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Research Article

# FORMULATION OF PROTRANSFERSOMAL GEL OF DICLOFENAC POTASSIUM AND ITS IN-VITRO CHARACTERIZATION

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**ABSTRACT**

The aim of present study was to formulate, evaluate, and perform stability studies of new vesicular drug carrier system protransfersome for transdermal delivery of Diclofenac potassium for effective and sustain deliver of drug. Protransfersome will be converted into the ultraflexible vesicles, transfersomes in situ by absorbing water from the skin. The Protransfersomal gel (PTG) were optimized by preparing 13 formulations using Span 60, Brij 35, Sodium deoxycholate and Tween 80 as edge activator. All protransfersome formulations were characterized and found that, in physical appearance PTG was yellowish semisolid compact mass and the mean vesicle size of transfersome varied from 1.24-7.48 µm. In SEM micrographs transfersome suspension showed that the vesicles have a uniform spherical shape. The formulation F1B1 have higher rate to produce transfersome than all formulations (12.331±0.246) X 103. Formulations with Surfactant Span 60: sodium deoxycholate (F1B1) in ratio 1:1 have good entrapment capacity, formulations containing combination of two surfactants have little advantage of having higher entrapment than in comparison with formulation containing single surfactant. Among all formulation, F1B1 shows highest elasticity of 34.4, Drug content of 98.42%, Zeta potential of -20.3mV. The drug permeation was continued upto 12 hrs. and 97.89% of drug had been released from the formulation representing the sustained release nature. Protransfersome may be a promising carrier for Diclofenac and other drugs, especially due to their simple production and facile up.

**KEY WORDS**: Protransfersome, Diclofenac potassium, Sodium deoxycholate, Brij 35, Span 60, Tween 80, Permeation.

## INTRODUCTION

Transdermal drug delivery systems (TDDS) are defined as self-contained, discrete dosage forms which, when applied to intact skin, deliver the drug(s), through the skin, at a controlled rate to systemic circulation.[1] The TDDS has various advantages over conventional ones such as avoidance of GI incompatibility, variable GI absorption, avoidance of first pass metabolism, improved bioavailability, reduced frequency of administration, improved patient compliance.[2] But barrier function of the stratum corneum still remains a problem, which makes the development of new transdermal drug delivery systems an interesting challenge. Vesicular systems have been widely studied as vehicles for dermal and transdermal drug delivery. Their benefits in enhancing drug

permeation have been well established.[3] Recently, various strategies have been used to augment the transdermal delivery of bioactives. Mainly, they include electrophoresis, iontophoresis, chemical permeation enhancers, microneedles, sonophoresis, and vesicular system like liposomes, niosomes, elastic liposomes such as ethosomes and transfersomes. Among these strategies transfersomes appear promising. Transfersomes are vesicles, which are self-optimized aggregates with ultra-flexible membrane that cross the skin under the influence of a transepidermal water activity gradient.[1] These vesicular transfersomes are more elastic than the standard liposomes and thus well suited for the skin penetration. They are able to transport both high and low molecular weight into the body noninvasively. These vesicles are much 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 may exhibit aggregation, fusion, leaking of entrapped drugs, or hydrolysis of encapsulated drugs, thus limiting the shelf life of the dispersion. To overcome the limitations (especially chemical and physical stability) of vesicular drug delivery systems like liposomes, niosomes, transfersomes, and pharmacosomes, the provesicular approach was introduced.[4, 16] Protransfersome developed for transdermal delivery possessed superior skin penetration ability and better stability. 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). 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.[1, 4] Diclofenac is a nonsteroidal anti-inflammatory drug (NSAID) is the most frequently prescribed drug, which is used in both acute and chronic symptoms of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and dysmenorrheal treatment because of its analgesic, antipyretic, and anti-inflammatory roles. Its anti-inflammatory effect is due to cyclooxygenase inhibition and the consequent reduction of prostaglandin synthesis which leads to unfavorable side effects specifically on the stomach via systemic administration. Therefore, some NSAIDs are administered transdermally to achieve local or systemic effect as an alternative for oral and parenteral administration.[5] The main aim of study was to develop a transdermal delivery vehicle for sustained systemic delivery of diclofenac pottasium using protransfersome system and to investigate the feasibility of using protransfersome as a transdermal drug delivery system for diclofenac potassium.

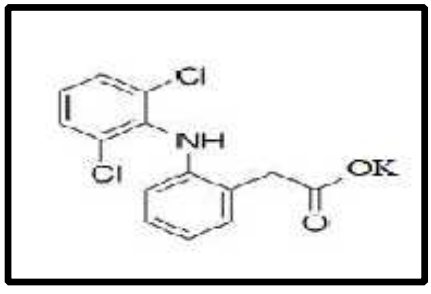


**Fig. no.1: Structure of Diclofenac Potassium**

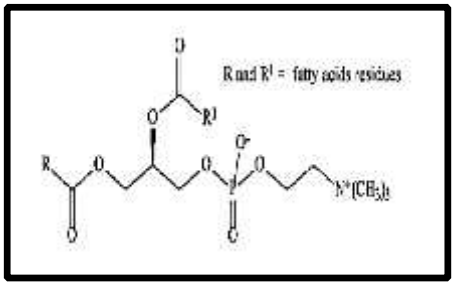


**Fig. no. 2: Structure of Soya lecithin**

## MATERIALS AND METHODS

**Materials:** Diclofenac Potassium were obtained as a gift sample from Ipca Laboratories Limited, Mumbai (India); Sodium deoxycholate, Isopropyl Alcohol from Progene life sciences, Pune (India); Brij 35, Soya Lecithin, Triton X-100 from Himedia Laboratories, Mumbai; Span 60, Tween 80 from

Research lab., Mumbai; Potassium dihydrogen phosphate, Sodium Hydroxide from Fisher scientific, Mumbai. All chemicals used were of analytical reagent grades.

**Preparation of Protransfersomal Gel (PTG) [1, 6]**

Protransfersomal gel (PTG) was prepared by using Soya lecithin (Phospholipid), sodium deoxycholate**/**Brij 35/span 60/Tween 80 (Edge activator) and isopropyl alcohol (Alcohol). Protransfersome gel was prepared by phase separation coacervation technique. All the ingredients i.e. Soya lecithin (PC), surfactant (EA) and Alcohol 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°C, until all the ingredients were dissolved. The Diclofenac Potassium in PBS (pH 7.4) was added 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 final composition contained (PC +EA: alcohol: aqueous phase) 2:1:2 w/w and drug concentration in the formulation is 1% w/w. The composition of formulations was shown in table no. 1(a) & 1(b).

**Table no. 1(a): Composition of PTG using different edge activator**

| Formulation Code | EA* | PC: S* | Solvent |
|---|---|---|---|
| F1 | Span 60 | 17:3 | Isopropyl Alcohol |
| F2 | Brij 35 | 17:3 | Isopropyl Alcohol |
| F3 | Sodium Deoxycholate | 17:3 | Isopropyl Alcohol |
| F4 | Tween 80 | 17:3 | Isopropyl Alcohol |

Where, PC- Phosphatidyl choline (Soya Lecithin); S or EA- Surfactant or Edge activator

**Table no. 1(b): Composition of PTG using different combination of edge activator**

| Formulation Code | PC: $S_{mix}$.* | $S_{mix}$ ratio | Surfactant | Solvent |
|---|---|---|---|---|
| F1A1 | 17:3 | 1:1 | Span 60 : Brij 35 | Isopropyl Alcohol |
| F1A2 | 17:3 | 1:2 | | Isopropyl Alcohol |
| F1A3 | 17:3 | 2:1 | | Isopropyl Alcohol |
| F1B1 | 17:3 | 1:1 | Span 60 : Sodium deoxycholate | Isopropyl Alcohol |
| F1B2 | 17:3 | 1:2 | | Isopropyl Alcohol |
| F1B3 | 17:3 | 2:1 | | Isopropyl Alcohol |
| F1C1 | 17:3 | 1:1 | Span 60 : Tween 80 | Isopropyl Alcohol |
| F1C2 | 17:3 | 1:2 | | Isopropyl Alcohol |
| F1C3 | 17:3 | 2:1 | | Isopropyl Alcohol |

Where, PC- Phosphatidyl choline (Soya Lecithin); $S_{mix}$- Surfactant mixture

**Characterization of Protransfersomal Gel:**

**Physical appearance [4]**

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.

**Encapsulation efficiency[1]**

The entrapment efficiency is determined after separating the unentrapped drug. Protransfersomes gel (100mg) is hydrated with 10 ml of phosphate buffer saline (pH7.4) using manual shaking for 5 minutes, to form transfersomal dispersion. For the separation of unentrapped drug the transfersomal dispersion is centrifuged at 15000 rpm for 30 minutes. The clear supernatant is filter off carefully to separate the unentrapped drug. The sediment (1ml) is resuspended in 1ml of Triton X-100 (0.1% v/v) is used to lyse the transfersomes after appropriate dilution of the sample with PBS (pH 7.4), absorbance is recorded.

% EE = (Amount entrapped / Total amount added) ×100 …….. (1)

**Vesicle size and shape[7, 8]**

Vesicle size and shape for each formulation was determined by optical microscope (Motic Image, Germany). 1gm of each formulation was spread uniformly on glass slide and observed under electron microscope for vesicular shape. Optical microscope also used to study size and shape under 45X optical lens.

**Viscosity Determination[9]**

Viscosities of the formulated PTG were determined using rotational viscometer (Fungi lab) using L4 Spindle at 12 rpm.

**Spreadability[10]**

Spreadability of formulations was determined by an apparatus suggested by Multimer et al. which was fabricated in laboratory and used for study. The apparatus consists of a wooden block, with a fixed glass slide and movable glass slide with one end tied to weight pan rolled on the pulley, which was in horizontal level with fixed slide.

**Procedure:** An excess of gel sample 1 gm was placed between two glass slides and a 1000g weight was placed on slides for 5 minutes to compress the sample to a uniform thickness. Weight (60g) was added to the pan. The time (seconds) required to separate the two slides was taken as a measure of spreadability. It was calculated using the formula: $S = m.l / t$ --------- (2)

Where, S - Spreadability in g.cm / sec

m - Weight tied to upper slide

l - Length of glass slide

t - Time in seconds

Length of glass slide was 7.5 cm and weight tied to upper slide was (60g) throughout the experiment.

**Drug Content Uniformity[9]**

Drug content uniformity is the degree of uniformity of the amount of active drug substance among containers, i.e., tubes containing multiple doses of the semisolid topical product. The uniformity of dosage is demonstrated by assay of top, middle, and bottom samples (typically 0.25–1.0 g) obtained from a tube cut open to withdraw respective samples for drug assay. An accurately weighed 1 gm quantity of the gel was transferred into a 100ml stopper volumetric flask and shaken vigorously with 100 ml methanol to extract the drug. The contents were filtered volume was made up to the mark with methanol. From the above solution, 1 ml was pipette in to a 10-ml volumetric flask and volume was made up to 10 ml with methanol. Finally, the UV absorbance of the resulting solution was measured at 274 nm against the blank solution of methanol.

**Rate of Hydration (Spontaneity) [11, 12]**

Spontaneity of transfersome formation is described as number of transfersome formed after hydration of protransfersomes for 20 minute. Approximately 20 mg of PTG 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. 2ml 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. The Transfersomes in 80 small squares are calculated using the following formula:

Total number of Transfersomes per cubic mm=Total number of Transfersomes counted × dilution factor × 4000… (3)

**Degree of Deformability [12]**

In the case of protransfersomes, the permeability study is one of the important and unique parameter for characterization. The deformability study is done against the pure water as standard. Protransfersomes preparation is passed through a large number of pores of known size (through a different microporous filters, with pore diameter between 50 nm and 450 nm, depending on the starting transfersomes suspension). Particle size is noted after each pass by optical microscopy. The degree of deformability can be determined using the following formula,

$$E = J \times (rv/rp)^2 \ldots\ldots\ldots (4)$$

Where, J= the amount of the suspension extruded during 5 min,
rv = the size of the vesicle,
rp = pore size of the barrier.

***In-vitro* drug permeation [1, 7]**

Permeation of drug 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°C ± 1°C by magnetic stirrer. Egg membrane was mounted between the donor and receptor compartment.

The donor compartment was filled with the 1 gm protransfersomal gel formulation. A 7.5 ml aliquot of 1: 99 (v: v) Isopropanol: pH 7.4 phosphate buffer was used as receptor medium to maintain a sink condition. At appropriate intervals 0.5ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution and analyzed by UV-Visible Spectrophotometer at 274nm.

**Scanning Electron Microscopy[13]**

The SEM photographs of drug loaded vesicles of optimized formulation were obtained by scanning electron microscope (Jeol, JSM 6360 Aº) using platinum sputter technique.

**Determination of Zeta-Potential: [14, 15, 16]**

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.

**Stability study [3, 7]**

Stability studies were carried out by storing the optimized formulation at various temperature conditions like refrigeration temperature (2°C±2°C), room temperature (27°C ± 2°C) and elevated temperature (45°C± 2°C) for a period of one month. Encapsulation efficiency and variation in the average vesicle diameter were determined before and after completion of one month.

## 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. Protransfersome gel was produced successfully using a simple method based on Phase separation coacervation technique. PTG were observed by light microscopy, photomicrographs were taken before

and after hydration by PBS 7.4. As shown, Fig. no. 3(a), lamellar structures of PTG. Fig. no. 3(b) vesicular structure of transfersomes formed upon hydration of protransfersomal gel.

**(a)** **(b)**

**Fig. no.3 (a) Lamellar structures of PTG; (b) Vesicular structure of transfersomes formed upon hydration of protransfersomal gel**

The formulated Protransfersomal gel were evaluated for drug encapsulation efficiency, Vesicle size and shape, Rate of hydration, Viscosity are shown in table no. 2

**Table no. 2 Characterization of Protransfersome Gel**

| Sr. no. | Formulation code | Encapsulation efficiency (%) | Vesicle size (µm) | Spontaneity (transfersome/ $mm^3$) | Viscosity (cps) |
|---|---|---|---|---|---|
| 1 | F1 | 75.27±1.20 | 1.87±0.78 | (11.19±0.694) X $10^3$ | 14500±0.78 |
| 2 | F2 | 69.78±1.54 | 3.74±1.56 | (8.62±0.883) X $10^3$ | 12503±2.08 |
| 3 | F3 | 70.67±1.56 | 2.49±0.44 | (11.03±0.634) X $10^3$ | 13504±0.33 |
| 4 | F4 | 71.91±0.52 | 4.67±1.22 | (9.125±0.662) X $10^3$ | 10632±0.32 |
| 5 | F1A1 | 68.53±1.53 | 2.80±0.45 | (10.93±0.512) X $10^3$ | 11362±1.08 |
| 6 | F1A2 | 62.01±0.68 | 6.54±1.56 | (9.017±0.883) X $10^3$ | 10408±1.03 |
| 7 | F1A3 | 70.16±1.13 | 7.48±1.89 | (9.534±0.221) X $10^3$ | 11900±0.88 |
| 8 | F1B1 | 77.68±1.05 | 1.24±0.55 | (12.33±0.246 ) X $10^3$ | 14800±0.78 |
| 9 | F1B2 | 74.15±3.73 | 1.56±0.56 | (10.24±0.452) X $10^3$ | 13807±0.56 |
| 10 | F1B3 | 78.88±1.95 | 1.86±1.84 | (8.54±0.231) X $10^3$ | 12858±0.52 |
| 11 | F1C1 | 68.00±2.04 | 3.11±1.26 | (10.96±0.163) X $10^3$ | 11500±2.42 |
| 12 | F1C2 | 65.00±1.28 | 5.61 ±0.78 | (7.672±0.883) X $10^3$ | 17808±2.38 |
| 13 | F1C3 | 69.22±1.81 | 3.33±2.06 | (8.336±0.441) X $10^3$ | 12188±0.68 |

Represents mean ± S.D. (n = 3)

The entrapment efficiency is the major factor to select the optimum formulation. The entrapment efficiency of formulations is given in table 2. The results of encapsulation efficiency indicated that formulation containing Span 60 (F1) had high encapsulation efficiency than formulations containing other surfactants (F1-F4). These results are related to the HLB values of these edge activators. They are 4.7, 15, 16 and 16.92 for Span 60, Tween 80, sodium deoxycholate and Brij 35 respectively. Based on these HLB values, the affinity for lipids was expected to be in the order of SP60 > TW80 > SDC>BJ35. This consideration explains the higher EE% encountered with SP60 as compared to TW80, SDC and Brij 35. The entrapment efficiency of the SP60 formulation was high because of the increase in the ratio of lipid volume in the vesicles as compared to the encapsulated aqueous volume. Further, on combination of two surfactant (F1A1 - F1C3) shows increase in entrapment efficiency than that of

formulation containing single surfactant. The formulation containing span 60: sodium deoxycholate (F1B3) (2:1) shows higher encapsulation efficiency (78.88±1.95). The prepared diclofenac protransfersome formulation was aimed for transdermal application. Thus, vesicle size of transfersomes are crucial parameters for transdermal permeation of such formulation. All batches showed a small mean size, well suited for transdermal permeation. Span 60 (F1) have lower size among all the tested formulations of surfactant (F1-F4). Span 60 hydrophobicity has been attributed to the decrease in surface energy with increasing hydrophobicity resulting in the smaller vesicles and span 60 have low HLB value 4.7 among the all tested formulations. As for vesicle size, increasing hydrophobicity of the surfactant monomer led to smaller vesicles; a result which might be anticipated since surface free energy decreases with increasing hydrophobicity. Due to lecithin increase hydrophobicity result in slightly decrease in vesicle size. It was observed that by combining two surfactant results in decreases in vesicle size (F1A1-F1C3). In study it was found that the vesicle size of transfersome formed from protransfersome containing Span 60: Sodium deoxycholate (F1B1) 1:1 shows smaller vesicle size with mean vesicle size 1.24±0.55. From the fig. no.4 Span 60: Sodium deoxycholate (F1B1) have lower size among all the tested formulations.

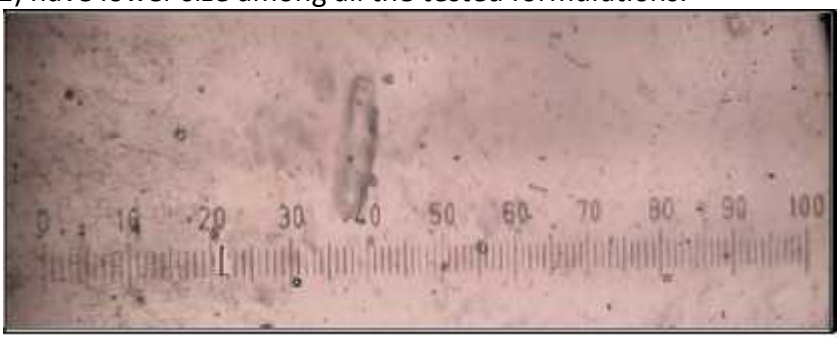


**Fig. no.4 Vesicle size of optimized formulation (F1B1)**

Spontaneity parameter influences the rate of permeation through skin. The results of study given in table no.2 shows that the spontaneity of protransfersome to form transfersome is greater for formulation containing two surfactant in combination (Span 60: Sodium deoxycholate) in comparison to formulation containing single surfactant. The formulation F1B1 have higher rate to produce transfersome than all formulations (12.331±0.246) X $10^3$. Viscosity is important parameter for characterization of gel as it affects the release of drug. Viscosity measurement revealed that all formulations show optimum consistency as shown table no.2. From figure no. 5, optimized formulation was analyzed for SEM, gives spherical vesicular shape.

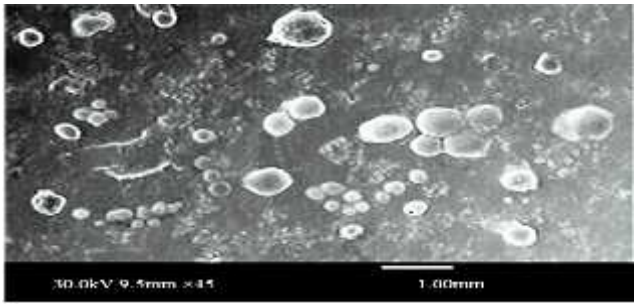

**Fig. no. 5: SEM image of vesicle shape**

The drug content of the prepared PTG gel was found to be within range of 82.31- 98.42 by UV Spectrophotometer. The results are shown in table no. 3.

**Table no. 3. Characterization of Protransfersome Gel by Drug content, Spreadability, Degree of Deformability studies (n = 3)**

| Sr. no | Formulation code | Drug content (%) | Spreadability (gm.cm/sec) | Degree of Deformability/ Elasticity |
|---|---|---|---|---|
| 1 | F1 | 97.38±1.54 | 18.97±0.46 | 26.5±1.6 |
| 2 | F2 | 89.76±1.18 | 16.93±0.44 | 17.1±1.4 |
| 3 | F3 | 96.51±1.01 | 18.09±0.98 | 24.0±1.2 |
| 4 | F4 | 91.53±1.17 | 15.77±0.87 | 19.6±1.4 |
| 5 | F1A1 | 85.17±0.84 | 17.55±0.76 | 22.7±1.1 |
| 6 | F1A2 | 84.23±0.36 | 15.08±0.79 | 15.1±1.4 |
| 7 | F1A3 | 82.31±2.43 | 18.90±0.85 | 17.1±1.6 |
| 8 | F1B1 | 98.42±0.78 | 19.08±0.97 | 34.4±1.4 |
| 9 | F1B2 | 97.38±1.54 | 16.07±0.90 | 27.5±1.6 |
| 10 | F1B3 | 98.12±0.68 | 17.09±0.34 | 22.2±1.3 |
| 11 | F1C1 | 96.51±1.01 | 18.08±0.66 | 15.7±0.5 |
| 12 | F1C2 | 95.01±0.88 | 20.09±0.99 | 16.8±1.3 |
| 13 | F1C3 | 97.86±0.45 | 17.14±0.93 | 17.8±0.9 |

Represents mean ± S.D. (n = 3)

Degree of Deformability of all formulation is recorded in table no. 3. The elasticity was maximum for F1B1 (34.4±1.4) formulation. The results indicate that the elasticity of vesicle is greater for formulation containing combination of two surfactant. Spreadability of all formulation is recorded in table no. 3 and it shows good spreadability of all formulation (F1-F1C3). Lesser the time taken for separation of two slides, better the spreadability. Zeta potential of the prepared Diclofenac loaded PTG was measured. PTG prepared by Span 60: Sodium deoxycholate (F1B1) 1:1 ratio showed higher stability, bearing a value of -20.3mV is shown in fig. 6

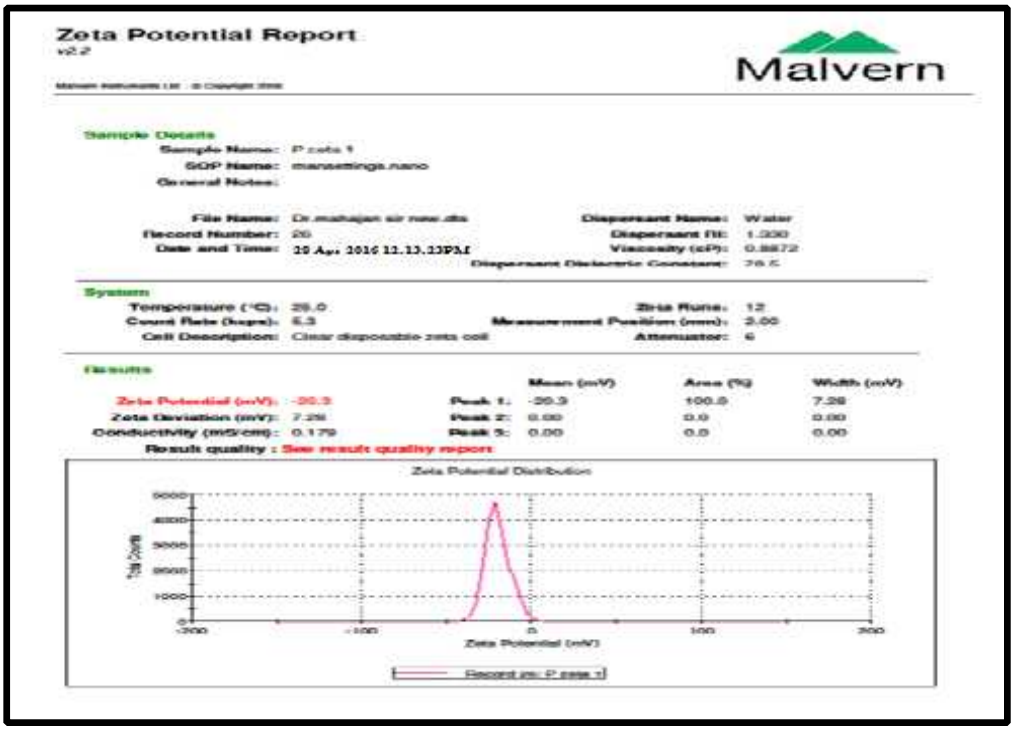


**Fig. no.6: Zeta potential of optimized formulation**

***In-vitro* permeation study:** The drug permeation was maximum for F1 formulation i.e. Span 60 among the all tested formulations (F1-F4). The diffusion study was continued up to 12 hours. From table no.4 and Fig. no.7 Span 60 (F1) have high release profile than other tested surfactant formulations, because transfersome prepared with another surfactant were significantly larger than those prepared with Span 60. The relationship observed between transfersome size and Span hydrophobicity has been attributed to the decrease in surface energy with increasing hydrophobicity resulting in the smaller vesicles. This would also explain the large vesicle size of transfersome prepared with other surfactant which has a much lower hydrophobicity than that Span 60. Hydrophobicity is also high (HLB = 4.7) so lipophilic drug can be easily penetrated through the skin. Protransfersome of Span 60 were smaller in size, demonstrated higher hydrophobicity and higher surface area due to low vesicle size so better permeability of drug also occurs. Further, it was observed that formulation containing combination of two surfactant results in decreases in vesicle size (F1A1-F1C3) due to that surface area increases leads to better permeability of drug. Vesicles with smaller diameter are believed to better permeate through the skin as smaller vesicles tend to fuse readily. From fig. no. 9 Span 60: sodium deoxycholate (F1B1) have high release profile than other tested formulations, because transfersome prepared with other surfactant or by combining two surfactants were significantly larger than those prepared with Span 60: sodium deoxycholate combination.

**Table 4: *In-vitro* drug permeation of PTG formulation F1-F1C3**

| Formulation | Time (hr) | % drug permeation | Formulation | Time (hr) | % drug permeation |
|---|---|---|---|---|---|
| F1 | 12 | 97.11±0.98 | F1B1 | 12 | 97.89±0.9 |
| F2 | 12 | 92.32±0.24 | F1B2 | 12 | 96.391±0.98 |
| F3 | 12 | 96.43±0.87 | F1B3 | 12 | 96.113±0.24 |
| F4 | 12 | 94.50±0.89 | F1C1 | 12 | 95.45±1.03 |
| F1A1 | 12 | 95.231±0.9 | F1C2 | 12 | 94.765±0.9 |
| F1A2 | 12 | 93.735±0.1 | F1C3 | 12 | 90.987±0.11 |
| F1A3 | 12 | 91.543±0.5 | | | |

Represents mean ± S.D. (n = 3)

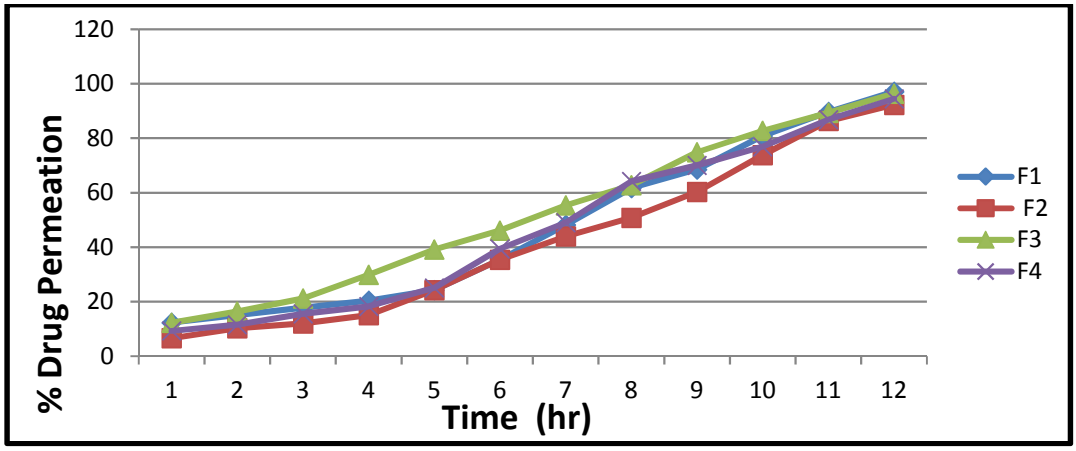


**Fig. no.7: *In-vitro* drug permeation studies of various PTG formulations (F1-F4)**

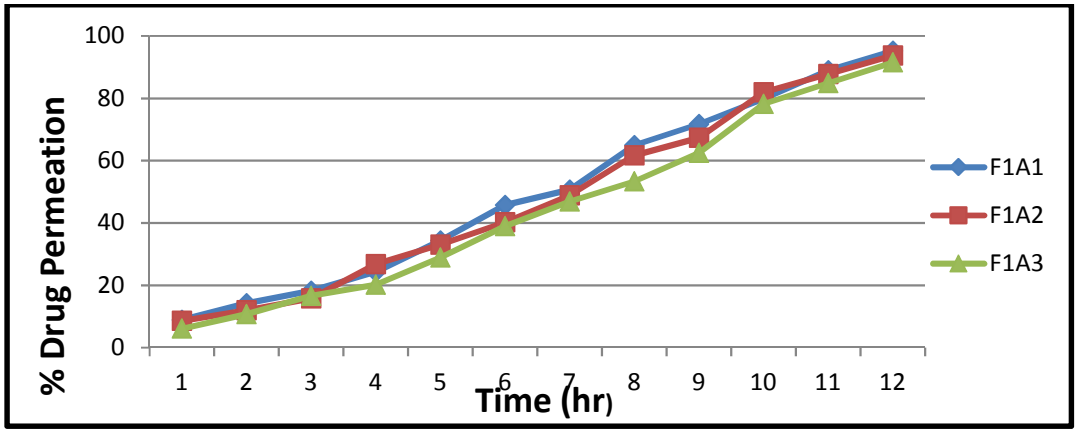


**Fig. no.8: *In-vitro* drug permeation studies of formulations F1A1-F1A3 using Span60: Brij 35 surfactant**

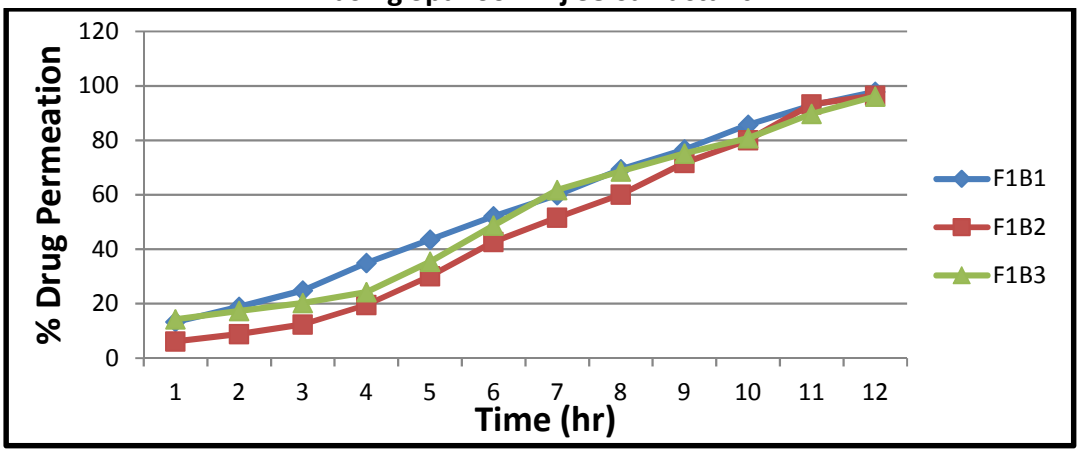


**Fig.no. 9: *In-vitro* drug permeation studies of formulations F1B1-F1B3 using Span 60: Sodium deoxycholate surfactant**

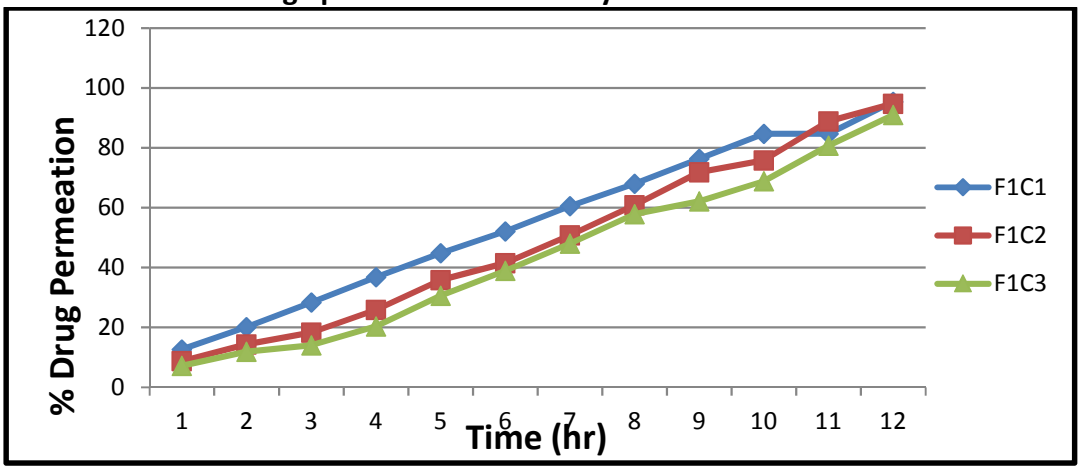


**Fig. no. 10: *In-vitro* drug permeation studies of formulations F1C1-F1C3 using Span 60: Tween 80 surfactant**

**Stability study**

The optimized formulation (F1B1) was found to be stable for period of one month; it can be observed that the PTG formulation showed no major alteration in relation to encapsulation efficiency and vesicle size (table 30).

**Table no. 30: Stability study of optimized formulation (F1B1)**

| Sr. No. | Temp. | Initial | | After 1 month | |
|---|---|---|---|---|---|
| | | Encapsulation efficiency | Vesicle size | Encapsulation efficiency | Vesicle size |
| 1 | 2°c | 77.68±1.05 | 1.24±0.55 | 77.39±0.36 | 1.78±1.45 |
| 2 | 25°c | 77.68±1.05 | 1.24±0.55 | 77.18±0.19 | 2.76±1.20 |
| 3 | 45°c | 77.68±1.05 | 1.24±0.55 | 77.08±0.54 | 3.64±0.95 |

## CONCLUSION

Protransferosome gel containing Diclofenac potassium was successfully formulated by phase separation coacervation method. Protransfersome gel was optimized by preparing total 13 formulations. All the formulations were optimized on the basis of evaluation parameters such as entrapment efficiency, vesicle size. Drug permeation was greater for Span 60 due to its low surface energy decreases, drug permeation increases. When Span 60 was combine with different surfactant in varying concentration its shows that the drug permeation get increases than that of using span 60 individually. It has been observed that optimized formulation F1B1 which contain surfactant Span 60: sodium deoxycholate in 1:1 ratio produce the protransfersomal gel with good consistency, spreadability, elasticity, viscosity, spontaneity and *In-vitro* drug permeation. It was found more promising among all formulation and has effective permeation rate. In SEM micrographs transfersome suspension showed that the vesicles have a uniform spherical shape. PTG shows zeta potential of -20.3mV. The results of *In vitro* anti-inflammatory study also revealed that the PTG formulation having span 60: Sodium deoxycholate showed the best inhibition of inflammation and sustained the drug release for a period of 12 h. From the stability studies, it was found that there was no significant change in the drug entrapment and vesicular shape. Protransfersomes may be a promising carrier for Diclofenac potassium, especially due to their simple production and facile scale up.

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