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Formulation and evaluation of diclofenac sodium transferosomes using different surfactants by thin film hydration method

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ABSTRACT

The present study aimed to prepare and evaluate Diclofenac sodium transferosomes for transdermal drug delivery. Transferosomes were prepared by thin film hydration method by varying the ratios of soya lecithin and surfactants in the organic phase. Three surfactants i.e. Span 20, span 60, span 80 were selected for the present study. Drug concentration was kept constant. With each surfactant 5 formulations were prepared. The prepared formulations were evaluated for vesicle size, morphology, zeta potential, drug content, entrapment efficiency and invitro skin permeation. It was investigated that among three surfactants span 60 was considered to be a better surfactant because of its small vesicle diameter and high entrapment efficiency with good stability. By using span 60, 5 formulations were prepared by varying the concentration of soyalecithin to surfactant. Among the 5 formulations of span 60, F10 formulation with 2:1 ratio of soyalecithin to surfactant was found to have the highest entrapment efficiency of 62.2%, drug content of 96.5%, vesicle size of 257.1nm, zeta potential of -25mv. The drug release was continued upto 12 hrs and 58.9% of drug has been released from the formulation representing the sustained release nature when compared to span 20 and span 80. The present study revealed successful preparation of Diclofenac sodium transferosomes, effect of type of surfactant and soyalecithin:surfactant ratio on entrapment efficiency, vesicle morphology and drug release was studied.

Keywords: Transferosome, Diclofenac Sodium, Soya Lecithin, Span20,60,80, Thin Film Hydration.

INTRODUCTION

Transdermal drug delivery systems (TDDS) offer a number of potential advantages over conventional methods such as injectables and oral delivery[1]. However, the major limitation of TDDs is the permeability of the skin; it is permeable to small molecules and lipophilic drugs and highly impermeable to macromolecules and hydrophilic drugs. The main barrier and rate-limiting step for diffusion of drugs across the skin is provided by the outermost layer of the skin, the stratum corneum[2]. Several strategies have been developed to overcome the skin's resistance, including the use of prodrugs, ion pairs, liposomes, microneedles, ultrasound, and iontophoresis [3-6].

Various types of liposomes (LPs) exist, such as traditional liposomes, niosomes, ethosomes, and transfersomes[11,1, 6]. Various LPs have been extensively investigated for improving skin permeation enhancement. Liposomes are promising carriers for enhancing skin permeation because they have high membrane fluidity. Previous reports indicate that liposomes can deliver a large quantity of hydrophilic drugs (e.g., sodium fluorescein, carboxyfluorescein), lipophilic drugs (e.g., retinoic acid, tretinoin), proteins, and macromolecules through the skin. Many factors influence the percutaneous penetration behavior of LPs, including particle size, surface charge, lipid composition, bilayer elasticity, lamellarity, and type of LPs[15,3,7]. Cevc's group introduced Transfersomes, which are the first generation of elastic vesicles. Transfersomes are prepared from phospholipids and edge activators. An edge activator is often a single-chain surfactant with a high radius of curvature that destabilizes the lipid bilayers of the vesicles and increases the deformability of the bilayers. Sodium cholate, sodium deoxycholate, Span 60, Span 65,Span 80, Tween 20, Tween 60, Tween 80, and dipotassium glycyrrhizinate were employed as edge activators.

Compared with subcutaneous administration, transfersomes improved in vitro skin permeation of various drugs, penetrated intact skin in vivo, and efficiently transferred therapeutic amounts of drugs[1,2,6].

Diclofenac sodium is a Nonsteroidal anti-inflammatory drug (NSAID) is the most frequently prescribed drug, which is used inboth 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. Several formulation approaches have been developed for NSAID's transdermal administration[11-14]. The conventional pharmaceutical dosage forms which are widely administered dermally aregels, creams, and ointments.

The objective of the present study was to prepare transferosomes of Diclofenac sodium by thin film hydration method and evaluate the effect of different surfactants span 20, 60, 80 and the effect of soya lecithin to surfactant ratio on vesicle morphology, entrapment efficiency and invitro drug release.

MATERIALS AND METHODS

Materials

Diclofenac sodium was supplied as a gift sample by NATCO pharmaceuticals. soyalecithin, span20,60,80 were purchased from SD Fine chemicals,Mumbai. Chloroform, ethanol and other chemical reagents used were of analytical laboratory grade.

Preparation of Diclofenac Sodium Transferosomes

Required quantities of Soya lecithin and surfactant were taken in a round bottom flask and dissolved in chloroform and ethanol by shaking. The thin film was formed by rotary evaporation by using rotary evaporator for 15 minutes at25^{0}C, 600mm/hg pressure and 100rpm.Vacume was applied for one hour to dry the film. Diclofenac sodium was dissolved in 10ml 7.4 pH phosphate buffer which was heated to 55c.Then the film was hydrated with the heated buffer by hand shaking for half an hour. Then the mixture was stirred for half an hour in orbital shaker. Then the transferosomes were observed under microscope. Transferosomal suspension was stored in refrigerator at 4^{0}C. Composition of transferosomes given in table 1.

Table1: formulation variables used in preparation of DFS transferosomes

S.no.	Formula-tion code	surfactant	Pc:surfactant ratio	Chloroform: Methanol(ml)	Diclofenac sodium(mg)
1	F1	Span20	1:1	6:4	50
2	F2	Span20	1:1.5	6:4	50
3	F3	Span20	1.5:1	6:4	50
4	F4	Span20	1:2	6:4	50
5	F5	Span20	2:1	6:4	50
6	F6	Span60	1:1	6:4	50
7	F7	Span60	1:1.5	6:4	50
8	F8	Span60	1.5:1	6:4	50
9	F9	Span60	1:2	6:4	50
10	F10	Span60	2:1	6:4	50
11	F11	Span80	1:1	6:4	50
12	F12	Span80	1:1.5	6:4	50
13	F13	Span80	1.5:1	6:4	50
14	F14	Span80	1:2	6:4	50
15	F15	Span80	2:1	6:4	50

Where DFS=Diclofenac sodium, Pc=phosphatidylcholine

CHARACTERIZATION OF DICLOFENAC SODIUM TRANSFEROSOMES

The transferosomal suspension obtained for all the formulations (F1- F15) was then characterized for particle size distribution and zeta potential to ensure that they were within Nano /micron size range and possessed optimum stability respectively. Further, they were evaluated for following parameters like entrapment efficiency, drug content and in vitro diffusion studies.

Mean Vesicle Diameter and Zeta Potential Measurement

The average vesicle size and size distribution of Diclofenac loaded transferosomes was determined by dynamic light scattering (DLS), using Malvern Zeta Sizer. The Zeta potential (Surface Charge) which indicates the stability of the transferosomes can be defined as electro kinetic potential that is determined by electrophoretic mobility. Sample was

prepared by diluting with water and corresponding zeta potential measured using Malvern Zeta Sizer.

Determining The Size And Surface Morphology Of The Transferosomes:
Scanning Electron Microscopy is used to determine the shape, size and surface morphology of the transferosomes. Suspension was made to obtain Photomicrographs of the Diclofenac loaded transferosomes using the SEM

EVALUATION OF DICLOFENAC SODIUM TRANSFEROSOMES
Drug Content
1 ml of Diclofenac loaded transferosomal suspension was taken and diluted with 10ml 7.4ph phosphate buffer. It was ultracentrifuged at 170000 rpm for 40 minutes at -4c. The pellet formed after centrifugation was disrupted with 10 ml methanol to come out the drug from vesicles. 1ml of this solution was taken and suitable dilutions were made and analysed by UV spectrophotometer at 276nm which gives the concentration of entrapped drug. The concentration of drug in supernatant and pellet collectively gives the amount of drug present in 1ml of suspension. % drug content was calculated by dividing with theoretical drug content present in 1ml of suspension.

% drug content=practical drug content/theoretical drug content ×100

Entrapment Efficiency
1 ml of Diclofenac loaded transferosomal suspension was taken and diluted with 10ml 7.4ph phosphate buffer. It was ultracentrifuged at 170000 rpm for 40 minutes at -4^0c. After centrifugation pellet was formed at the bottom of centrifuge tube.1 ml of supernatant was collected and suitable dilutions were made and analyzed by UV spectrophotometer at 276 nm. % entrapment efficiency was calculated by the following formula.

% entrapment efficiency=Total drug added-unentrapped drug/ Total drug added

Invitro Drug Diffusion Study
Diffusion studies were carried out using franz diffusion cell by using dialysis membrane. 1 ml of transferosomal suspension was taken in donor compartment and 25 ml of 7.4ph phosphate buffer was taken in receiver compartment. Alliquotes of 5ml of samples were withdrawn at definite time intervals from the sampling port and replaced with the buffer to maintain sink conditions. The samples were analysed by UV spectrophotometer at 276 nm. The % of drug release in a time period of 12 hours was reported.

RESULTS

Characterization Of Transferosomes
Surface Morphology:-The prepared transferosomes were spherical in shape (figure 1). The size distribution of the prepared transferosomes along the mean diameter was measured using particle size analyzer. The average vesicle size of the prepared Diclofenac loaded transferosomes was recorded. It was found to be minimum for F10.The vesicle size of all the formulation ranged between 257.1-340.2nm and the report of mean particle diameter of the optimized formulation was given in fig 2.

Figure1: Photomicrograph of F10 Formulation

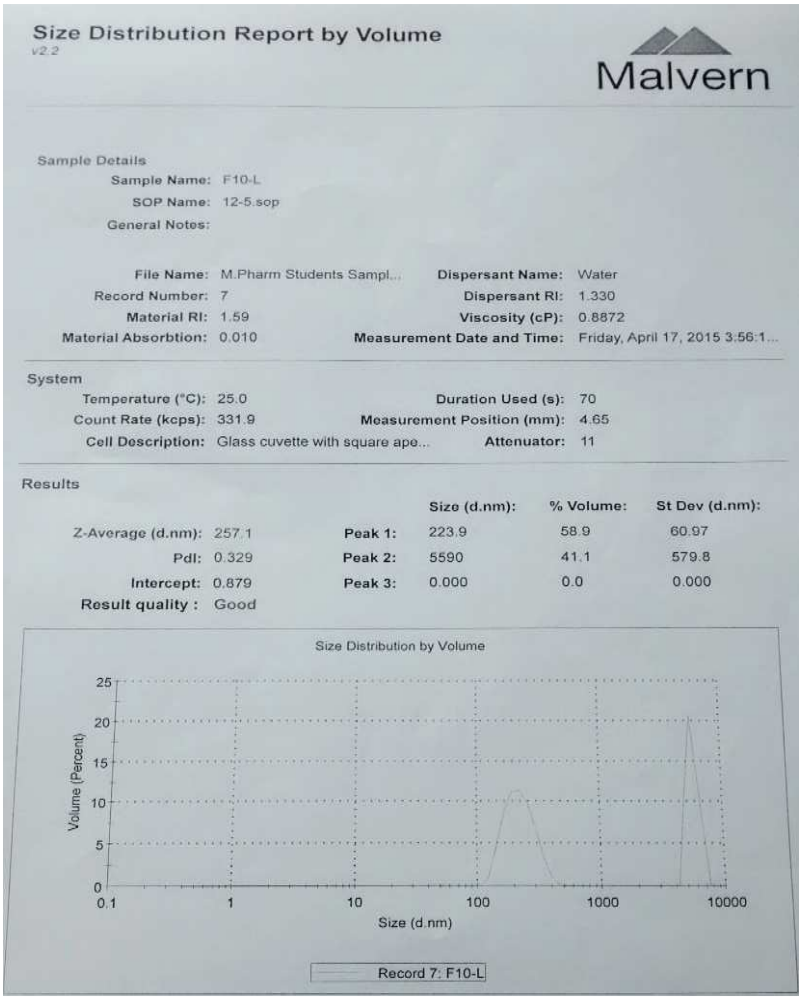

Figure2: Mean vesicle diameter of optimized formulation

Zeta Potential:- Zeta potential of the prepared diclofenac loaded transferosomes was measured using zeta meter. transferosomes prepared by span 60 (F10) 2:1 ratio showed higher stability, bearing a value of -25 mV when compared to all other formulations.

Evaluation of transferosomes:-

The effect of different surfactants and soyalecithin:surfactant ratio was studied upon parameters like entrapment efficiency, drug content, in vitro diffusion studies etc. The drug encapsulated in the transferosomal vesicles tends to leak out during storage. A significant loss of Diclofenac was observed during storage at 4c for a period of 3 months and there was decrease in the drug content value.

Product yield:-The yield obtained for all the formulations prepared by thin film hydration technique was optimum. They were evaluated for above mentioned characters and results obtained were as follows.

Drug Content of the Formulations:-

The drug content for all the 15 formulations was evaluated and it varied between 90.2% to 96.5%. Among all the formulations the transferosomes prepared by span 60 (F10) 2:1 ratio was superior with highest drug content of 96.5%. Followed by span 80 (F15) formulation with 95.6% followed by span20 (F5) with drug content of 95.1% respectively.

The order of drug content

F10 > F15 > F5 > F3 > F13 > F4> F12 > F14 > F8>F1>F6>F2>F11>F7>F9

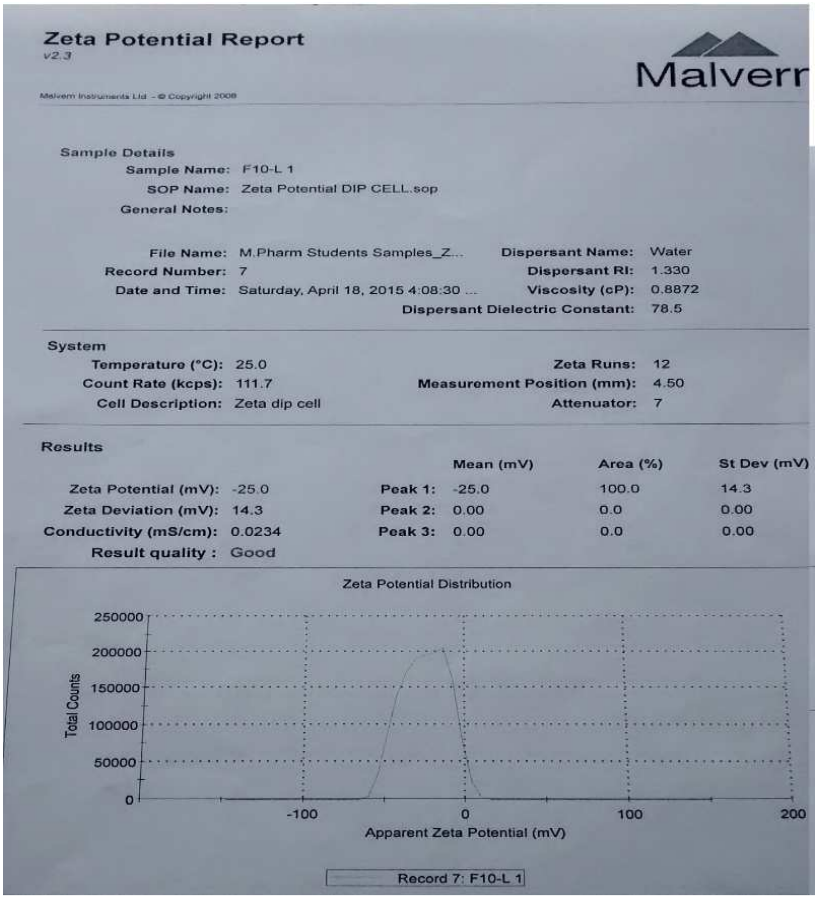

Figure3: zeta potential of optimized formulation

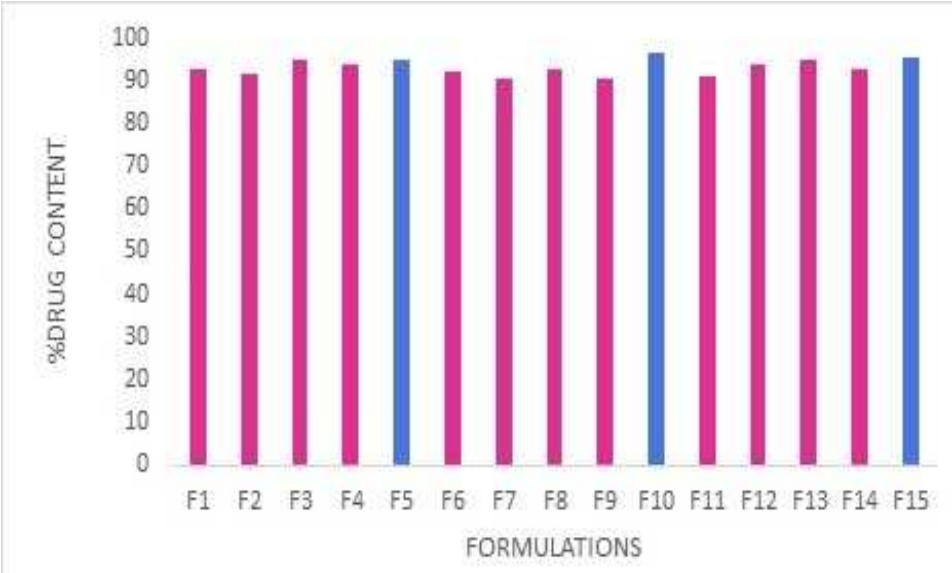

Figure 4: Drug Content Profile of all 15 Formulations

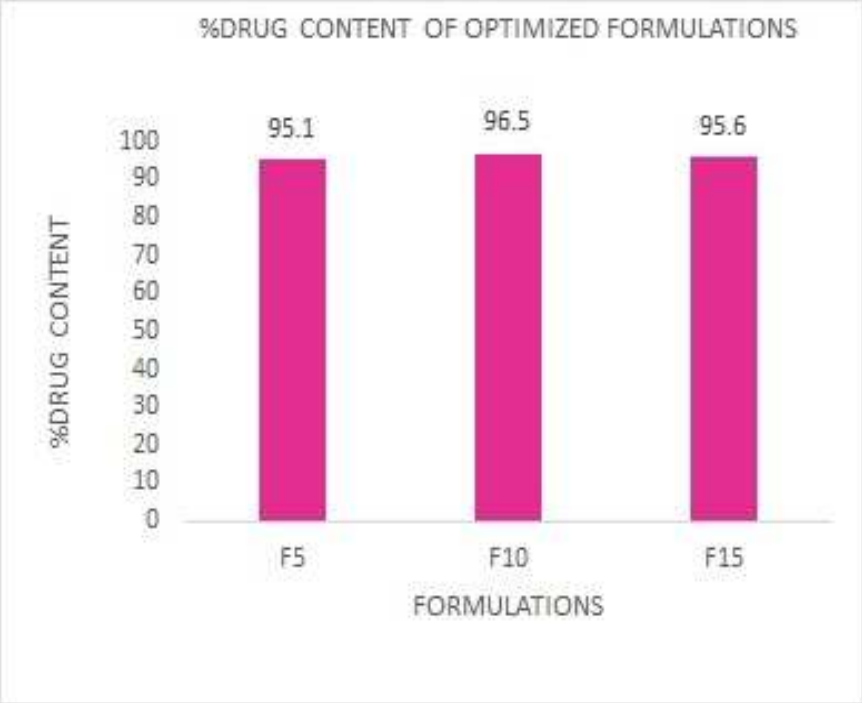

Figure 5: Drug Content Profile of the three optimized Formulations

Entrapment Efficiency:-

The Entrapment Efficiency for all the 15 formulations was evaluated and it varied between 32.6% to 62.2% Among all the 15 formulations the highest entrapment efficiency was found to be for span 60 (F10)2:1 ratio with entrapment of 62.2% followed by span 80 (F15) 2:1 ratio with the entrapment efficiency of 61.2% followed by span 20 (F5) 2:1 ratio with entrapment efficiency of 60 % respectively.

The order of entrapment efficiency

F10 > F15 > F5 > F8> F6 > F13 > F3 > F7> F1>F11>F2>F4>F9>F12>F1

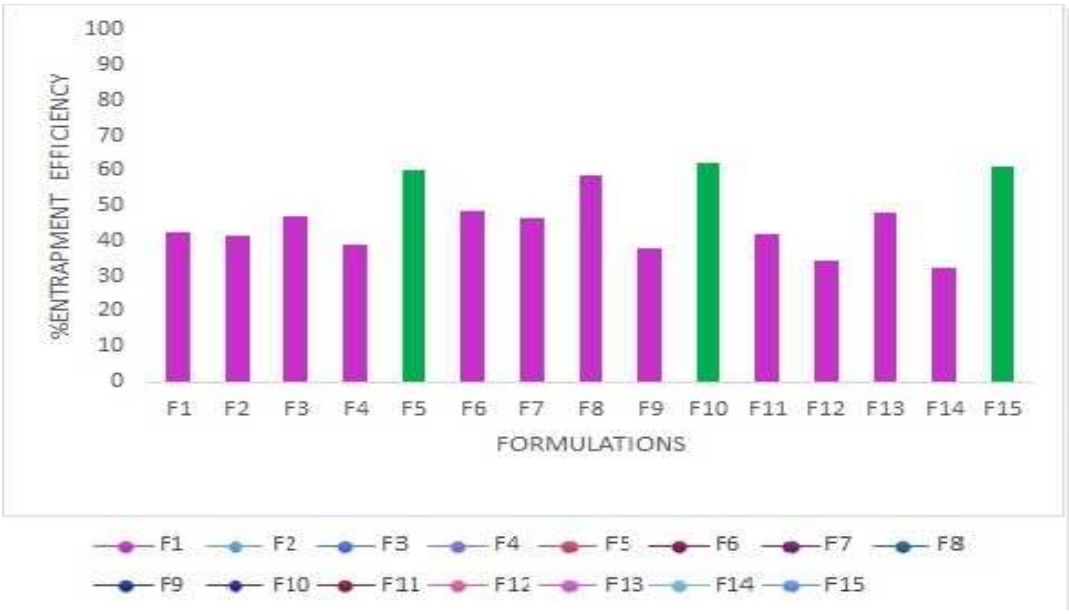

Figure 6: Entrapment Efficiency Profile of 15 Formulations

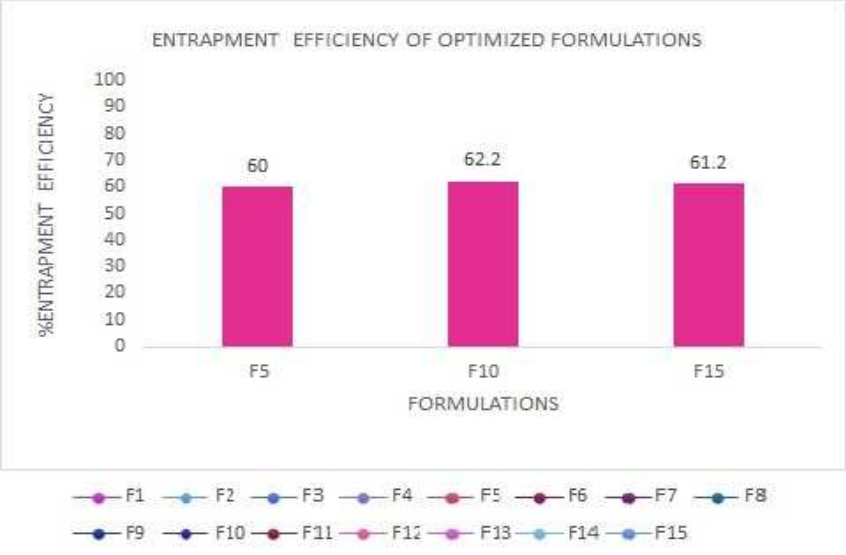

Figure7: Entrapment Efficiency Profile of the three optimized Formulations

In vitro Drug Diffusion Studies:-

In vitro drug diffusion studies were performed using Franz diffusion cell to determine the sustained release nature of the formulations. The diffusion studywas continued up to 12 hours. For F10 formulation the drug release was found to be sustained with the release of 59.8% for span 60 2:1 ratio, 64.5% for span 80 2:1 ratio (F15) formulation and 83.4% for span 20 2:1 ratio (F5)formulation.whereas the other formulations were sustained up to 8 -11 hours.

The order of in vitro controlled drug release up to 12 hours
F10>F15>F13>F8>F5>F1>F3>F11>F1>F7>F12>F9>F2>F14>F4

Comparative Invitro drug release kinetic data of optimized formulations(F10,F15,F5) given in the following figures. F10,F15 formulations followed zero order kinetics and F5 followed first order kinetics with supercase II transport mechanism.

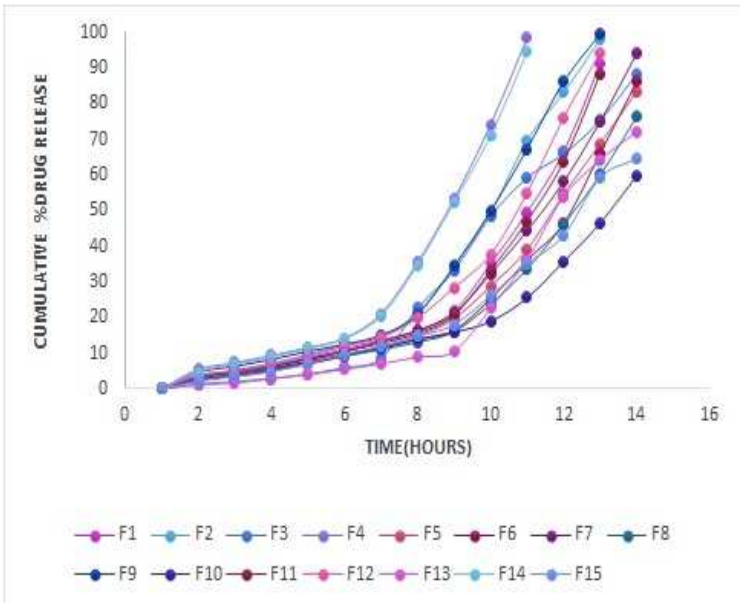

Figure 8:-Comparative Cum % Release Vs Time profile of 15 formulations

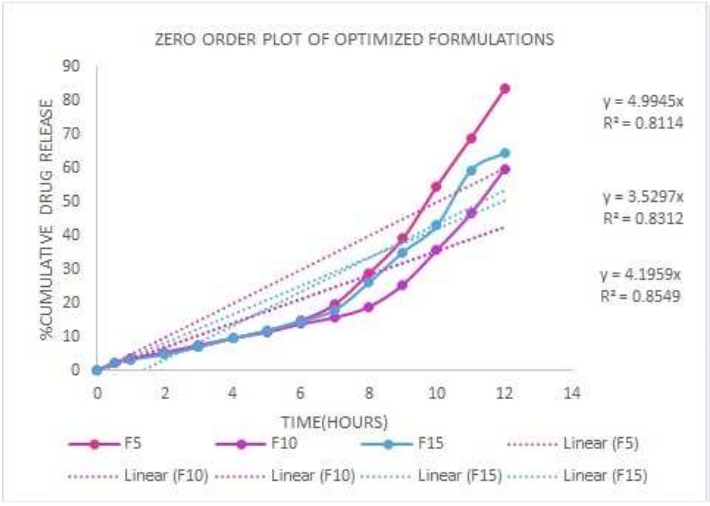

Figure 9:-Zero Order Plot of Optimized Formulations (F10, F15 and F5) Span 60,80,20

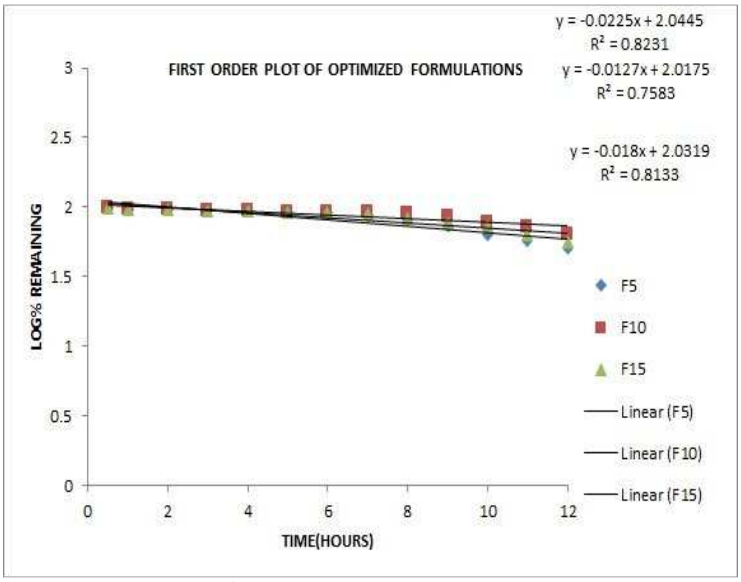

Figure10: First Order Plot of Optimized Formulations (F10, F15 and F5) Span60, 80, 20

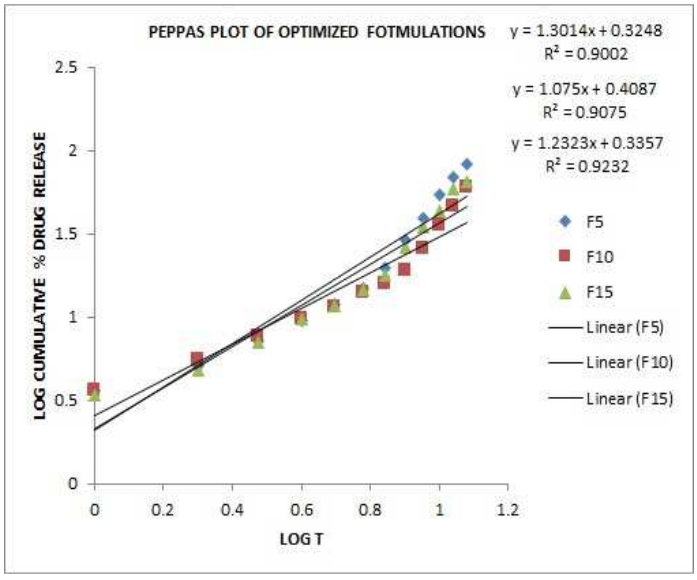

Figure11: Peppas Plot of Optimized Formulations (F10, F15 and F5) Span 60, 80,20

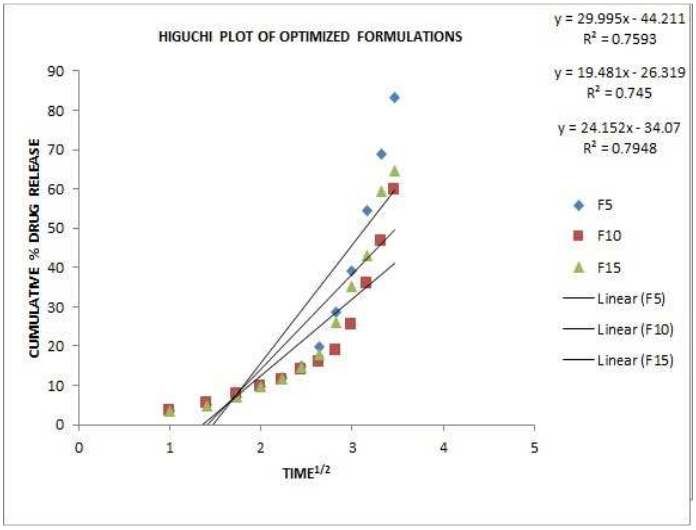

Figure12: Higuchi Plot of Optimized Formulations (F10, F15 and F5) Span 60, 80, 20

DISCUSSION

Diclofenac sodium transferosomes were prepared by thin film hydration method. The main factors affecting the size and shape of the vesicle is the concentration of soyalecithin and HLB value of surfactants.

Increase in the concentration of soyalecithin increases the entrapment efficiency of vesicles, thus preventing the drug from leakage.

Data in figure 7 reveals that the entrapment efficiency for transferosomes prepared by span 60 was superior when compared to span 20 and span80.This may be due to many factors like hydration temperature used for making transferosomes, phase transition temperature of surfactant, Alkyl chain length of the surfactant, HLB value of the surfactant, Saturation and unsaturation of alkyl chain length. The hydration temperature should be above the gel to liquid phase transition temperature which makes the transferosomes less leaky and possess high entrapment efficiency. Among all the surfactants span 60 was superior as it has the highest phase transition temperature of 50c and hence exhibit highest entrapment efficiency when compared to span 20 and span80. The length of alkyl chain length have a major effect on the permeability of prepared transferosomes, As the length of surfactant chain increases the entrapment efficiency increases and decrease in chain length decreases entrapment and among all the surfactants span60 found to better. Lower the HLB higher will be the entrapment, vesicle size, stability etc. The HLB value of span 60, 80, 20 was found to be 4.7, 4.3, 8.6 respectively. Eventhough span 80 is having the least HLB value but the entrapment efficiency was not found to be optimum it may be mainly because of existance of unsaturated alkyl chain length. Span 80 and span 60 possess same alkyl chain length but span 60 is having saturated chain length as well as higher phase transition temperature 53c whereas span80 is having unsaturated alkyl chain length with least phase transition temperature of -12^0C.

Hence the order of surfactant with higher entrapment, Drug content and less Particle size is as follows:-
SPAN 60 > SPAN 80 > SPAN 20.

In tranferosomal formulations, the studies showed that the rate of drug release depends on the percentage of drug entrapment efficiency. From the non-ionic surfactants used (span 60, span 80, span 20) the highest sustained release was obtained for span 60 (F10) 2:1 ratio.

The ratio of soya lecithin to surfactant was optimized for all the three surfactants. With increased concentration of soya lecithin compared to surfactant increases entrapment efficiency. it was found that the entrapment efficiency was decreased when the concentration of surfactant was more than soya lecithin. Finally 2:1 ratio of soyalecithin to surfactant was optimized which given best results. Because the well entrapped drug released in a controlled manner.
In the present study the effect of type of surfactant on formulation was evaluated. It was found that span 60 was best suitable for the preparation of Diclofenac sodium transferosomes because span 60 having long alkyl chain length and saturated alkyl chain compared to other spans. Phase transition temperature of span 60 was high (53c).So the entrapment efficiency was more.

Transferosomes prepared with span 60 were good in terms of stability. Transferosomes prepared with span 20 and 80 were getting separated upon storage.

Out of the 5 formulations of span 60, F10 formulation of 2:1 ratio was found to be best formulation because of its good entrapment efficiency(62.2%), drug content(96.5%) and 59.8% sustained drug relese in a time period of 12 hours.

CONCLUSION

Diclofenac sodium is a first line drug in the treatment of rheumatoid arthritis. for transdermal delivery. Transferosomes of diclofenac sodium was successfully prepared using three surfactants by thin film hydration method. The ratio of soya lecithin to surfactant was optimized. it was concluded that span 60 was best suitable surfactant for the preparation of diclofenac sodium transferosomes because of its good entrapment efficiency and stability. Five formulations were prepared by varying the lipid to surfactant ratio using span 60 as surfactant. On comparison 2:1 ratio was showing sustained drug release property. From the results it can be concluded that span 60 was considered as a better surfactant because of its high entrapment efficiency, dug content and good stability. Among the 15 formulations F10 with 2:1 lipid to surfactant ratio was found to be optimized formulation because of its highest entrapment efficiency of 62.2%, drug content of 96.5%, less vesicle size of 257.1nm, zeta potential-25mV and sustained drug release property.

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