deformable than unmodified liposomes. This characteristic makes transfersomes, to squeeze through pores in the stratum corneum. This makes transfersome a most efficient transdermal vehicle but as liposome its aqueous dispersion

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may exhibit aggregation, fusion, leaking of entrapped drugs, or hydrolysis of encapsulated drugs, thus limiting the shelf life of the dispersion[4].

Protransfersome**,** a product in gel form may avoid many of the problems associated with aqueous dispersions of transfersome and minimize problems of physical stability (aggregation, fusion, leaking). The additional convenience of the transportation, distribution, storage, and dosing would make 'Protransfersome gel' a promising industrial product. Protransfersome gel on hydration form multilamellar transfersome suspension.

Ketoprofen is an anionic non-steroidal anti-inflammatory drug (NSAID). It is a derivative of propionic acid and widely used in the management and treatment of patients with rheumatic disease[5-8]. Ketoprofen is used for musculoskeletal and joint disorders such as ankylosing spondylitis, osteoarthritis, and rheumatoid arthritis. NSAIDs are very widely prescribed but they have a poor tolerability profile, with a range of potential adverse effects. Ketoprofen when given orally, its adverse drug reactions are mild upper gastrointestinal complaints such as nausea, dyspepsia or epigastric discomfort[9, 10].

The main aim of study was to develop a transdermal delivery vehicle for sustained systemic delivery of ketoprofen using protransfersome system and to investigate the feasibility of using protransfersome as a transdermal drug delivery system for ketoprofen.

MATERIALS AND METHODS

Material: Ketoprofen was provided by Torent Labs Ltd, Mumbai, India. Soya lecithin was a kind gift from Natterman phospholipid GMBH, Germany, and contained 93±3% phosphotidylcholine. All other chemicals used throughout this investigation were of analytical grade and no additional purification was carried out. Doubled distilled water was used through out the study.

Preparation of Protransfersome gel: Protransfersome gel was prepared by phase separation coacervation technique. The method used by Jain N. K. et al, (2005) was redesigned to prepare Ketoprofen protransfersome. Sodium cholate, soya lecithin, and cholesterol in a specified ratio were taken in a dry, clean, wide mouth small test tube. A dose calculated weighed amount of drug was added to it and than a measured amount of solvents also added to test tube to dissolve the ingredients. The open end of test tube was covered with a lid to prevent loss of solvent from it and warmed over water bath at 67 ± 3^{o} C for about 5 minute until the surfactant mixture was dissolved completely. Then the aqueous phase (phosphate buffer saline (pH 7.4)) was added and warmed on a water bath till a clear solution was formed. The clear solution formed was cooled to room temperature to convert it to a gel known as protransfersome gel[11].

Optimization of formulation: Various process variables, which could effect preparation and properties of the protransfersome gel formulation, were identified and studied.

Following two process variables of protransfersome gel formulation were selected for optimization of formulation.

Ratio of surfactant, lecithin and cholesterol

Type of solvent

Type of surfactant (EDGE Activator)

Total 18 formulations were designed on the basis of these variables. The formulation code and respective variables used in the preparation are given in table 1 and 2. The effect of these variables was observed on the properties of formulation.

Table 1. Different variables used in the preparation of protransfersome With Sodium deoxycholate

Sl. No.	Formulation code	PC:S:C	Solvent
1	PTdC1-E	16:3:1	Ethanol
2	PTdC1-B	16:3:1	Butanol
3	PTdC1-I	16:3:1	Isopropanol
4	PTdC2-E	18:2:0	Ethanol
5	PTdC2-B	18:2:0	Butanol
6	PTdC2-I	18:2:0	Isopropanol
7	PTdC3-E	17:3:0	Ethanol
8	PTdC3-B	17:3:0	Butanol
9	PTdC3-I	17:3:0	Isopropanol

PC:S:C = Phosphotydil choline : surfactant (Sodium deoxycholate) : Cholesterol

Table 2 Different variables used in the preparation of protransfersome With Brij 35

Sl. No.	Formulation code	PC:S:C	Solvent
1	PTBJ1-E	16:3:1	Ethanol
2	PTBJ1-B	16:3:1	Butanol
3	PTBJ1-I	16:3:1	Isopropanol
4	PTBJ2-E	18:2:0	Ethanol
5	PTBJ2-B	18:2:0	Butanol
6	PTBJ2-I	18:2:0	Isopropanol
7	PTBJ3-E	17:3:0	Ethanol
8	PTBJ3-B	17:3:0	Butanol
9	PTBJ3-I	17:3:0	Isopropanol

Where, S = surfactant, L = lecithin (Brij 35), C = cholesterol

Characterization of Protransfersome Gel

Physical appearance: The prepared gel was viewed by naked eye to characterize color and physical state of gel. Protransfersome gel was also viewed by optical microscope at 40 X magnification, to observe crystal characteristics of gel by spreading a thin layer of protransfersome gel on a slide and placing the cover slip on it[12].

Vesicle size and size distribution: Hydrated protransfersome gel as transfersome suspension was evaluated for transfersome size.

Vesicle size of transfersome was also analyzed by diffraction light scattering method (Malvern Master Sizer). For this protransfersome was hydrated with phosphate buffer saline with slight agitation[13].

Vesicle shape and surface characteristics: Hydration of Protransfersome gel was performed with phosphate buffer saline (pH 7.4) with slight agitation to produce transfersome. A drop of transfersome suspension was placed on a slide and after placing cover slip observed under microscope. Photomicrographs were taken at 100 X magnification.

To further evaluate the surface characteristics of vesicle transmission electron microscopy (TEM; CM12 Philips, USA) were performed on PTdC3-E formulation. The transfersome suspension was negatively stained with a 1%

Table 3 Characterization of Protransfersome Gel

Formulation code	Vesicle size (nm)	Spontaneity (transfersome/mm^3)	Encapsulation efficiency
PTdC1-E	1172	(8.54±0.231 X 10^3	86.32±1.42
PTdC1-B	1121	(10.24±0.452) X 10^3	86.14±2.43
PTdC1-I	1096	(10.9375±0.512) X 10^3	88.75±1.25
PTdC2-E	1153	(10.187±0.347) X 10^3	79.58±2.75
PTdC2-B	1070	(10.96±0.163) X 10^3	83.08±1.66
PTdC2-I	952	(11.03±0.634) X 10^3	87.13±1.46
PTdC3-E	1034	(10.762±0.558) X 10^3	81.42±3.66
PTdC3-B	996	(11.106±0.694) X 10^3	85.71±3.93
PTdC3-I	938	(12.331±0.246) X 10^3	89.26 ±1.49
PTBJ1-E	1374	(7.29±0.221) X 10^3	79.25±0.90
PTBJ1-B	1246	(7.672±0.883) X 10^3	80.08±1.66
PTBJ1-I	1192	(8.336±0.441) X 10^3	80.97±1.25
PTBJ2-E	1217	(8.187±0.312) X 10^3	68.88±2.62
PTBJ2-B	1181	(8.406±0.662) X 10^3	72.88±2.62
PTBJ2-I	1125	(9.017±0.883) X 10^3	74.15±3.73
PTBJ3-E	1169	(8.62±0.883) X 10^3	78.02±2.42
PTBJ3-B	1106	(9.125±0.662) X 10^3	78.88±1.95
PTBJ3-I	1079	(9.534±0.221) X 10^3	83.54±2.62

aqueous solution of phosphotungustic acid (PTA). Transfersome suspension was dried on a microscopic carbon coated grid for staining. The excess solution was removed by blotting. After drying the specimen was viewed under the microscope at 13.5, 22 kV old enlargement[14].

Rate of Hydration (spontaneity): Spontaneity of transfersome formation is described as number of transfersome formed after hydration of Protransfersomes for 20 minute. Approximately 20 mg of protransfersomegel was transferred to bottom of a small stoppered glass vial and spread uniformly around the wall of the glass vial with the help of the glass rod. Two ml phosphate buffer saline was added carefully along the walls of the vial and kept aside without agitation. After 20 minute suspension was withdrawn and diluted to 10 ml. one drop of diluted suspension was placed on Neubaur's chamber. The number of transfersomes eluted from protransfersome was counted at RBC subdivisions on Neubaur's chamber[14].

Drug Entrapment efficiency: To evaluate loading capacity of protransfersome system for Ketoprofen, an accurately weighed quantity of protransfersome gel (100 mg) was dispersed into 10 ml phosphate buffer saline to produce transfersome suspension. The transfersome suspension was centrifuged at 18000 rpm in cooling centrifuge at 20 C for 30 min to separate ketoprofen containing transfersome from unentrapped drug. Then the sediment of vesicle was resuspended in 1 ml 30% PEG-400 and 1 ml 0.1% Triton X100 solution was added to it. Resulting solution was filtered and diluted to 100 ml with phosphate buffer saline and analyzed for drug content spectrophotometrically[14].

RESULTS AND DISCUSSION

The prepared Protransfersome formulations were viewed by naked eye to characterize color and physical state of gel. The results of physical appearance are Yellowish semisolid compact mass.

The prepared Ketoprofen Protransfersome formulation was aimed for transdermal application. Thus vesicle size and vesicle size distribution of transfersomes are crucial parameters for transdermal permeation of such formulation.

Effect of ratio of surfactant, phospotydylchloine and cholesterol has an immense effect on vesicle size and distribution. All batches showed a small mean size, well suited for transdermal permeation. The mean particle size in by diffraction light scattering varied from 938-1374 nm ranges. In study it was found that vesicles formed by formulations with deoxycholate surfactant are larger than that formed by formulations with Brij 35. The results are shown in Table 3.

In study it was found that the vesicle size of transfersome formed from protransfersome containing S: L: C ratio 3 (S:LC::17:3:0) produces smallest vesicle in comparison to ratio 2 (S:LC::18:2:0))and ratio 1 (S:LC::17:2:1). When groups are divided according to solvent used the group of isopropanol produces smallest vesicle in comparison to group of butanol and ethanol

The photomicrographs of formulations are presented in Figure 1, TEM micrographs (Fig 2) of Ketoprofen loaded transfersome suspension showed that the vesicles have a uniform spherical shape with a smooth surface and they are uniformly distributed. It also confirms the vesicle size range, which was obtained by DLS vesicle size analysis.

Spontaneity parameter influences the rate of permeation through skin. The results of study given in Table 3 shows that the spontaneity of protransfersome to form transfersome is greater for formulation containing S: L: C ratio 3 (S:LC::17:3:0) in comparision to ratio 2 (S:LC::18:2:0)) and ratio 1(S:LC::17:2:1). The formulation PTdC3-I have higher rate to produce transfersome than all formulations (12.331±0.246) X 10^3.

The entrapment efficiency is the major factor to select the optimum formulation. The entrapment efficiency of formulations is given in table 2

If the formulations are evaluated according to Ratio of Phosphatydyl choline, edge activator and cholesterol, the formulations with cholesterol (Ratio 1) have good entrapment capacity in comparison to ratio 2 & 3. The formulations with isopropanol have a little advantage of higher entrapment in comparison with others either with butanol or ethanol.

Drug release study

Fig 1 Photomicrograph of PTdC3-I

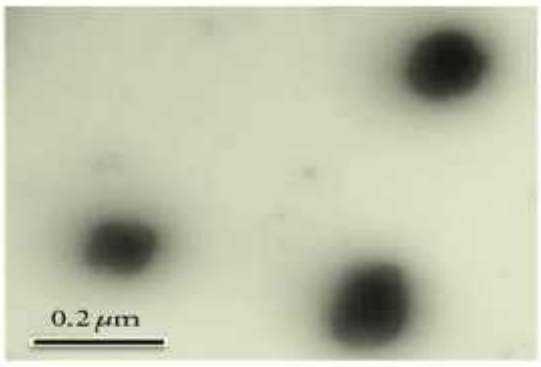

Fig 2 TEM micrograph of PTdC3-E

Table: 4 Data of cumulative amount release from protransfersome gel (100mg) at various time point of sample collection through cellophane membranes

Sl. No.	Sample collection time (hr)	Cumulative amount released(µg) PTdC2-I	PTdC3-E	PTdC3-B	PTdC3-I	PTBJ3-B	PTBJ3-I	H. alc. sol^n
1	0.5	12	16	22	17	13	14	12
2	1	61	64	79	83	34	42	27
3	1.5	97	93	104	126	42	51	49
4	2	159	145	190	212	74	83	78
5	3	202	193	245	252	103	132	92
6	4	245	248	309	310	142	157	112
7	5	315	309	385	403	198	216	135
8	6	388	382	417	464	237	258	166
9	7	416	422	492	548	294	326	203
10	8	445	478	528	624	345	379	245
11	12	589	561	692	781	460	474	274
12	24	812	764	881	948	547	552	342
13	36	994	915	1065	1123	618	649	406
14	48	1164	1112	1224	1287	698	765	471

MATERIALS AND METHODS

Permeation of Ketoprofen from different formulation was studied using a locally fabricated Franz glass diffusion cell having Cellophane membrane, 200 µm thickness. The effective permeation area of donor compartment exposed to receptor compartment was 2.54 cm^2 and he receptor compartment was filled with 30 ml of pH 7.4 PBS at temperature 37± 0.5ºC, stirred at 600 rpm. A weighed amount of protransfersome gel (100 mg containing 2.0 mg ketoprofen) was placed on donor compartment with the help of a glass rod. Sample were withdrawn through the sampling port of the diffusion cell at predetermined intervals over 48 hours and analyzed spectrophotometrically. An equal volume of fresh pH 7.4 PBS maintained at 37 ±0.5ºC was replaced into the receptor compartment after each sampling [13,14,15].

The release through cellophane membrane from hydro-alcoholic solution of drug (1 mg/ml of Hyd. HCl in 5%v/v ethanol in water) was also performed by using above mention procedure.

DATA ANALYSIS

The cumulative amount of Ketoprofen permeated through Cellophane membrane was plotted as a function of time. The slope of the linear portion of the plot was derived by regression. The flux (permeation rate) was calculated from slope.

RESULTS

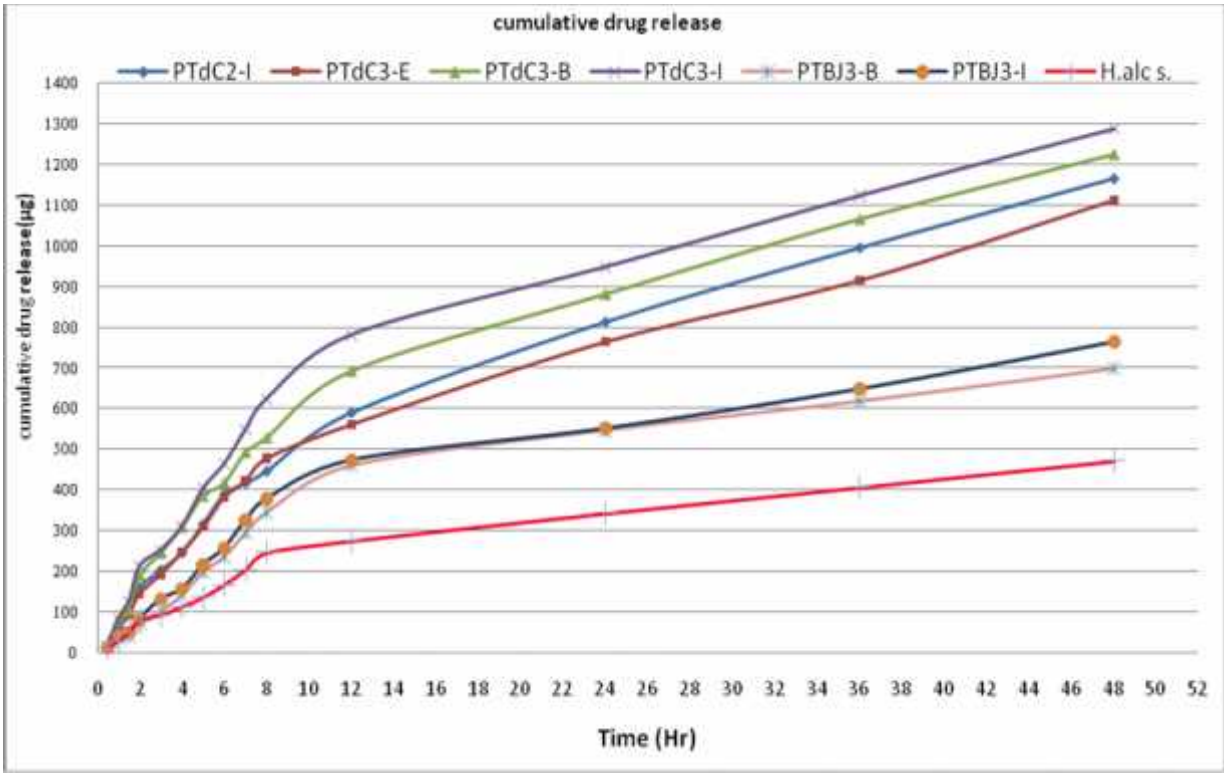

Fig.3 Graphical presentation of cumulative drug release

Drug diffusion study was conducted through cellophane membrane with 4 selected optimized formulations (PTdC2-I PTdC3-E PTdC3-B, PTdC3-I, PTBJ3-B, PTBJ3-I) and hydro-alcoholic solution of drug. The cumulative amount of Ketoprofen release from protransfersome formulations through an artificial membrane at various sampling time are presented in Table 4

The result indicate that the % cumulative release in 48hr (Table 3) was found ranging in 34.9 to 64.35, s PTdC3-I formulation has shown highest percentage release in 48 hr. protransfersome with cholesterol have lower release rate in comparison with others.

The data obtained from permeability studies using cellophane membrane with ketoprofen protransfersome revealed a flux of 5.71 to 9.99 µg/cm^2/hr.

The data of release of ketoprofen from hydro-alcoholic solution revealed that protransfersome formulations have shown significantly increased % release and flux with comparison to same concentration of hydro-alcoholic solution of ketoprofen.

Summary and Conclusion

Protransfersome of ketoprofen was prepared with a purpose of achieving a high local concentration of the active ingredient at the effected site, with as low plasma concentration as possible. Protransfersome gel was optimized by preparing total 18 formulations using various combinations of phosphotydil choline, solvents, cholesterol and Sodium deoxycholate or Brij 35 as edge activator.

Formulations containing cholesterol shown high entrapment but has significantly low permeability rat and spontaneity and formulations with isopropyl alcohol have low vesicle size and high permeation rate. PTdC3-I formulation found more promising among all formulation and has effective permeation rate. TEM images of

transfersome suspension from PTC3-I showed that the vesicles have a uniform spherical shape with a smooth surface and they are uniformly distributed.

In study it was observed that protransfersome is a promising tool for transdermal delivery of drugs with sustain and effective release rate.

REFERENCES

1. Pandey S., Goyani M., Devmurari V., Fakir J., Transferosomes: A Novel Approach For Transdermal Drug Delivery, Scholars Research Library Der Pharmacia Letter, 2009, 1 (2) 143-150
2. Heather AE Benson Transfersomes for transdermal drug delivery Expert Opinion on Drug Delivery, Vol. 3, November 2006, Pages 727-737
3. Fang j., Hong T.C et al., Effect of liposomes and niosomes on skin permeation of enoxacin, international journal of pharmaceutics, volume 219, may 2011, 61-72
4. Ceve G. , Blume G., Lipid vesicle penetration into intact skin owing to the transdermal osmotic gradients and hydration force. Biochim. Biophys. Acta, 1104 (1992), 226-232
5. G.G. Riverside. Ketoprofen. In Analytical Profiles Of Drug Substances Volume 10, K. Florey (Ed.), Academic Press, London, Uk, 1981, Pp. 443-471.Therapheutic Drugs Volume 2, C. Drollery (Ed.), Churchill Livingstone, London, UK, 1991, Pp. 25-27.
6. The British Pharmacopoeia, the Pharmaceutical Press, London, UK, 2002, Pp. 1003-1004.
7. Clarke; S Lsolation and Ldentification of Drugs 2nd Edition, A.C. Moffat (Ed.) the Pharmaceutical Press, London, UK, 1986, Pp. 697-698.
8. The Pharmaceutical Codex 12th Edition, W. Lund (Ed.), the Pharmaceutical Press, London, UK, 1994, Pp. 933-935.
9. G.G. Riverside. Ketoprofen. In Analytical Profiles Of Drug Substances Volume 10, K. Florey (Ed.), Academic Press, London, Uk, 1981, Pp. 443-471.
10. Ketoprofen: a review of its pharmacologic and clinical properties. T. G. Kantor, M.D. Pharmacotherapy 6 (1986)93-103.
11. Bhavana V., Ajay J. K., Jain N.K., Proniosome based transdermal delivery of levonorgestrel for effective contraception, Journal of controlled release 54 (1998), 149-165.
12. Gupta V, Agrawal RC, Trivedi P. Reduction in cisplatin genotoxicity (micronucleus formation) in non target cells of mice by protransfersome gel formulation used for management of cutaneous squamous cell carcinoma., Acta Pharm., 61(1), (2011), 63-71.
13. Jain S., Sapre R., Tiwary AK, Jain N. K., Proultraflexible Lipid Vesicles for Effective Transdermal Delivery of Levonorgestrel: Development, Characterization, and Performance Evaluation. *AAPS PharmSciTech*. 6(3), 2005, E513-E522.
14. S. Jain, R. Sapre, R. B. Umamaheshwari and N. K. Jain, Protransfersomes for effective transdermal delivery of norgestrel preparation and in vitro characterization, Indian J. Pharm. Sci. 65 (2003) 152–161.
15. Jalon E.G., Josa M., et al., Determination by high performance liquid chromatography of ketoprofen in vitro in rat skin permeation sample. journal of chromatography, A 870, (2000), 143-149.