Diclofenac Sodium (NSAID) Proniosomal Formulation for Enhanced Transdermal Delivery

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Abstract- **The objective of the thesis was to assess proniosomes of DFS to increase its permeation through skin for enhanced transdermal drug delivery in systemic circulation avoiding hepatic first-pass metabolism. Results indicated that all the formulations were found to be smooth and spherical in shape and average particle size (17.52±1.54 to 25.23±1.02; size influenced by agitation). Drug loading efficacy (%) of proniosomes was 90.78±0.96 to 95.92±1.36. Entrapment efficacy was maximum for formulation R4.**

The *in-vitro* drug release study was performed in phosphate buffer solution at pH 7.2 and by changing the combination of different types of surfactants the % drug release were found in the range of 85.12±3.82, 80.46±2.54, 83.87±2.92, 79.12±2.62 and 76.25±2.80 for formulations R1, R2, R3, R4, R5 and R6 respectively. Finally, present study conclusively demonstrated that drugs having poor water solubility showed enhanced bioavailability by transdermal route via proniosomes.

Keywords: **Drug release, drug loading, entrapment, first pass metabolism, nanotechnology, niosomes, proniosomes, skin, surfactant, transdermal.**

Introduction

Kumar *et al*., 2012, illustrated that encapsulation of API in a vesicle is example of vesicular drug delivery system (VDDS) and there are many VDDS systems like cubosomes, liposomes, phytosomes, niosomes, transferosomes, proniosomes etc. were developed (Radha *et al*., 2013).

Kamani *et al*., 2012, in medication targeting, APIs can be specifically and precisely targeted to the site of action without interfering with other tissues. Immunoglobin, serum proteins, synthetic polymers and liposomes are used as carriers to target the medicine. Adella *et al*., 2014, reported that controlled release drug products maintaindrug concentration at target site.

Sunitha *et al*., 2020, advancement in nanotechnology ((modify matter on a nano scale range / functional systems at atomic level) has made tremendous revolutionary improvements in the innovative areas of drug delivery (development of new formulations), diagnostics, nutraceuticals and biomedical for implants. Waghmode and Shruti (2012), nanotechnology research played a important role for various types of NDDS like liposomes, microparticles, niosomes and proniosomes. (Chien, 2009)

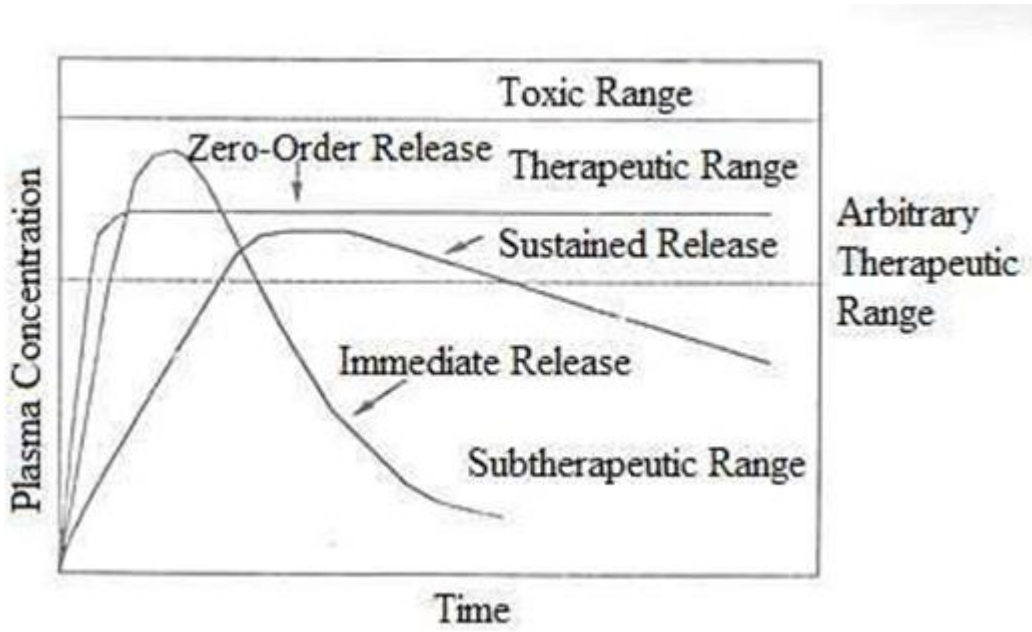


Figure 1.1: Plasma Drug Profiles.

Transdermal Drug Delivery System (TDDS)

Skin as Route of TDDS

Jacob (1970), reported that skin is the largest organ of the body, accounting for more than 10% of body mass (surface area is 3000 inch^2 / approximately 1.8m^2)and receives one third of the circulating blood. Mehta *et al*., 2004, permeation involves partitioning into and transport through the cutaneous layers (stratum corneum) and epidermis (stratum basale). (Barry (2001)

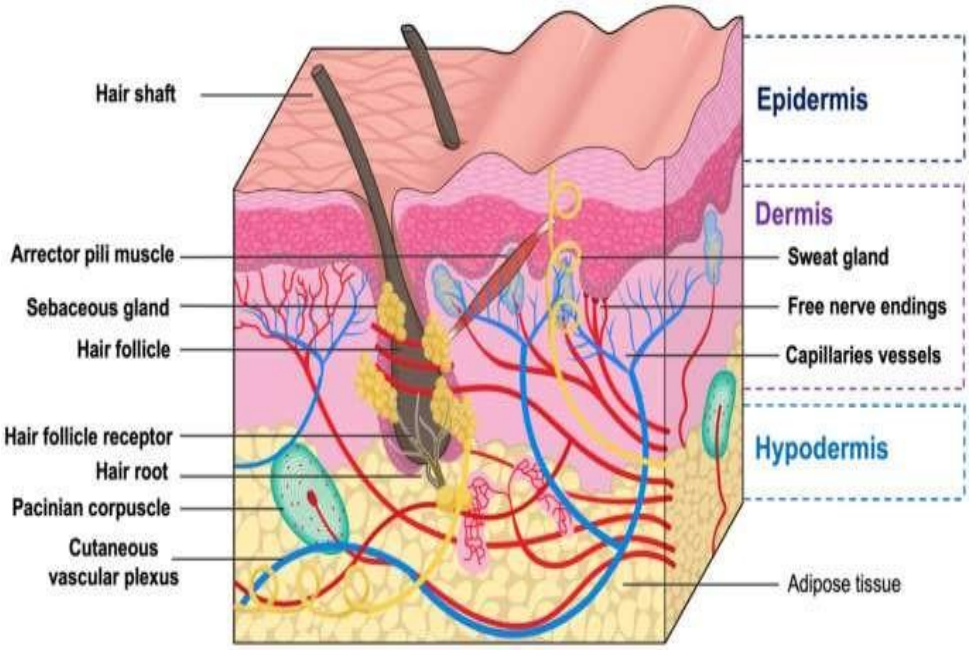


Figure 2: Skin Structure.

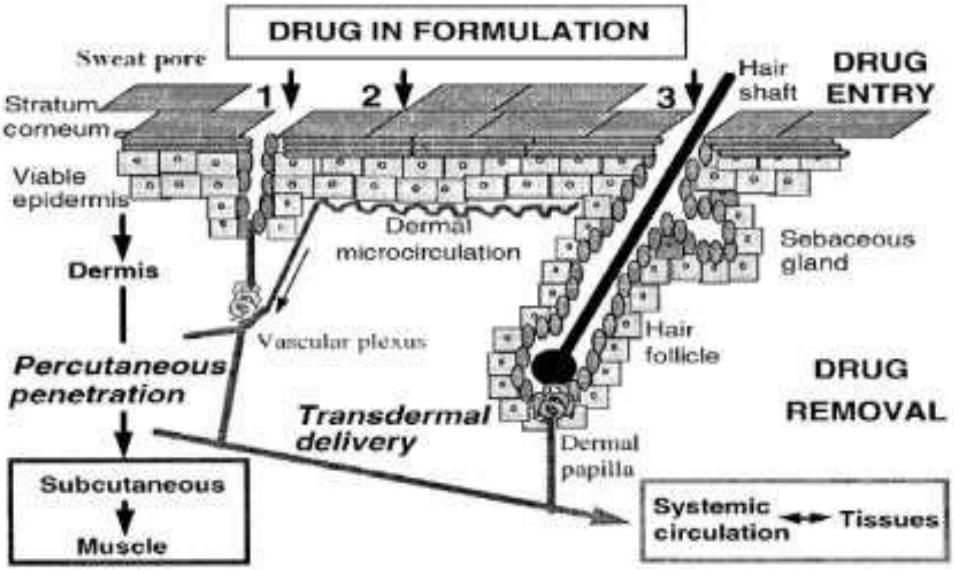


Figure 3: Mechanism of drug through by transdermal delivery.

Route of Drug Delivery in Skin

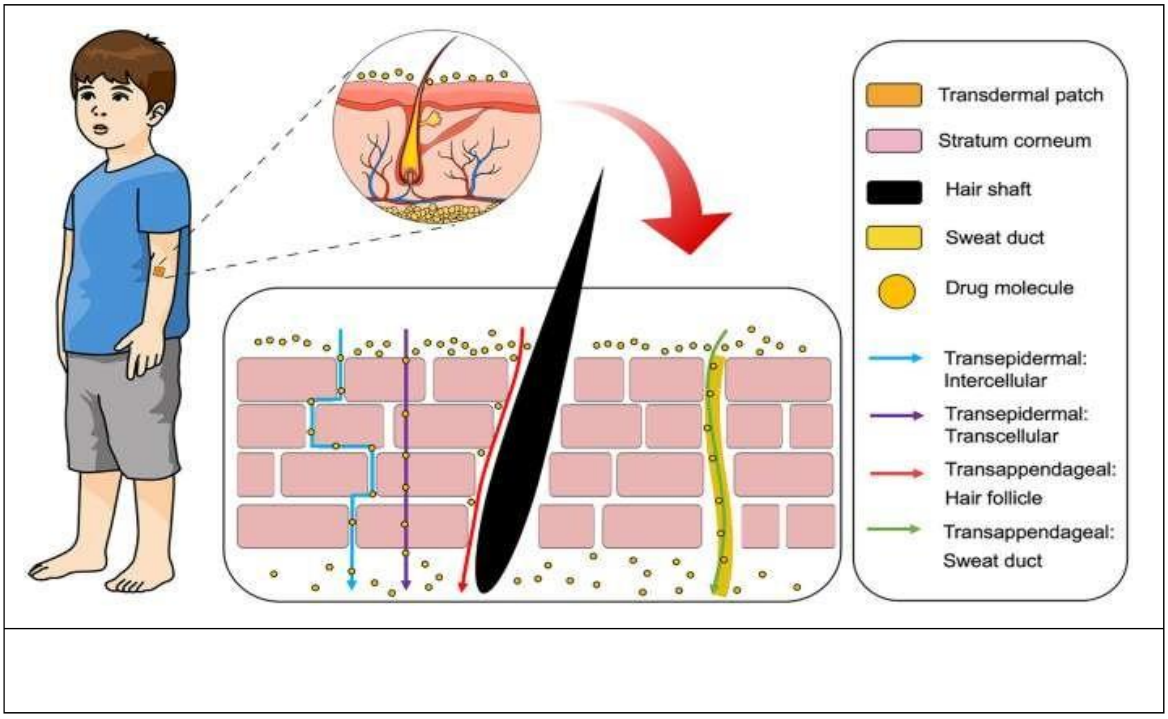


Figure 4 : Transdermal drug delivery mechanisms.

Permeation Enhancement by TDDS Formulation

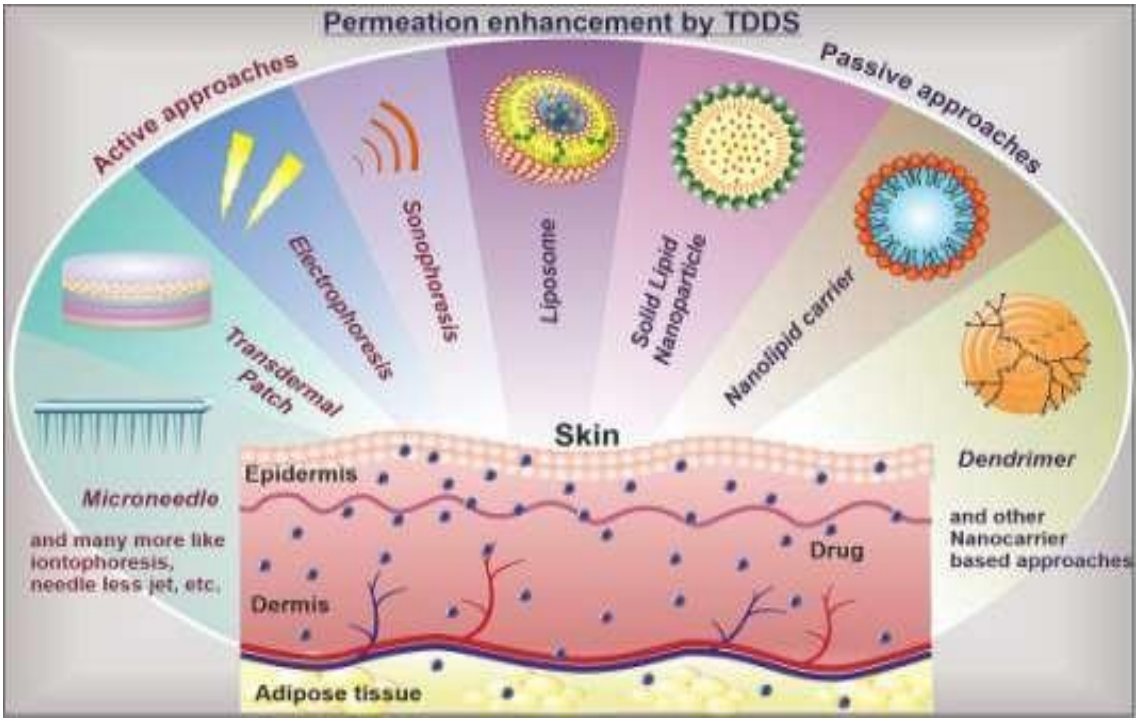


Figure 5: Permeation Enhancement by TDDS.

Technologies for enhancing TDDS

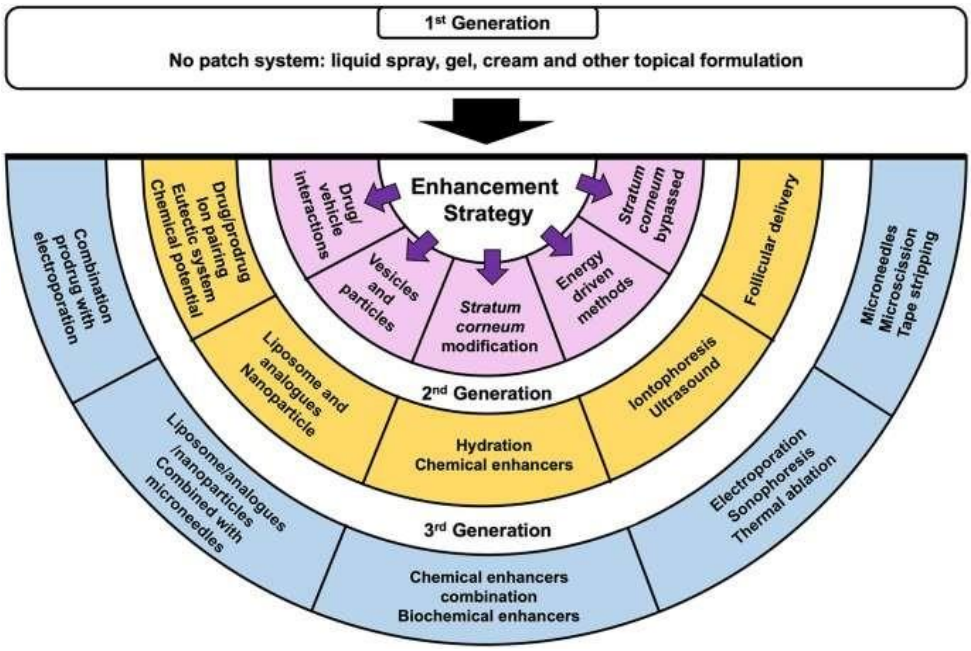
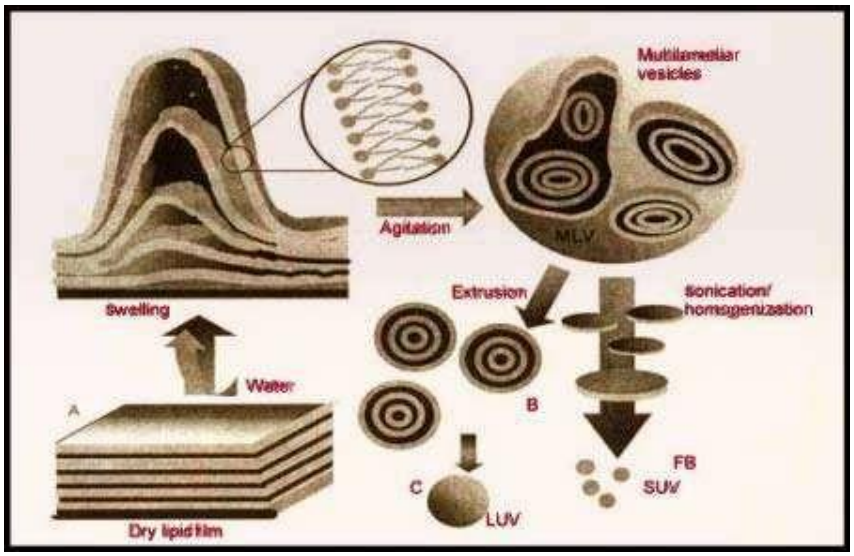


Figure 6: Technologies for enhancing TDDS.

Vesicular Drug Delivery System (VDDS)

Chengjiu *et al*., 1999, VDDS (liposomes, transferosomes, pharmacosomes, niosomes / proniosomes) is a novel approach (spherical vesicles) becomes the vehicle (improved bioavailability of poorly soluble drugs) of choice in dermal and transdermal drug delivery. (Kakr *et al*., 2010; *Abdul et al*., 2019)

Figure 7 : Vesicle formation.



Mittal *et al*., 2020, uni lamellar vesicles/or more multilamellar vesicles in presence of water (Walve *et al*., 2011); hydrophilic drugs and lipophillic drugs drugs can be entrapped into the vesicles; Maryam *et al*., 2017)

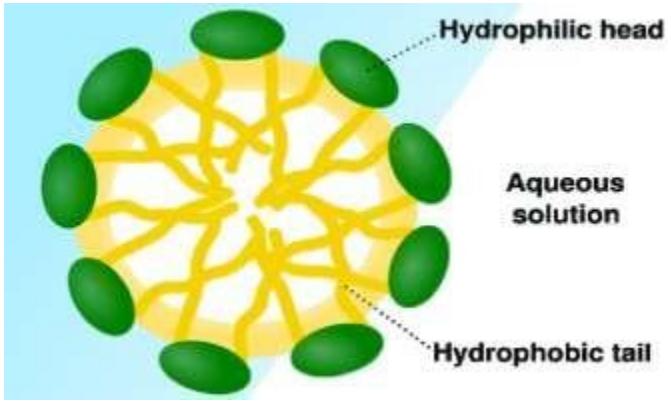


Figure 8: Vesicle structure.

Yasam *et al*., 2014, lipids and surfactants used to prepare vesicles (phospholipids or non-ionic surfactants). They are liposomes, niosomes or non-ionic surfactants vesicles. (Ammar *et al*., 2011; Ahmad *et al*., 2017)

Proniosomes (Akhilesh *et al*., 2012)

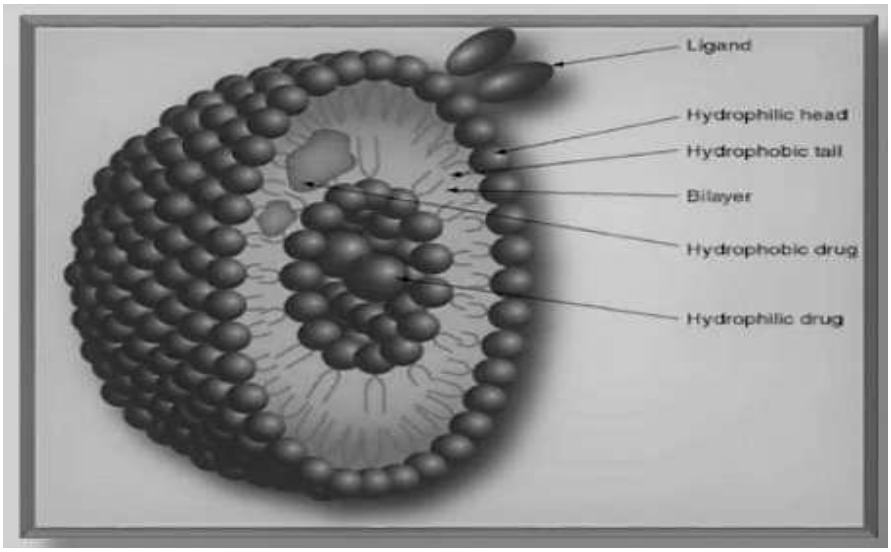


Figure 9: Structure of Proniosomes.

Akhilesh *et al*., 2012, proniosomes are dry formulation of water soluble carrier particles coated with surfactant; rehydrated to form niosomal dispersion immediately before use on agitation in hot aqueous media within minutes; physically stable during storage and transport.

Types of Proniosomes
Two types (on the basis type of carrier and method of preparation):

(a) Dry Granular Proniosomes
(i) Sorbitol based proniosomes

Sorbitol based proniosomes are dry formulation (sorbitol as a carrier) coated with non-ionic surfactant (used as noisome by the addition of hot water followed by agitation; Kakr *et al*., 2010)

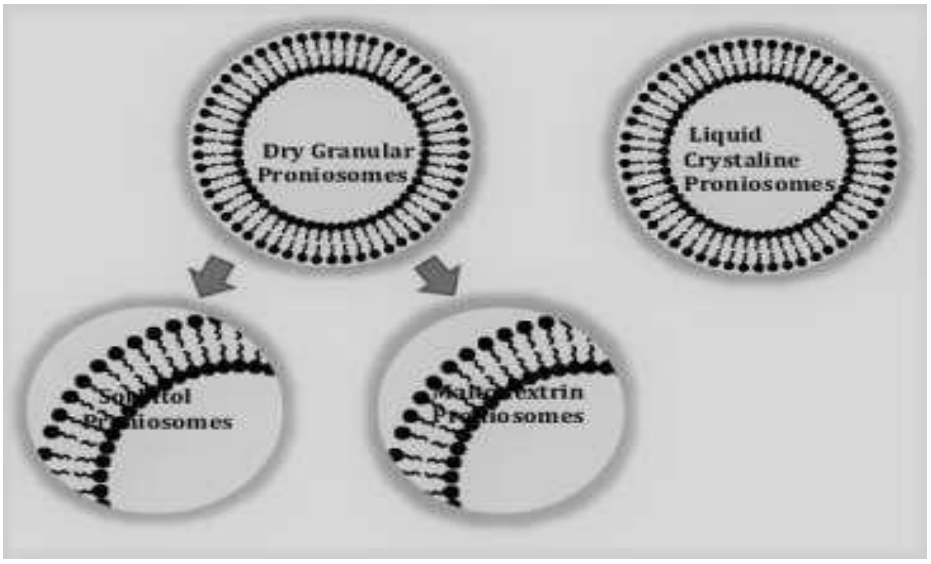
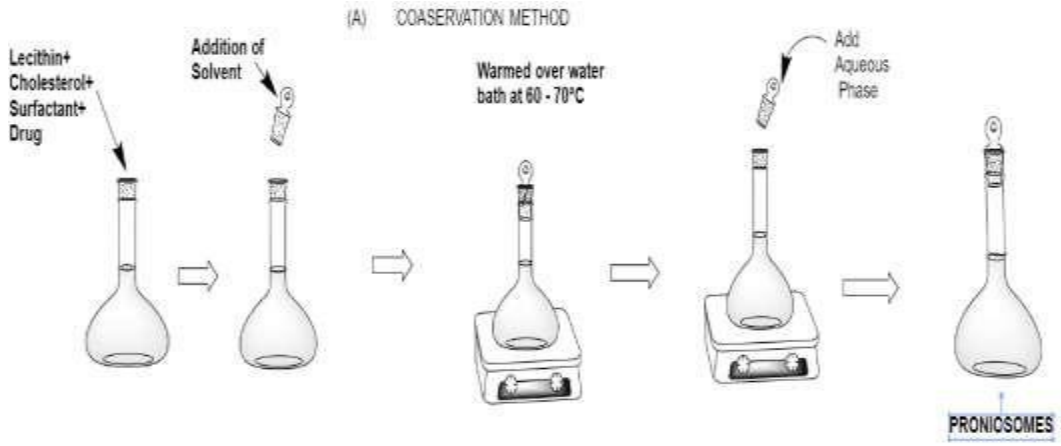


Figure 10 : Types of Proniosomes.

Preparation of Proniosomes

i. Hand shaking method (Walve *et al*., 2011)
ii. Slurry method (Walve *et al*., 2011)
iii. Slow spray coating method (Mishra *et al*., 2011; Sankar *etal*., 2010)



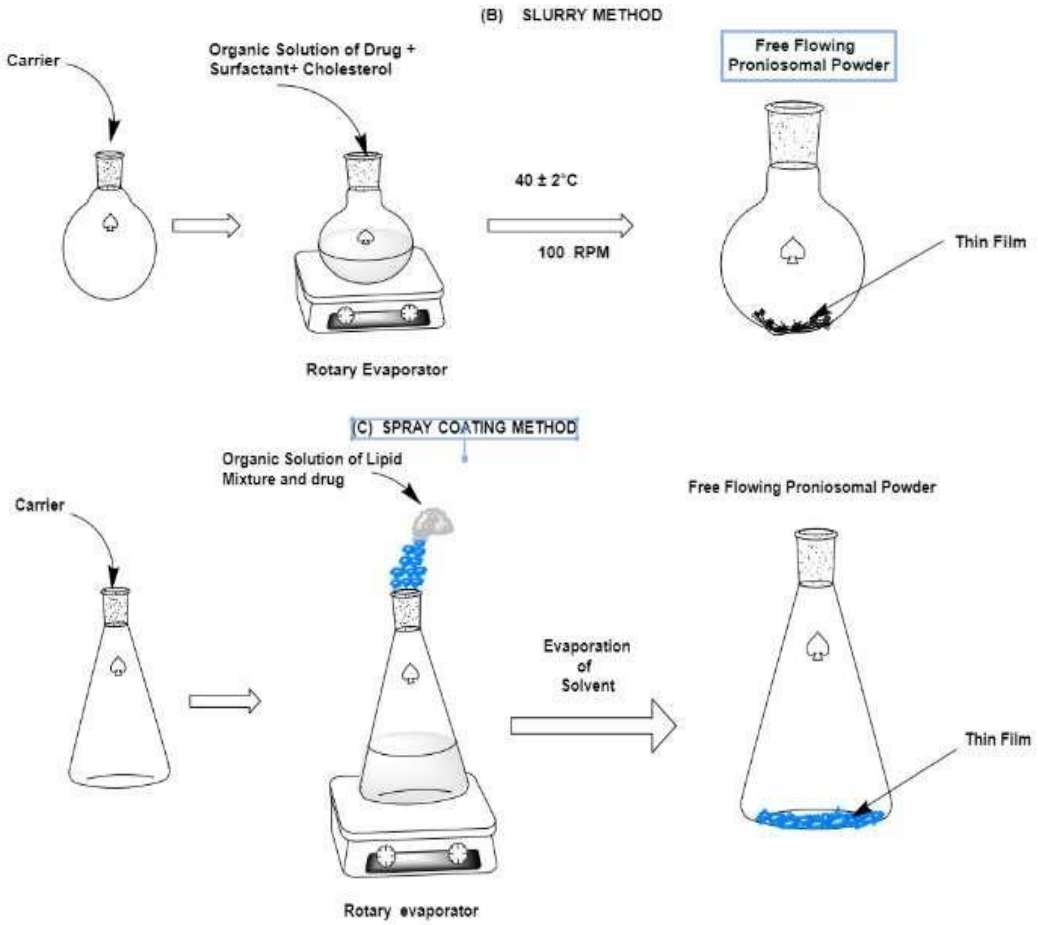


Figure 11: Various Methods of Preparation of proniosomal preparations.

Characterization of Proniosomes

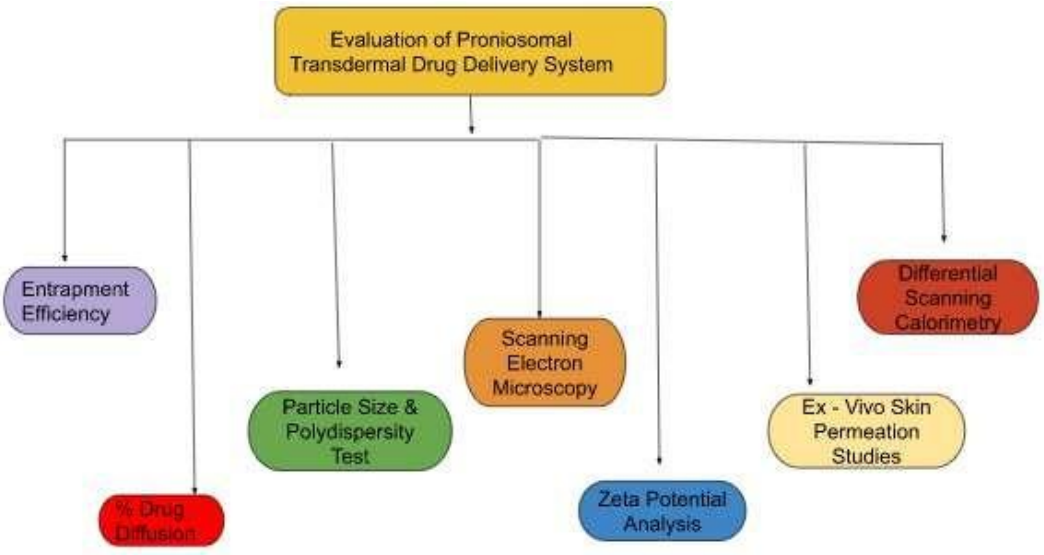


Figure 12: Evaluation of Proniosomal Transdermal Drug Delivery System.

Proniosomes Advantages (Arunothayanun *et al*., 2000)

- Act as penetration enhancers (drug diffusion);
- Overcomes problems of noisome / liposomes;
- Enhance recovery rate of skin barrier.

Applications of Proniosomes

Drug targeting

(i) Proniosomes in Cardiology (Gupta *et al*., 2007)
(ii) Proniosomes in diabetes (Azeem *et al*., 2009)
(iii) Proniosomes in hormonal therapy (Vora *et al*., 1998)
(iv) Proniosomes in delivery of peptide drugs (Akhilesh *et al*., 2012)
(v) Uses in studying immune response (Chandra and Sharma (2008)
(vi) Niosomes as carriers for hemoglobin (Kakr *et al*., 2010)

Other applications

(i) Sustained release (Biju *et al*., 2006)
(ii) Localized drug action (Oommen *et al*., 1999)

Materials and Methods

Diclofenac sodium is an aryl acetic acid derivative (water insoluble) and a potent NSAID therapeutically used for the several of inflammatory conditions like chronic rheumatoid arthritis and osteoarthritis (inhibition of prostaglandin (PG) synthesis. In the present work, an attempt was made to develop a proniosomal drug delivery system of diclofenac sodium (poorly water-soluble). In current scenario, very creative and innovative research projects have been undertaken using nanotechnology (magnanimous growth in drug delivery technologies) for deliver the drug at a desired rate and period of time. VDDS (liposomes, transferosomes, pharmacosomes, niosomes / proniosomes) function as sustained release systems solve the problems of drug insolubility, instability and rapid degradation. Proniosomes (dry-niosomes; comparatively more stable than niosomes) enhance bioavailability. Niemic *et al*., 1995, both hydrophilic and hydrophobic substances can be embedded in niosomal vesicles (Arunothayanum *et al*., 2000). Non-ionic surfactants increases both permeability and fluidity of biological membranes so drugs having poor water solubility show enhanced bioavailability by transdermal route via proniosomes (Florence *et al*., 1993). The aim of the present study was to develop the proniosomal drug delivery system of diclofenac sodium and to assess the ability of transdermal preparation thus avoiding hepatic first pass metabolism.

Polymer Profiles

Cholesterol

Cholesterol is the principal sterol (a steroid with a hydoxy group in position-3) and is a 27 carbon compound with a unique structure with a hydrocarbon tail, a central sterol nucleus made of four hydrocarbon rings, and a hydroxyl group. Central sterol nucleus or ring is a feature of all steroid hormones.

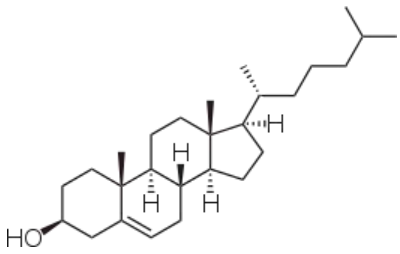


Figure 13: Chemical Structure of Cholesterol.

Phosphatidylcholine

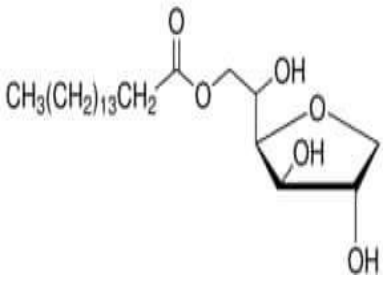
Phosphatidylcholine is a chemical contained in eggs, soybeans, mustard, sunflower, and other foods and found naturally in the body in all cells, source of choline in the body and used for ulcerative colitis. Walkey *et al*., 1998, phosphatidylcholines are composed of two fatty acids covalently linked to a glycerol moiety by ester bonds in the sn-1 and sn-2 positions. The third carbon of glycerol is esterified to phosphorylcholine.

Sorbitan Laureate

Sorbitan laurate (naturally derived or synthetic) is used as a surfactant, cleansing agent, emulsifying agent, stabilizing agent, lubricating agent,dispersing agent, and as a food additive;

i. Used to enhance the overall texture of skin care formulas;
ii. Fiber lubricant & softener in the textile industry;
iii. Wetting agent and dispersant for pigments;

Sorbitan Monopalmitate (Span 40)



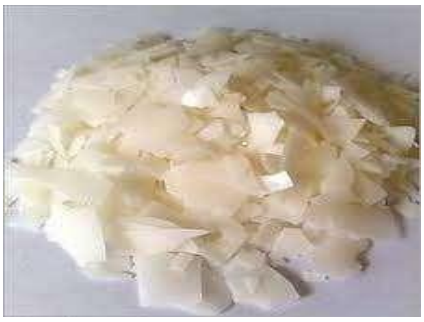


Figure 14a: Structure of SorbitanMonopalmitate.
Figure 1 4b: Flakes of SorbitanMonopalmitate.

Pre-formulations Studies

The overall objective of pre-formulation testing is to generate information useful to the formulation and development of stable dosage form with good bioavailability.

Identification of Samples drug - Diclofenac sodium (DFS) by FTIR

DFS + KBr dispersion pellets FTIR Spectroscopy 4000-400cm^{-1}.(Shimatzu 8400 PC based). Drug sample (DFS) peaks were compared with standard DFS drug (Figure 15).

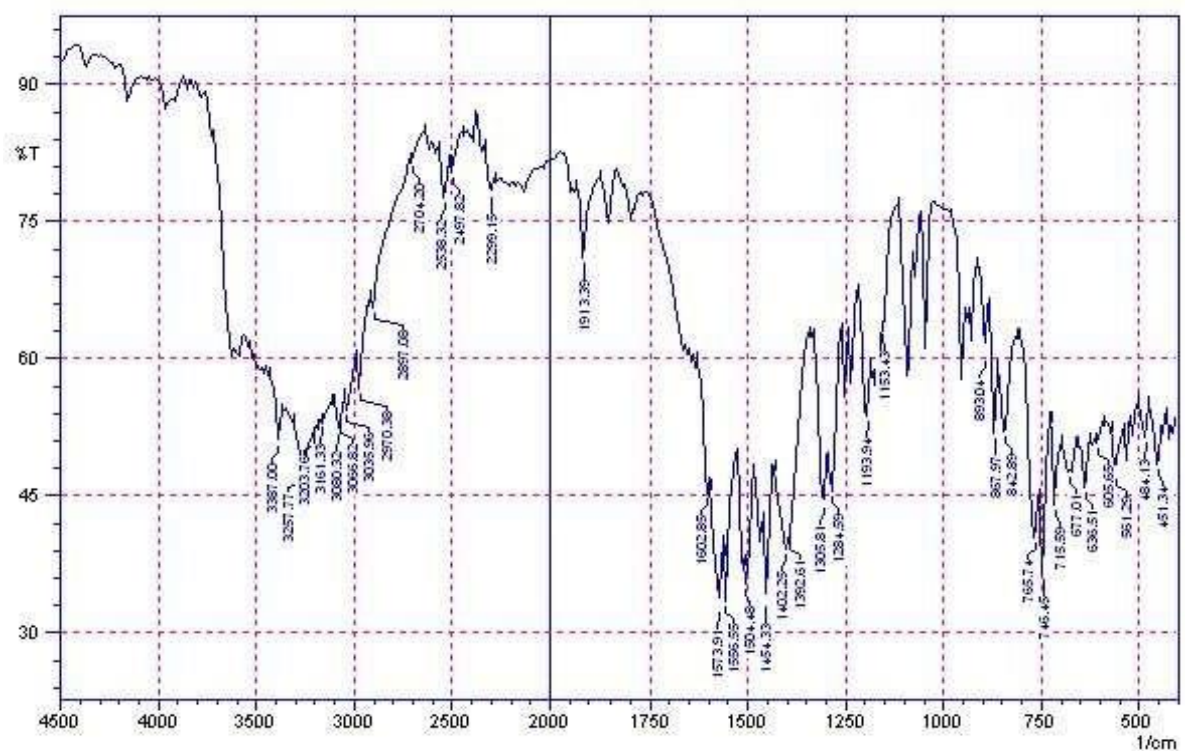


Figure 15: IR spectra of DFS (/ Pure).

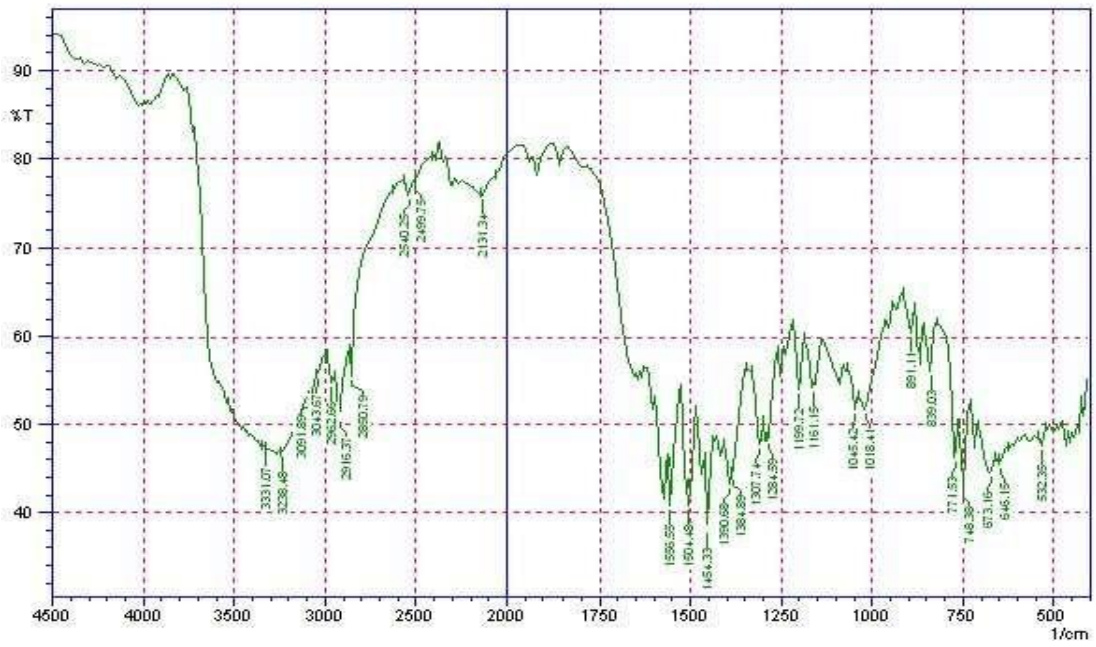


Figure 16 : IR spectra of Diclofenac sodium (Drug Sample).

Table 1: Interpretation of DFS FTIR Spectra

Functional Group	Wave number / Peak (Cm^{-1})
N-H	3210.66 - 3447.91
CH	1405.37 - 1452.30
C=C	1518.53 - 1576.04
C-C	844.99 - 1094.82
C-NH	1178.42 - 1284.28
Cl	622.96 - 755.33

Identification of Drug (DFS) by Ultraviolet (UV) Spectroscopy

In order to determine λmax of diclofenac sodium (DFS) stock solution in ethanol / water was prepared and scanned / analysed for absorbance at wavelength between 200-400 nm using Double Beam UV Spectrophotometer - Model 2202, Systronics, India. Stock solution B (10 µg/ml) was scanned between between 200- 400 nm using to determine the absorption maxima (λmax) using Double Beam UV Spectrophotometer - Model Systronics -2202) (Figure 5). Observed λmax of Diclofenac sodium (DFS) was 275.6 nm.

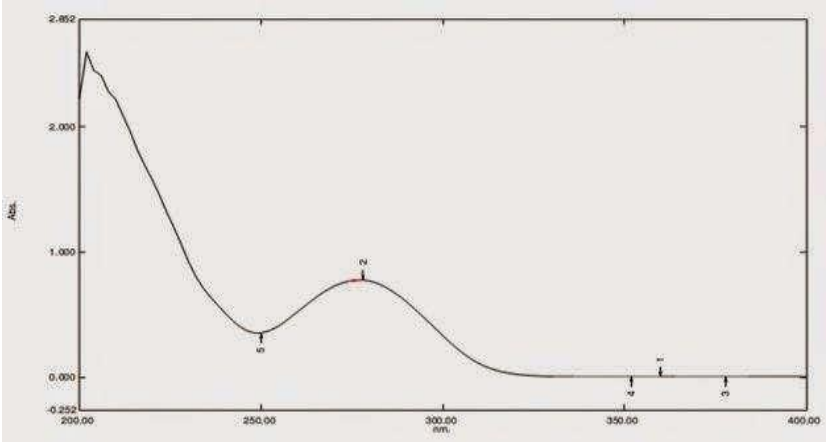


Figure 17: Absorption maxima (λmax) of Diclofenac sodium (DFS).

Melting Point Analysis of Diclofenac sodium (DFS)

Melting point (mp) is the temperature at which the pure liquid and solid exist equilibrium. MP of the Diclofenac sodium (DFS) was determined byusing thieles tube method (Table 2).

Table 2: Melting point of Diclofenac sodium (DFS)

S. No.	Reported	Observed
1.	280^0C	278^0C & 281^0C

Solubility Determination / Analysis of Diclofenac Sodium (DFS)

Table 3: Solubility of diclofenac sodium in different solvents.

Solvent	Solubility (µg/ml)
Distilled water	56.31 (poorly soluble)
Ethanol	980.21 (Freely Soluble)
Methanol	968.48 (Freely Soluble)
1-octanol	935.16 (Soluble Freely)
Acetone	272.82 (Slightly Soluble)

Physical Appearance Analysis of Diclofenac Sodium (DFS)

Colour : White Powder
Nature : Crystalline Powder (Solid)
Odour : Characteristic odour
Taste : Alkaline taste

Partition Coefficient Determination of Diclofenac Sodium (DFS)

Table 4: Partition Coefficient of DFS.

Solvent System	Reported	Observed
n-octanol / water	1.46	1.418
n-octanol / PBS (pH : 7.2)	1.34	1.127

Compatibility Studies of Diclofenac sodium

Peaks of physical mixtures were unchanged / similar peaks like pure DFS.

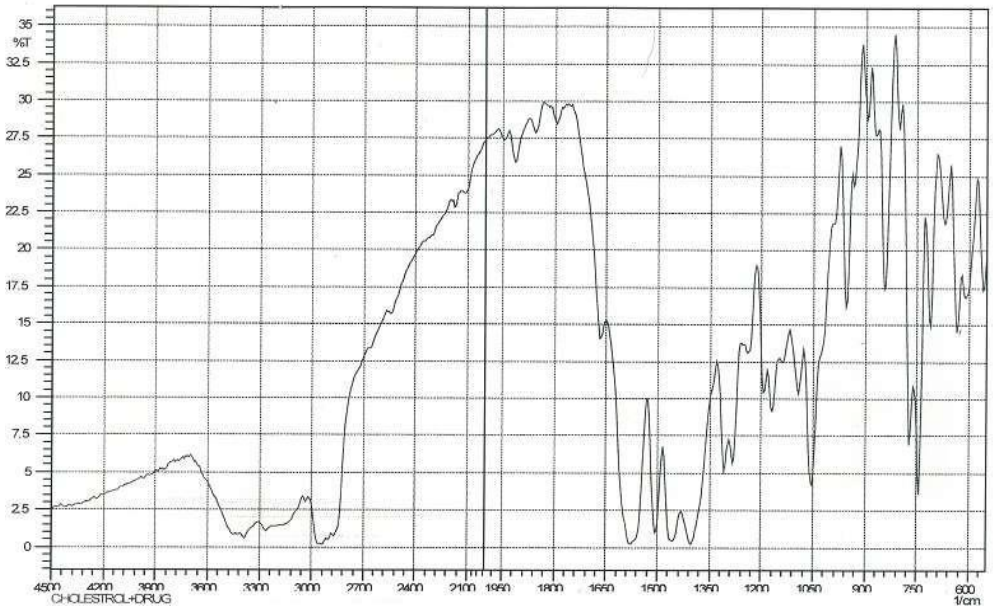


Figure 18 : FTIR spectra of Cholesterol + Diclofenac sodium (Drug).

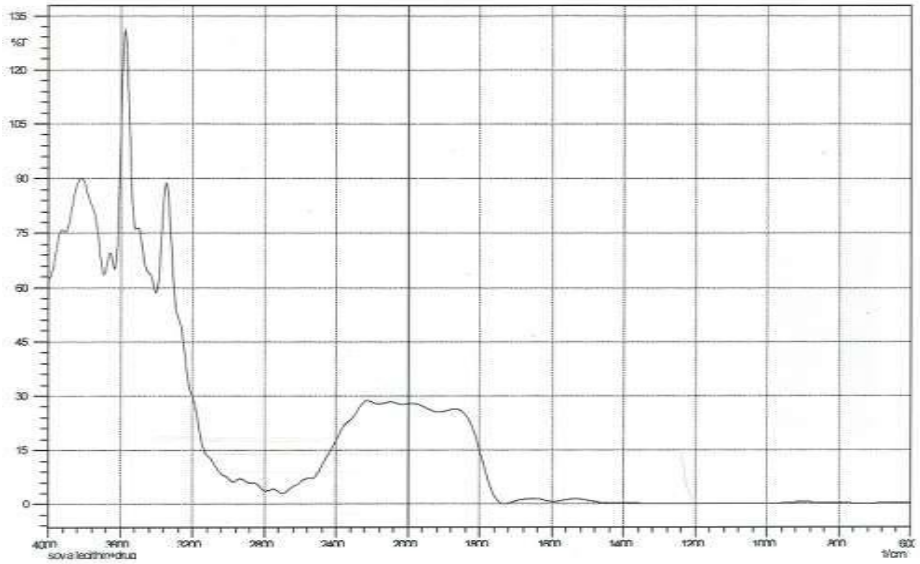


Figure 19: FTIR spectra of Lecithin + Diclofenac sodium (Drug).

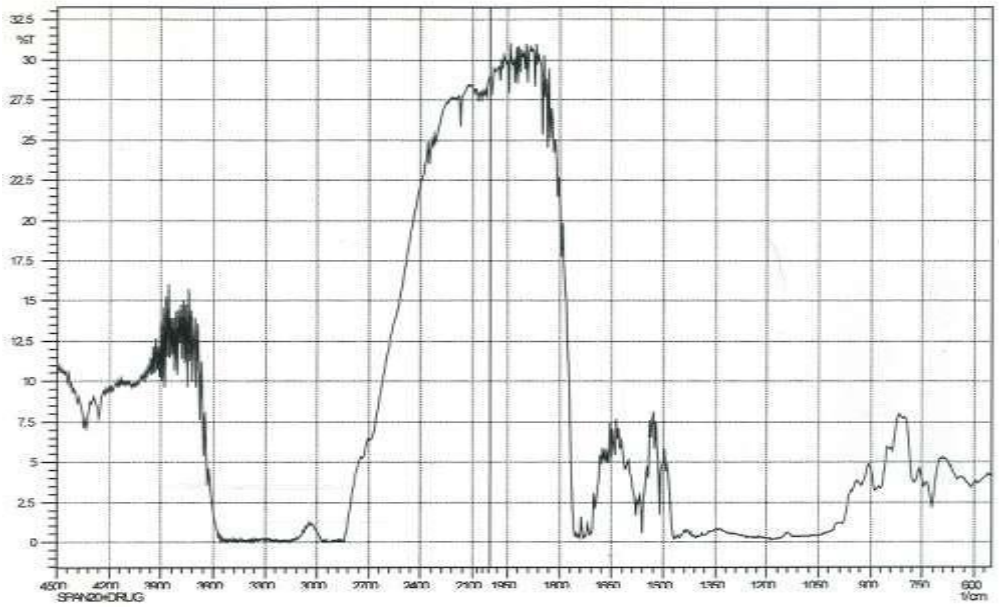


Figure 20 : FTIR spectra of Span 20 + Diclofenac sodium (Drug).

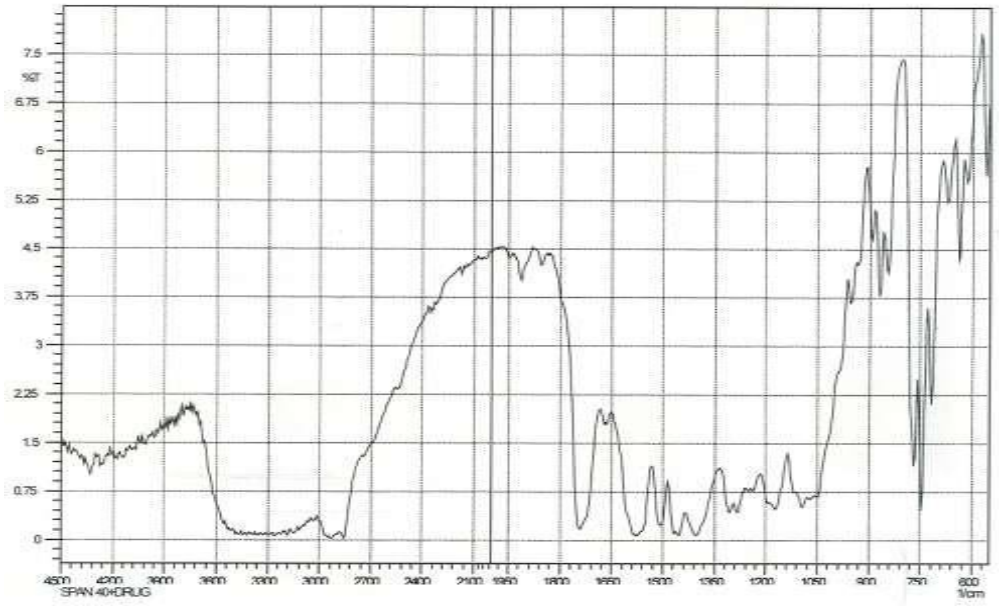


Figure 21: FTIR spectra of Span 40 + Diclofenac sodium (Drug).

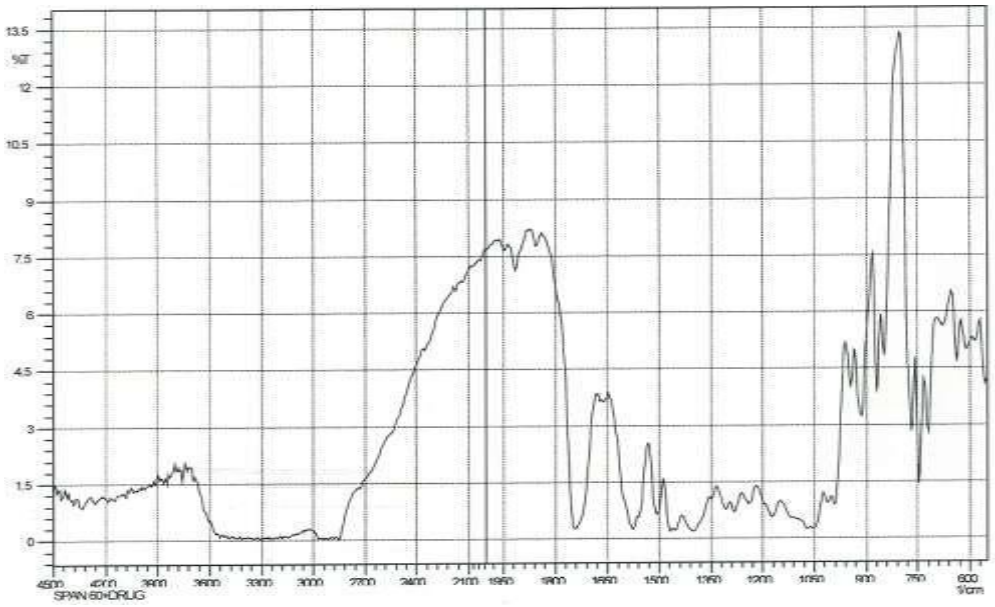


Figure 22: FTIR spectra of Span 60 + Diclofenac sodium (Drug).

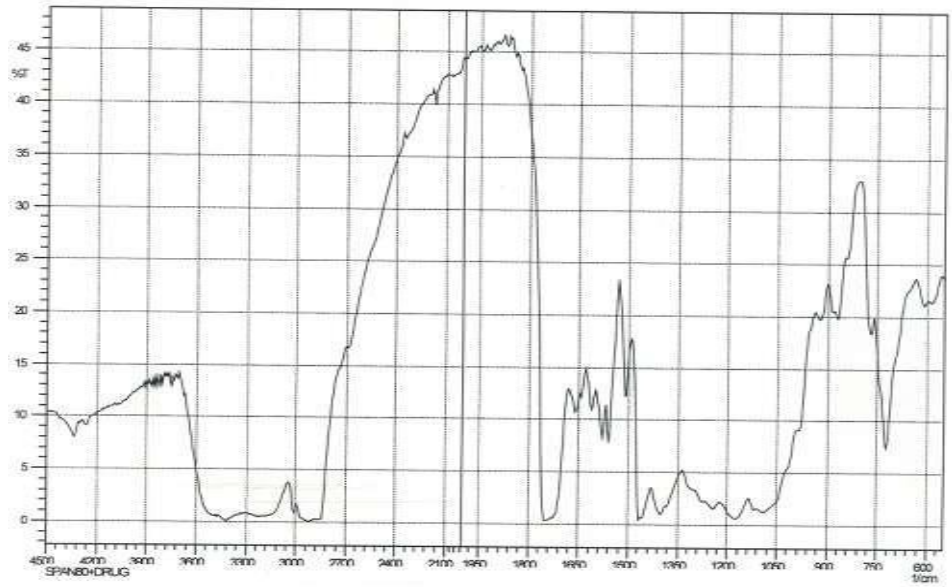


Figure 23: FTIR spectra of Span 80 + Diclofenac sodium (Drug).

Preparation of Calibration / Standard Curve

Different aliquots (dilutions) of Stock solution B (10 µg/ml) were prepared to get 2, 4, 6, 8 and 10 µg/ml concentration and absorbance was recorded at 276 nm against blank (Systronics- 2202, Double Beam UV Spectrophotometer). Same procedure was repeated with phosphate buffer (pH 7.2) and 0.1N HCl solution (pH 1.2). (Table 5; Figure 24-26).

Table 5 : Calibration Curve of DFS.

S. No.	Concentration DFS (µg/ml)	Absorbance (nm)		
		D.W.	PBS	0.1N HCl
1.	2	0.021	0.046	0.034
2.	4	0.046	0.081	0.062
3.	6	0.072	0.123	0.093
4.	8	0.095	0.157	0.126
5.	10	0.119	0.202	0.152

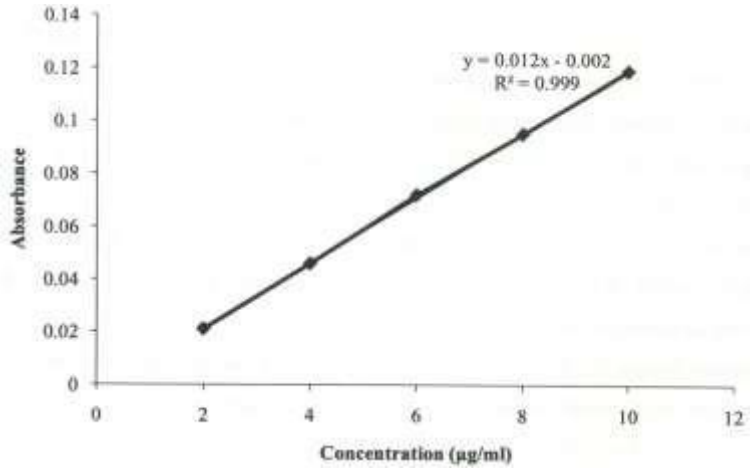


Figure 24: Calibration Curve of DFS in D.W.

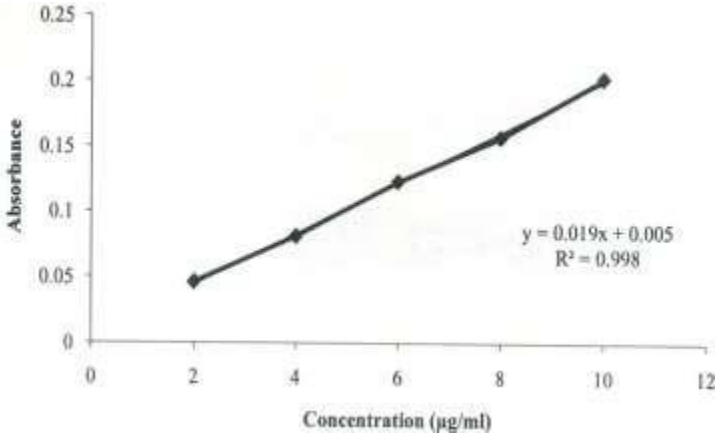


Figure 25: Calibration Curve of DFS in PBS (pH 7.2)

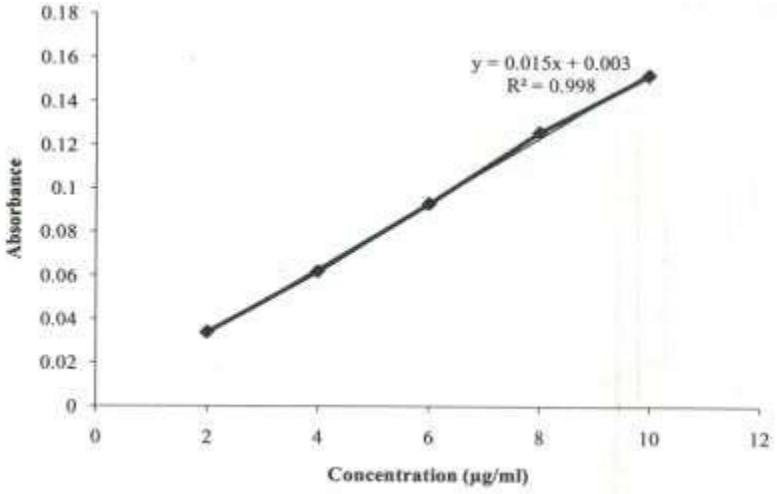


Figure 26: Calibration Curve of DFS in 0.1N HCl (pH 1.2)

Preparation of the Formulation

Method of Preparation: Coacervation Phase Separation Method

Accurately weighed quantity of surfactant, carrier (lecithin), cholesterol and drug DFS were taken in clean and dry glass vial (5 ml) and solvent was added with simple mixing. Vials were closed with cap and heated on water bath for 5 min (untilthe surfactant dissolved completely). The mixtures were allowed to cool at room temperature till the dispersion got converted into proniosomes.

Table 6: Composition of Proniosomal Gel Formulations.

Formulation Code	Surfactant Type	Ratio(mg)	Soya Lecithin (mg)	Cholesterol (mg)	Drug (DFS) (mg)	Ethanol(ml)
R1	S20:S40	250:250	500	100	20	0.5
R2	S20:S60	250:250	500	100	20	0.5
R3	S20:S80	250:250	500	100	20	0.5
R4	S40:S60	250:250	500	100	20	0.5
R5	S40:S80	250:250	500	100	20	0.5
R6	S60:S80	250:250	500	100	20	0.5

Characterisation of Proniosomes

Scanning Electron Microscope Analysis (SEM Analysis)

The surface morphology and structure were visualized by SEM (Evo-40, Zeiss, Germany) at AIRF, JNU, New Delhi.

Figure 27: SEM Analysis of Proniosomes loaded with DFS (R1)

Figure 28: SEM Analysis of Proniosomes loaded with drug DFS (R4)

Transmission Electron Microscope Analysis (TEM)

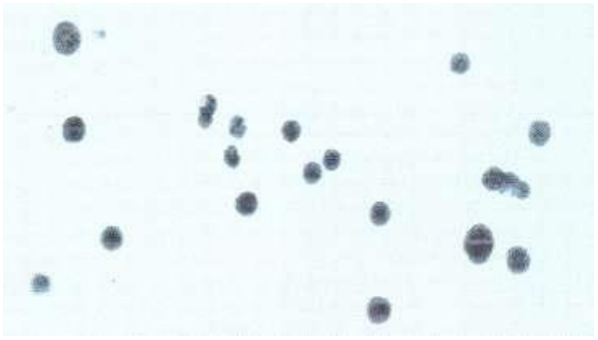


Figure 29: TEM Photograph of Proniosomes loaded with drug DFS (R1)

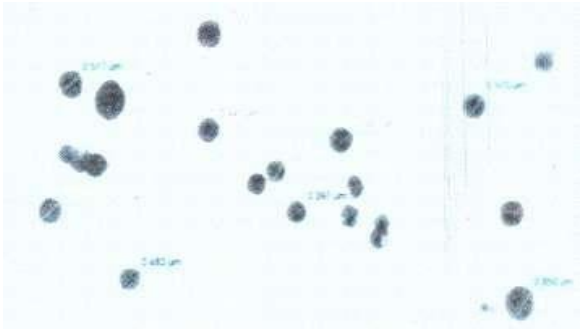


Figure 30: TEM Photograph of Proniosomes loaded with drug DFS (R4)

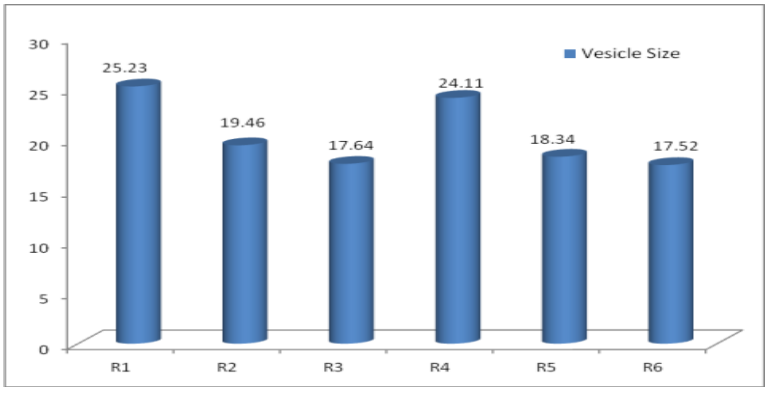
Particle Size Analysis

Particle size was determined by laser scanning light using Malvern LaserAnalyser Instrument at NIPER, Mohali.

Table 7: Vesicle Size Analysis of Different Formulations.

S. No.	Formulation Code	Vesicle Size ± SD (μm)
1.	R1	25.23 ± 1.03
2.	R2	19.46 ± 1.86
3.	R3	17.64 ± 1.58
4.	R4	24.11 ± 1.72
5.	R5	18.34 ± 1.64
6.	R6	17.52 ± 1.54

Figure 31: Vesicle Size Analysis of Different Formulations.



Percentage Entrapment Efficiency

Table 8: Entrapment Efficiency of various Proniosomal Formulations.

S. No.	Formulation Code	Drug Loading (%)
1.	R1	94.86 ± 1.83
2.	R2	93.34 ± 1.02
3.	R3	90.79 ± 0.96
4.	R4	95.92 ± 1.36
5.	R5	93.45 ± 1.08
6.	R6	91.62 ± 1.72

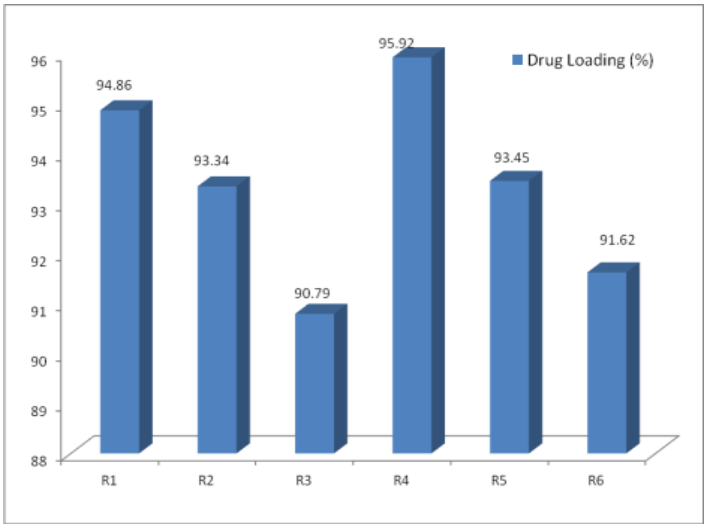


Figure 32 : Entrapment Efficiency of various Proniosomal Formulations.

***In-vitro* Drug Release Studies**

Table 9: Various parameters for *in-vitro* drug release study.

S. No.	Material	Quantity
1.	Membrane	Semi-permeable membrane
2.	Release Media	Phosphate buffer (pH 7.2)
3.	Temperature	37 ± 2^0C
4.	Volume of medium used	40 ml
5.	Absorbance wavelength of DFS	275.6
6.	Assembly used	Diffusion cell
7.	Time	30 hrs
8.	Area of diffusion Cell	1 cm^2

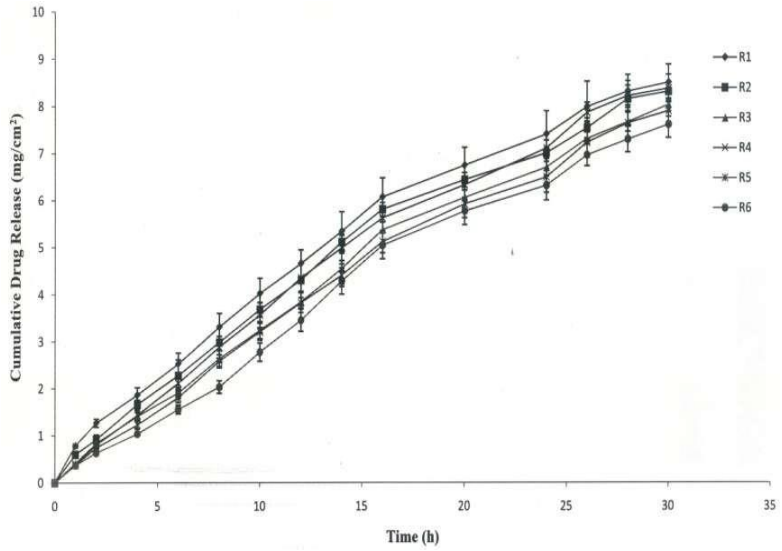


Figure 33 : Comparative Cumulative Drug Release of Different Formulations.

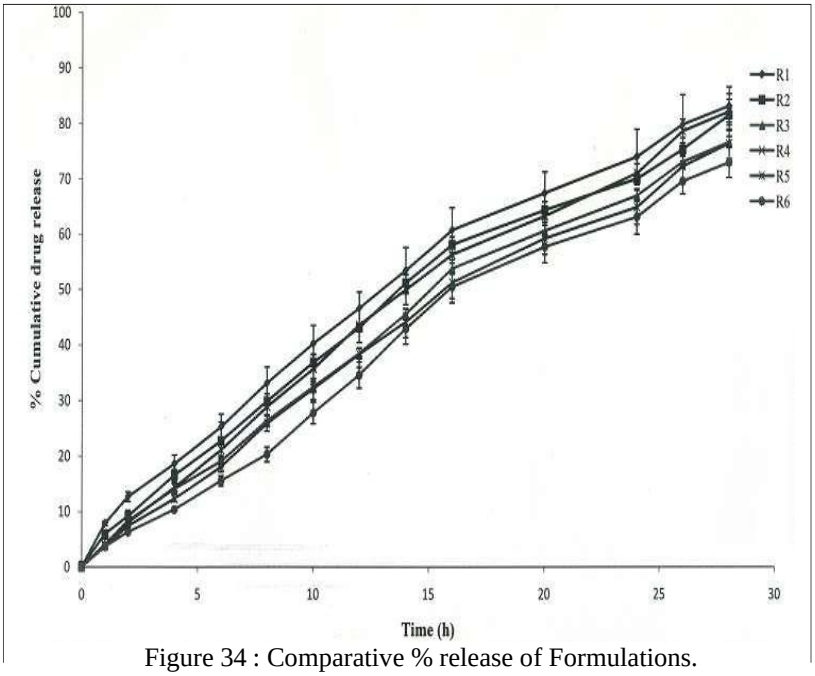


Figure 34 : Comparative % release of Formulations.

Release Kinetics Modeling

Table 10 : Mathematical models for drug release.

models	Equation
"0" order	Qt=Qo+Kot
Ist order	In Q=In Qo+K1t
Higuchi	Qt=KH√t
Korsmeyer-peppas	Qt/Q∞=Kktn

Table 11: Different release mechanism of "n" value.

S. No.	"n" value	Mechanism
1.	0.5	Fickian Diffusion (Higuchi Matrix)

2.	$0.5 < n < 1$	Anomalous Transport (First order)
3.	1	Case-II Transport (Zero order release)
4.	$n > 1$	Super case-II Transport

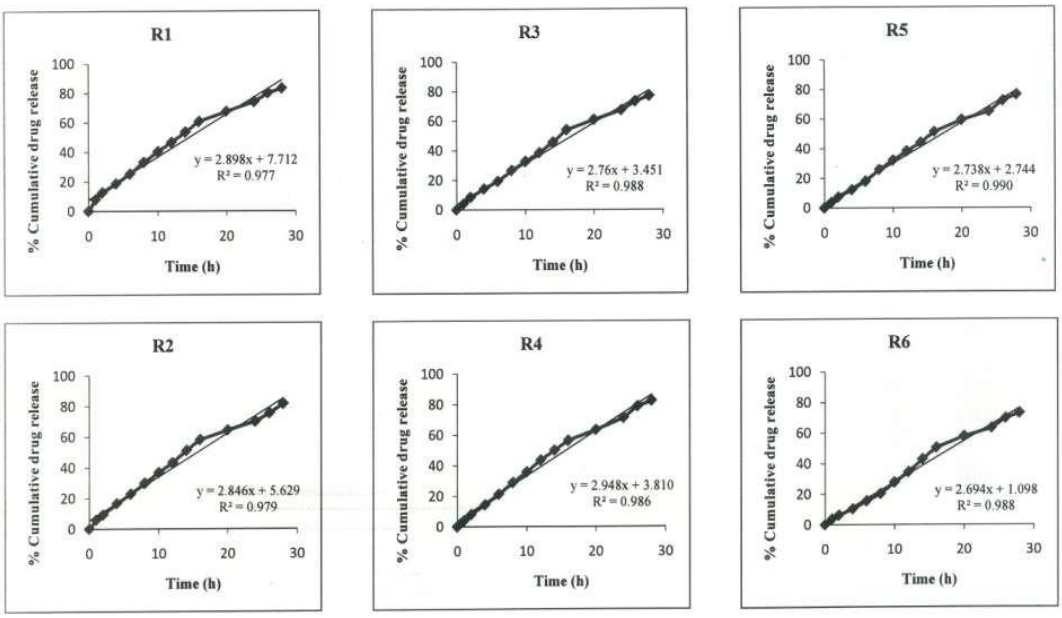


Figure 35 : Release Kinetics (Zero Order) for Different Formulations.

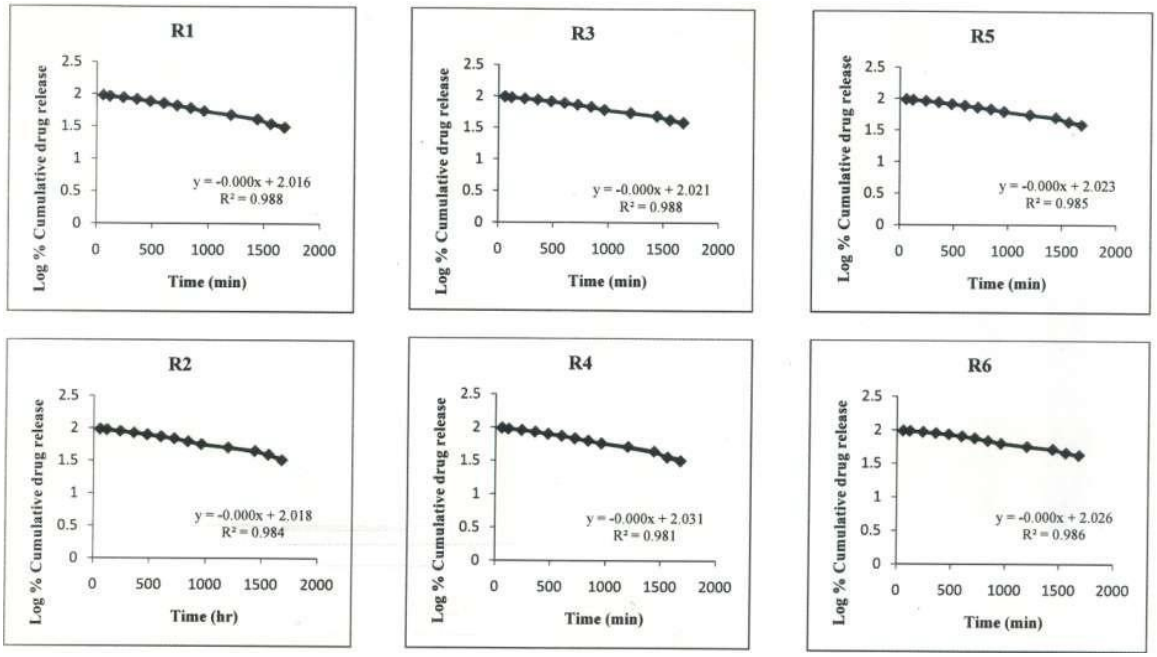


Figure 36 : Release Kinetics (First Order) for Formulations.

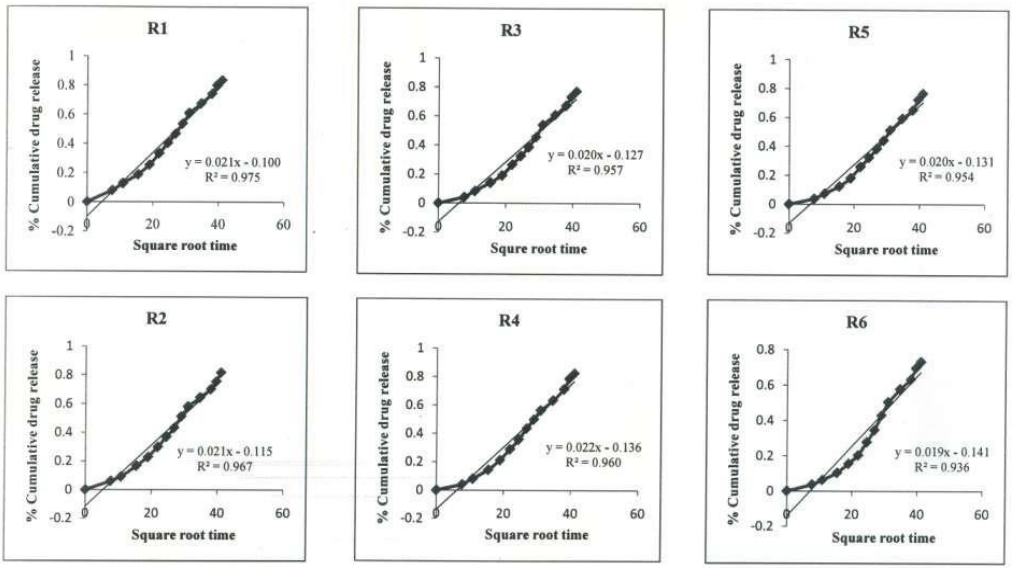


Figure 37: Release Kinetics (Higuchi) for Formulations.

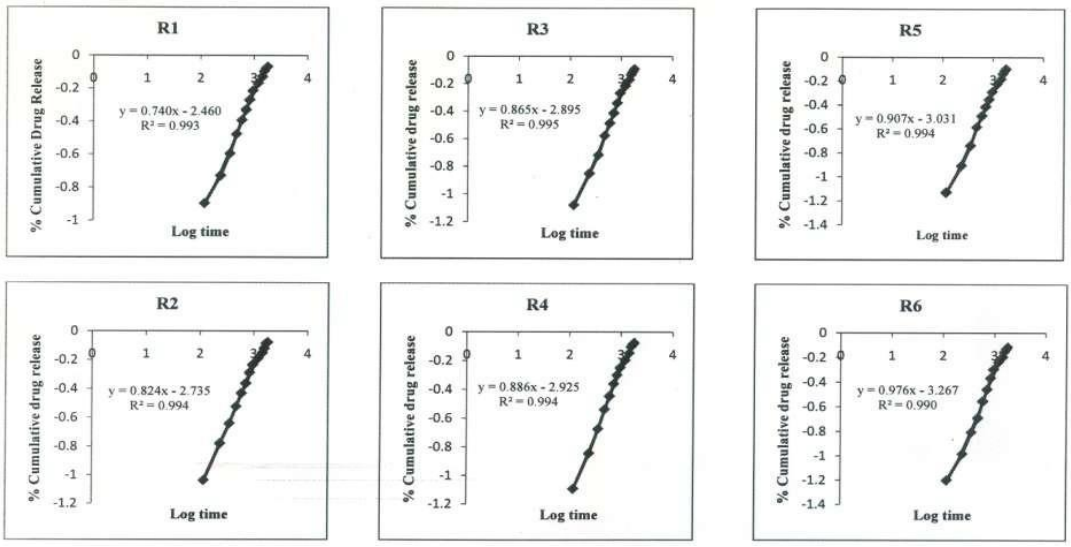


Figure 38: Korsmeyer-peppas drug release for Different Formulations

Table 12 : Release modeling data for different DFS formulations.

S. No.	FormulationCode	"0" Order	First Order	Higuchi	Korsmeyer-Peppas	
		R^2	R^2	R^2	R^2	n
1	R1	0.977	0.988	0.975	0.993	0.740
2	R2	0.979	0.984	0.966	0.994	0.824
3	R3	0.988	0.988	0.957	0.995	0.865
4	R4	0.986	0.981	0.960	0.994	0.886
5	R5	0.990	0.985	0.954	0.994	0.907
6	R6	0.988	0.986	0.936	0.990	0.976

Stability Studies

According to ICH guidelines, diclofenac sodium (DFS) containing nano- particles (proniosomes) were stored at 25±2^{0}C/60%±5% RH, 45±2^{0}C/75%±5% RH for 4 weeks. Freshly prepared nano-particles were stored at 4±2^{0}C used as control. Samples were kept for 28 days for stability analysis and after 28 days, drug loading of nano-particles were compared with those of the control formulations.

Table 13: Storage stability of Formulation R1 and R4 after 4 weeks.

Time (weeks)	Drug retained (%)					
	2-8^{0}C		25±2^{0}C		45±2^{0}C	
	R1	R4	R1	R4	R1	R4
0	100	100	100	100	100	100
1	98.6	98.9	95.8	96.9	91.3	92.6
2	98.2	98.3	93.6	94.3	85.7	87.3
3	97.8	97.2	90.2	93.6	79.2	81.6
4	97.6	97.1	87.2	89.4	71.3	76.5

Result and Discussion

In present research investigation, an attempt was made to use vesicular drug delivery system (VDDS) to design an optimum proniosomal transdermal formulation diclofenac sodium by proniosomal (dry-niosomes) formulation (characterized by safety and high therapeutic efficacy) to increase bioavailability (to reduce the daily dosing schedule of diclofenac sodium with subsequent improvement in patient compliance and drug safety). Pure diclofenac sodium (DFS) procured from a commercial source and sample drug (diclofenac sodium sample gifted by Alkem Pharmaceuticals Pvt. Ltd., Baddi, Himachal Pradesh) were analysed for various parameters in different studies.

FTIR analysis was performed for confirmation of their identity and purity. Sample drug showed absorption peaks which were similar to reference standard drug. Absorption peaks (Cm^{-1}) at 3209.66-3446.91 (N-H Stretching), 1400.37- 1456.30 (CH bend in plane), 1504.53-1572.04 (C=C), 840.99-1089.82 (C-C Stretching), 1180.47-1296.21 (C-NH) and 624.96-750.33 (Cl) were found and both sample drug and reference drug were pure diclofenac sodium (DFS).

The UV absorption maximum (λmax) for Diclofenac sodium (DFS) was found to be 275.6 nm. DFS mp was found to be 280-281^{0}C. DFS was found soluble in ethanol (980.21 µg/ml) and methanol (968.48 µg/ml); soluble in 1-octanol (935.16 µg/ml), Very slightly soluble in acetone (272.82 µg/ml) and poorly soluble in water (56.31 µg/ml). Besides, DFS was found to be white crystalline powder with alkaline taste and characteristic odour. The partition coefficient of DFS was analysed and was found to be 1.418 (reported 1.46 in n-octanol/distilled water) and 1.127 (reported 1.34 in n- octanol/phosphate buffer pH -7.2). In FTIR spectrum of cholesterol, absorption peaks were found at 3448.17 (OH), 2979.81 (C-H stretching), 1470.24 (C=C aromatic) and 1377.41 cm^{-1} (CH bending). In FTIR spectrum of Lecithin, absorption peaks were found at 2948.29 (CH stretching), 1747.31 (C=O) and 1428.23 cm^{-1} (CH bending). FTIR spectrum of Span 20 showed absorption peaks at 3606.81 (OH), 2835.12 (CH-CH stretching), 1755.91 (C=O) and 1467.31 cm^{-1} (CH-CH bending). Span 40 FTIR spectrum showed absorption peaks at 3635.50 (OH), 2987.31 (CH-CH stretching), 1766.69 (C=O) and 1445.62 cm^{-1} (CH-CH bending). Span 60 FTIR spectrum showed absorption peaks at 3635.50 (OH), 2835.12 (CH-CH stretching), 1722.29 (C=O) and 1467.39 cm^{-1} (CH-CH bending).

Span 80 FTIR spectrum showed absorption peaks at 3347.31 (OH), 2979.27 (CH-CH stretching), 1755.29 (C=O) and 1463.81 cm^{-1} (CH-CH bending). FTIR spectrum of Cholesterol + DFS (Drug) showed absorption peaks at 3390.53 (N-H Stretching or OH), 2891.03 (CH-CH Stretching), 1701.13 (C=O), 1671.37 (C=C Aromatic), 1578.15 (N-H bending), 1472.42 (C-H bending), 1310.90 (C-N), and 744.65 cm^{-1} (C-Cl). FTIR spectrum of Lecithin + Drug (DFS) showed absorption peaks at 3308.39 (NH Stretching), 2946.49 (CH-CH Stretching), 1748.31 (C=O), 1605.85 (C=C Aromatic), 1428.31 (CH bending), 1428.31 (NH bending), and 744.65 cm^{-1} (C-Cl). FTIR spectrum of Span 20 + Drug (DFS) showed absorption peaks at3556.47 (NH Stretching), 2972.52 (CH-CH Stretching), 1746.31 (C=O), 1580.27 (C=C), 1560.21 (NH bending), 1476.61 (CH bending), 1013.52 (C-N) and 722.46 cm^{-1} (C-Cl). FTIR spectrum of Span 40 + Drug (DFS) showed absorption peaks at3390.42 (N-H or OH), 2936.71 (CH-CH Stretching), 1744.49 (C=O), 1582.55 (C=C), 1560.98 (NH bending), 1445.62 (CH bending), 1093.98 (C-N) and 747.68 cm^{-1} (C-Cl). FTIR spectrum of Span 60 + DFS showed absorption peaks at 3340.56 (N-H or OH), 2979.81 (CH-CH Stretching), 1744.49 (C=O), 1575.36 (C=C), 1546.87 (NH bending), 1473.56 (CH bending), 1071.61 (C-N) and 747.62 cm^{-1} (C-Cl). FTIR spectrum of Span 80 + DFS showed absorption peaks at 3412.80 (N-H or OH), 2936.15 (CH-CH Stretching), 1748.06 (C=O), 1575.36 (C=C), 1511.67 (NH bending), 1463.81 (CH bending), 1072.31 (C-N) and 722.46cm^{-1} (C-Cl). In FTIR spectral analysis, it was found that there was no change in absorption maxima of functional groups in pure drug as well as drug polymer mixture hence drug and polymer were compatible to each other.

Standard plot of concentration vs absorbance was plotted which formed a straight line (obeyed Beer's Law). Coacervation phase separation method was used for formulation of vesicular proniosomes of DFS and various ratio of surfactant, lecithin, cholesterol, drug and ethanol were used for the formulation of proniosomes. Different proniosomal formulations were prepared (R1, R2, R3, R4, R5, and R6) by varying concentration of cholesterol, lecithin, spans (20, 40, 60 and 80). All formulations were evaluated for various parameters. Among all these proniosomal formulation, R6 formulation showed smallest vesicle size and R4 formulation confirmed highest entrapment efficiency. The visualization by SEM and TEM showed that formulated proniosomes has a lamellar vesicular structure and vesicular characteristics were shown by the carrier.

Size of formulated vesicles were reduced on agitation of the dispersion which range between 17.64 ± 2.01 µm to 25.23 ± 1.03 µm. Largest size was found to be in formulation R1 (25.23 ± 1.03 µm) whereas smallest in formulation R6 (17.64 ± 2.01 µm) due to presence of Span 60. Span 40 leads to larger size vesicles of the proniosomes due to higher HLB value (7.65±1) of theformulation with higher entrapment efficiency of drug.

The drug (DFS) entrapment efficiency of proniosomes was found in the range of 90-95% and it was due to lipophilic nature of DFS. Entrapment efficiency was maximum in R4 (95.92%; because Span 40 and Span 60 were used alongwith soya lecithin and cholesterol). Soya lecithin contains saturated fatty acids and Span 40 produced larger vesicle size with higher DFS (drug) entrapmentefficiency. *In-vitro* drug release studies were performed using semi-permeable dialysis membrane. The order of drug release in different formulations was R1> R2>R4>R3>R5>R6. According to this drug release order of different formulations, R1 formulation showed the maximum drug release i.e. 85.11% and R6 showed the minimum drug release i.e. 76.25% after 30 hrs. Drug release from R1 was highest due to composition with S20 and S40 resulted into better in-flux properties than S60 and S80 (R6). Hence, the formulation containing S20 and S40 was selected as best according to various parameters related to release profiles of diclofenac sodium (DFS). With the decrement of the alkyl chains of various Spans, a significant increase ($P<0.05$) was observed in the *in-vitro* release of diclofenac sodium from proniosomal formulations. It was found that 83.27%, 81.65%, 76.79%, 82.28%, 76.51% and 73.11% diclofenac sodium (DFS) was released from R1, R2, R3, R4, R5 and R6 formulations respectively. It has clearly indicated that DFS release from the prepared proniosomal formulations was increased with shortening of the alkyl chain length of Span (Span 20, 40, 60, 80) used. The R1 formulation was the best proniosomal formulation. For the

different proniosomal formulations drug permeation profiles were studied in triplicate and mean data values were used finally. Mathematical modeling is increasingly used to investigate the release profile of bioactive compounds in polymeric systems, since it can provide important information about the release mechanism. In analysis of the mechanism of release of DFS from proniosomes, zero order, first order, Higuchi and Korsmeyer-Peppas models were employed. Non-fickian transport.

The stability study was carried out for formulation R1 and R4 at different temperature like refrigeration temperature (2-8^{0}C), room temperature (25±3^{0}C)and at 45^{0}C for 4 weeks and after evaluation of each work data, the data showsthat at 2-8^{0}C formulation R1 has remained 97.6% and formulation R4 was remained 97.1%; at 25±3^{0}C, R1 has remained 87.2% and formulation R4 was remained 89.4%. At 45^{0}C, 71.3% and 76.5% were remained for formulation R1 and R4 respectively. So, there was nominal degradation at room temperature (stable) whereas degradation took place at high temperature (45^{0}C) after 4 weeks. After 4 weeks, best stability was found at refrigeration temperature (2-8^{0}C). So proniosomalformulation should be stored at 2-8^{0}C (refrigerator). Formulation R1 showed better storage stability than formulation R4 (either at room temperature or at refrigeration temperature).

Conclusions

In FTIR analysis, DFS sample showed absorption peaks similar to reference DFS drug and confirmed their identity and purity. Further, in UV analysis, λmax of DFS was found to be 275.6 nm and melting point was found to be 280-281^{0}C. Partition coefficient of DFS was found to be 1.418 in n-octanol/distilled water and 1.127 in n-octanol/phosphate buffer pH -7.2. In compatibility studies, FTIR spectral analysis confirmed no change in absorption peaks of functional groups in pure drug as well as drug, surfactant and other polymer mixture. Hence drug, surfactants and polymer were pure and compatible to each other. Subsequently, calibration curve analysis at 275.6 nm standard plot of DFS dilutions vs absorbance formed a straight line indicated drug samples obeyed Beer's Law. Further, Coacervation phase separation method was used for formulation of vesicular proniosomes of diclofenac sodium drug (DFS) and various ratio of surfactants, lecithin, cholesterol, drug and ethanol were used for the formulation of proniosomal formulations R1, R2, R3, R4, R5, and R6. All formulations were evaluated for various parameters like vesicle size, SEM and TEM analysis, entrapment efficiency. Among all these proniosomal formulation, R6 formulation showed smallest vesicle size (17.64 ± 2.01 μm; reduced on agitation of the dispersion; presence of Span 60; increasing hydrophobicity of the surfactant lead to a smaller vesicle size) and formulation R1 (25.23 ± 1.03 μm) showed largest vesicle size (Span 40 leads to larger vesicle size due to high HLB value; Soya lecithin contains saturated fatty acids). Drug entrapment efficiency of lipophilic DFS proniosomes was found in the range of 90-95% and maximum in formulation R4 (95.92%; because Span 40 and Span 60 were used alongwith soya lecithin and cholesterol).

After 30 hrs. in drug release analysis, order of drug release in different formulations was R1> R2>R4>R3>R5>R6. Due to composition with S20 and S40 and better in-flux properties, R1formulation indicated the maximum drug release(85.11%; best proniosomal formulation) and R6 containing S60 and S80 confirmed minimum drug release (76.25%). It was found that 83.27%, 81.65%, 76.79%, 82.28%,76.51% and 73.11% DFS was released from formulations R1, R2, R3, R4, R5 and R6 respectively. The data of various drug release kinetics models revealed that formulations R1, R2, R3, R4, R5 and R6 followed Korsmeyer-Peppas model with r^2 value of 0.993, 0.994, 0.995, 0.994, 0.994, 0.0990 and n value of 0.740, 0.824, 0.865, 0.886, 0.907, 0.976 respectively. Release can be concluded as by super case 2 transport mechanism (non-fickian transport). Finally, after 4 weeks in stability studies, formulation R1 showed better storage stability than formulation R4 (nominal degradation either at room temperature or at refrigeration temperature). Degradation of Formulation R1 and R4 found at high temperature (45^{0}C) after 4 weeks. So proniosomal formulation should be stored at 2- 8^{0}C.

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