Design and development of diacerein transfersomal gel by employing natural surfactant using factorial design

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Abstract:

Background: Diacerein is an anthraquinone derivative belonging to class 2 BCS drug. The drug is well known for its application in the treatment and management of osteoarthritis. When taken orally the drug has several limitations such as diarrhoea, gastrointestinal disturbance, and discoloration of skin and eyes. This needs the development of alternative dosage forms to improve bioavailability. The present research work aimed to prepare ultradeformable vesicles that are transferosomes of diacerein using Acacia concinna as a natural surfactant which effectively deliver the drug and provides symptomatic relief in the management of osteoarthritis. A full factorial design was employed to optimize the formulation. Transfersomes were then converted into gel using carbopol 934 by simple homogenization technique.

Results: The diacerein-carried transferosomes were optimized for vesicle size, entrapment efficiency and percent drug diffusion resultingina spherical size of 250±1.8nm, entrapment efficiency of 79.33±0.28%, and %drug diffusion of 94.35% for 24 hours. The zeta potential of these vesicles was - 36.8±0.98, this suggests good electrostatic repulsion among the vesicle molecules.

Conclusion: The research work helped to know the effect of natural surfactant on the ultra-deformable vesicles of diacerein using Acacia concinna which resulted in improved permeation of drug into deeper layers of skin and helps to manage therapy.

Keywords: diacerein, transferosomes, acacia concinna, full factorial design, natural surfactant

Background: Osteoarthritis (OA) is one of common arthritis found in older adults affecting their day-to-day activities. The disease is associated with intermittent but intense pain in any joints of the human body but is often seen in knees, hips, and lower back causing disability among individuals. Osteoarthritis mainly occurs due to degeneration of cartilage associated with it but unfortunately, self-healing is poor which makes treating osteoarthritis more challenging [1, 2, 3].

Skin is the largest organ of our body hence is one of the leading pathways for transporting many drugs via the transdermal (TD) route. The major drawback of the transdermal route is the stratum corneum (SC) which acts as a major barrier and limits the entry of many drug molecules. The TD is one of the non-invasive pathways for transporting drugs into the systemic circulation and hence offers the advantage over the drawbacks associated with oral administration of drugs Hepatic bypass gastric irritation, prolonging drug release, constant plasma concentrations, and increasing patient compliance [4]. Many transdermal drug delivery systems have been developed so far such as transdermal patches, gels, micro needling, and electrophoresis. Transfersomes (FTs) are novel vesicular systems that can improve efficiency by delivering the drug to the target site with its local administration. Hence these ultradeformable vesicles can deliver the entrapped drug by squeezing and penetrating themselves into much deeper layers of intact skin with better plasma concentrations. These novel vesicles can release most of the entrapped drug across the skin thus reducing dose-related toxicity associated with conventional dosage forms [5].

Diacerein, an anthraquinone derivative, is a stimulator of proteoglycon production. It mainly helps in the formation of soft connective tissue around the joints thus helping in reducing pain and swelling of affected areas. The drug shows its anti-inflammatory effects by inhibiting interleukin 1 beta, a protein that is involved in the information and destruction of cartilage. The rhein moiety of diacerein has severe diarrhoea and intestinal disturbance which limits its oral usage in older adults [6, 7]. Though diacerein has good oral bioavailability, it is associated with severe side effects which suggest the TD route as an alternate and preferred route with sustained effects. The study of diacerein suggests that the drug is practically insoluble in water and many organic solvents involved in pharmaceutical preparations, requiring the need for surfactant i.e.

edge Activator in preparation in the correct concentration. The edge activator not only helps in dissolving the drug in the solvent used in the preparation but also helps in crossing the barrier properties of the skin [8, 9].

Nowadays extracts from natural sources are in great use due to their efficiency and safety. Acacia concinna is a medicinal plant widely grown in southern Asia. The extract of Acacia concinna has surface active properties along with antimicrobial properties thus making it a suitable edge activator in the preparation of FTs [10].

The present research is aimed to prepare ultra-deformable transfersomeal vesicles of diacerein using Acacia concinna, a natural surfactant. 2^3 Factorial designs were considered for optimization and the resultant transferosomes were converted into a suitable gel for its application on skin and finally evaluated for its efficiency. This research would be an attempt to prepare an alternative Pharmaceutical preparation for the delivery diacerein with improved permeability and bioavailability.

Methods:

Materials

Diacerein was gifted by Rakshit Pharmaceuticals Limited, Hyderabad. Phosphatidylcholine (Phospholipon 90H) was a gift sample from Nattermann, Germany. Acacia concinna (surfactant) prepared in laboratory Span 20 gifted by Lobachemie, Mumbai. Tween 80 sigma aldrich Germany. Cholesterol was obtained from Vav life sciences Mumbai as a gift sample.

Extraction of Acacia concinna

The pods of CA were collected and freed of dirt and microorganisms by washing them with distilled water. The cleaned pods were dried in a hot air oven at 60^0C for 3 days. The pods were then separated from the seeds and ground to powder. To prepare the solution of CA the powder was dissolved in deionized water, stirred for 6h, and sonicated for 10 min; the resulting solution was filtered with Whatman filter paper. For the present investigation 10 mg of powder dissolved in 100 ml of deionized water, resulting in a 0.195M stock solution. Further dilutions were done according to the requirement [11].

Determination of surface tension and critical micelle concentration

The surface tension (SFT) of the resulting solution Acacia concinna was determined by "Drop count method" using a stalagmometer and the following equation was used to calculate surface tension.

$$\frac{\gamma 1}{\gamma 2} = \frac{\rho 1 n 2}{\rho 2 n 1}$$

γ1= surface tension of water, γ2= surface tension of AC, n1= number of drops of water, n2= number of drops of AC, ρ1= density of water, ρ2= density of AC

For the determination of critical micelle (CMC) concentration, a graph is plotted between concentrations of Ac versus corresponding surface tension. The sudden change in the trend of the linear line represents the CMC [12].

Preformulation studies

Drug-excipients compatibility study

Fourier transform infrared spectroscopy (FTIR) (Perkin Elmer) was used to note any compatibility issue among the diacerein and excipients. 400-4000 cm^{-1} was chosen spectral region. The spectral scan was compared with the standard FTIR spectrum[13].

Differential Scanning calorimeter (DSC) was also used to further investigate the possible drug and excipients compatibility. The DSC curve for pure diacerein and Diacerein with CA was taken into consideration to analyze the compatibility [14].

Preparation of Diacerein FTs

The thin film hydration method was employed to prepare these diacerein-loaded transfersomes (FTs) using Rota Evaporator (RE) equipment. The formulation composition of diacerein and other excipients is given in table 1. The precise quantities of Diacerein, cholesterol, PL90H, and surfactant were added to chloroform: methanol (1:2) in Round Bottomed Flask (RBF). The RBF

was attached to RE for solvent evaporation and the formation of a thin film. The formed thin film hydrated at 70 0C by adding a 20 ml phosphate buffer (pH7.2). The hydrated vesicles underwent further size reduction by sonication for 5 min [15, 16, and 17].

Formulation Code	Diacerein	AC	Span 20	Tween 80	PL90H	Cholesterol
DTF 1	50	-	-	-	150	30
DTF 2	50	75	-	-	150	30
DTF 3	50	-	75	-	150	30
DTF 4	50	75	75	-	150	30
DTF 5	50	-	-	75	150	30
DTF 6	50	75		75	150	30
DTF 7	50	-	75	75	150	30
DTF 8	50	75	75	75	150	30

Table: 1 composition of diacerein FTs

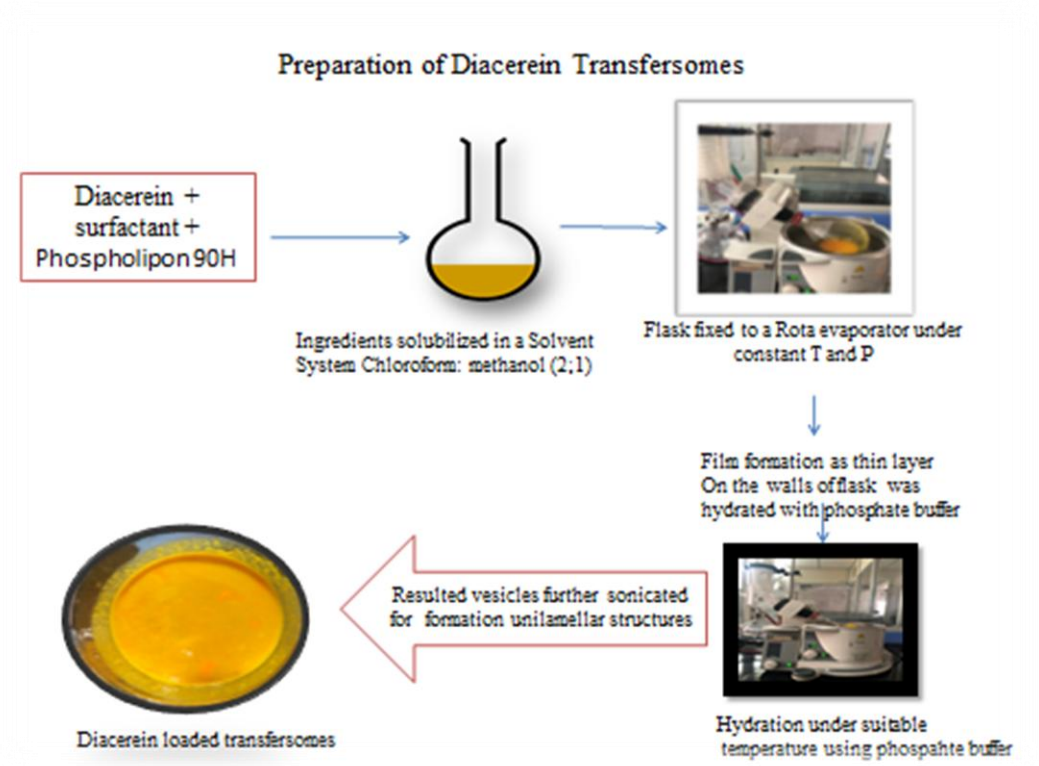

Figure 1 Preparation of Diacerein FTs

Experimental Design (ED)

A 2^3 (three factors at two levels) factorial designs were used to optimize the formulation. The concentrations of three surfactants - Acacia concinna (A), span 20 (B), and Tween 80 (C) were taken as independent variables. The vesicle size, percent entrapment efficiency (%EE), and percent drug release were selected as dependent variables. The concentrations of surfactant at two levels were considered for batch optimization [17]. A total of 8 factorial batches were prepared as provided by Design Expert software and formulation codes were given as shown in Table 2

Factors		**Levels (mg)**	
Surfactant	**Code**	**Low**	**High**
Acacia Concinna	**A**	**0**	**75**
Span 20	**B**	**0**	**75**
Tween 80	**C**	**0**	**75**

Table: 2 the level of independent factors as per the Design expert

Characterization of Diacerein-loaded FTs

Vesicle size, shape, and Zeta potential Diacerein loaded FTs

The Transmission Electron Microscope (TEM) (JEOL JEM-F200)is used to get information ofthe vesicle size and shape of diacerein FTs by producing two-dimensional pictures. The diluted sample of diacerein FTs was placed directly on the grid microscopic slide followed by immediate drying using filter paper at room temperature. The samples were then examined at appropriate magnification. The surface charge of diacerein TFs helps to understand the stability of the preparation. The Malvern zetasizeris used to measure zeta potential. A high negative zeta potential is required to prevent the aggregation of vesicles. The sample of Diacerein FTs was prepared by suitable dilution and filtration using 10 ml distilled water, the sample the subjected to zetasizer [17, 18, 19, 20].

Percent entrapment efficiency Diacerein loaded FTs

The percent entrapment efficiency (% EE) of diacerein TFs was determined using a centrifuge (Remi C 852). Weight equivalent to one unit dose was added to 20 ml phosphate buffer (pH 7.4), and centrifuged at 10000 rpm for 30 min. The clear supernatant layer containing dissolved drug

was collected and the free drug concentration was estimated spectroscopically using UV spectrophotometer (JASCO INC U.S) at 412 nm. The % EE for each formulation was determined in triplicate and calculated using following equation [17, 18, 19, and 20].

$$\%\ \text{Entrapment efficiency}\ \frac{\text{Total drug} - \text{Unentrapped drug}}{\text{Total drug}} \times 100$$

Percent drug release (%DR) Diacerein loaded FTs

In vitro diffusion method (elctrolab automatic diffusion cell) apparatus used to determine. 25 ml of phosphate buffer (pH7.4) was added to the receptor compartment of the diffusion cell. The acceptor chamber separated from the donor compartment with 0.45µ PES membrane and the temperature was maintained at 37±0.5^{0}C. The study was conducted for 12h by continuously stirring at 100rpm. The sample was withdrawn at determined time intervals (0, 2, 4, 6, 8, 10, and 12 h) while maintaining sink condition [17, 18, 19, and 20].

Figure: 2 electro lab automatic diffusion cells

Formulation of Diacerein transfersomal gel

The transfersomal gels (FTs-G) of the optimized batch containing diacerein were prepared using carbopol 934 as a gelling agent. Different concentrations of carbopol 934 were prepared using demineralized water. A homogenization technique (15000 rpm for 15 min) was employed to get

homogeneous dispersion of diacerein FTs gel. The concentration of gelling agent along with the formulation code given in table 3 [17] .

Formulation	Carbopol 934 Concentration (%) (w/v)
DTFG1	1.00
DTFG2	0.95
DTFG3	0.80

Table 3: Formulation of Diacerein transfersomal gel.

Evaluation of diacerein FTs-G

The viscosity and spreadability of diacerein FTs-G

A calibrated Brookfield (LV) cone and plate viscometer is used to measure the viscosity of diacerein FTs gel. The cone rotated at a constant angular speed of 50 rpm at 25^0C. Each measurement was performed in triplicate.

This work investigates the spreading of Diacerein FTs gel when applied to the skin. 0.5 g of diacerein FTs gel was placed between two glass slides (previously engraved circle 1cm). 500 g of weight was placed on the upper slide for 300 s. The diameter of the gel spread on the glass slide was measured [17, 20]

Skin irritation of diacerein FTs-G

The skin irritation test was carried out on male Wister rats. The diacerein FTs were applied on shaved skin of the rat and examined for any itching and irritation [17, 20].

Ex-vivo permeation of diacerein FTs-G

The permeation of diacerein FTs-G through Wistar rat skin was measured ex vivo using a Franz diffusion cell. The male albino Wistar rats weighing 180–200 g were used to collect skin. (The study was conducted with the approval of 1373/PO/Re/10/CPCSEA). The animals were warehoused with free access to water and food. The rats were sacrificed by inhaling excess ether.The epidermal layer of the rat's abdomen was collected, cleaned gently with water, and wrapped in aluminium foil stored at -20°C (can be within 14 days). The rat skin was brought to room temperature before use and placed between the compartments. The receptor compartment was filled with phosphate buffer (pH 7.4). The donor compartment is filled with formulation

fixed over the receptor compartment with the help of a clamp. The study was conducted for 12 h at 37 ^{0}C with constant stirring on a magnetic stirrer. Sampling was done from the receptor compartment at a determined time interval and diacerein content was measured spectroscopically. From the data ex vivo study, the flux of diacerein, cumulative permeation across the skin, and permeability coefficient were calculated [17, 20].

Stability study of diacerein FTs-G

The optimized FTs-G formulation was sealed in the glass vials and stored at 4°C and room temperature, observed for a period of 6 months for any sign of aggregation or sedimentation or leakage of drug from the vesicles. The sample was tested after six months for viscosity, the particle size and permeation across skin. [17, 20].

Results

The percent yield of acacia concinna

The aqueous extract of acacia concinna pods was found to be 9.2 g per 100 g.

Surface tension and critical micelle concentration of concinna

The determination of CMC is a gold standard for any surfactant, especially when the surfactant is used in drug delivery systems or any industrial applications. At CMC surfactant it forms aggregate in which the drugs get occluded. The SFT property of surfactant can be used to find CMC. The surface tension does not reduce once CMC is reached. SFT linearly depends on the concentration of the surfactant. Change in this linearity indicates a CMC point. The prepared AC was found CMC at 4.4×10^4M.

Preformulation studies

FTIR spectrum of diacerein and diacerein with acaciaconcinna was taken in account to investigate any possible drug-excipient interaction. Figure 3 illustrate spectrum of pure diacerein. The spectrum exhibits all absorption bands related to its characteristic function groups such as, 3327.56 cm^{-1}(broad) O–H stretching for (-COOH),1766.44 $cm^{-1}$for C=O (ester group),1678 $cm^{-1}$for carbonyl group,743 $cm^{-1}$for m-substituted benzene ring and at 703$cm^{-1}$for benzene ring. Figure 4 illustrate the FTIR spectrum diacerein with acaia conacinna. The spectrum retain all important peaks releated to function groups of diacerein such as 3327 cm^{-1}O-H group, 1622 cm^{-1} C=C group,2919 cm^{-1}C-H group at 1732 cm^{-1} C=O group, 1013 cm^{-1}C-O group. Indicating no significant interaction between diacerein and acacia concinna.

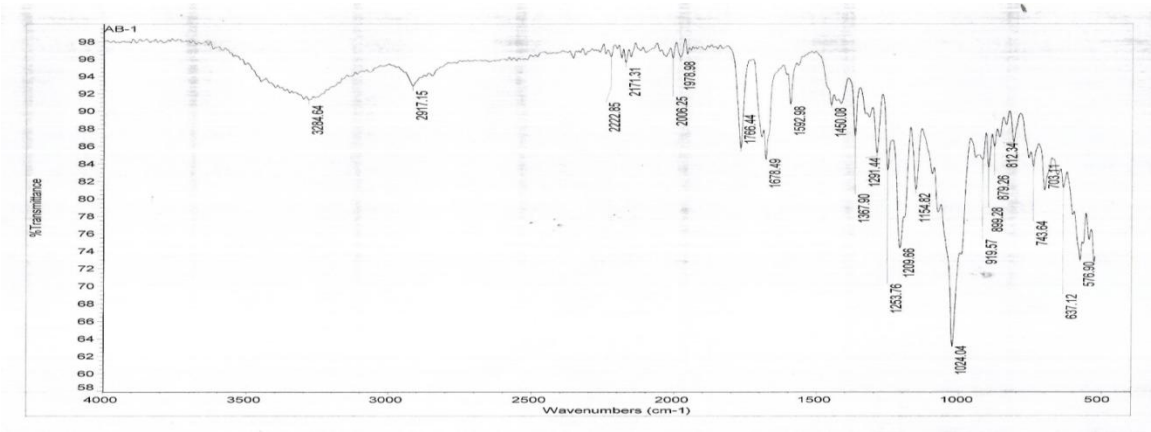

Figure 3 FTIR spectrum of pure diacerein

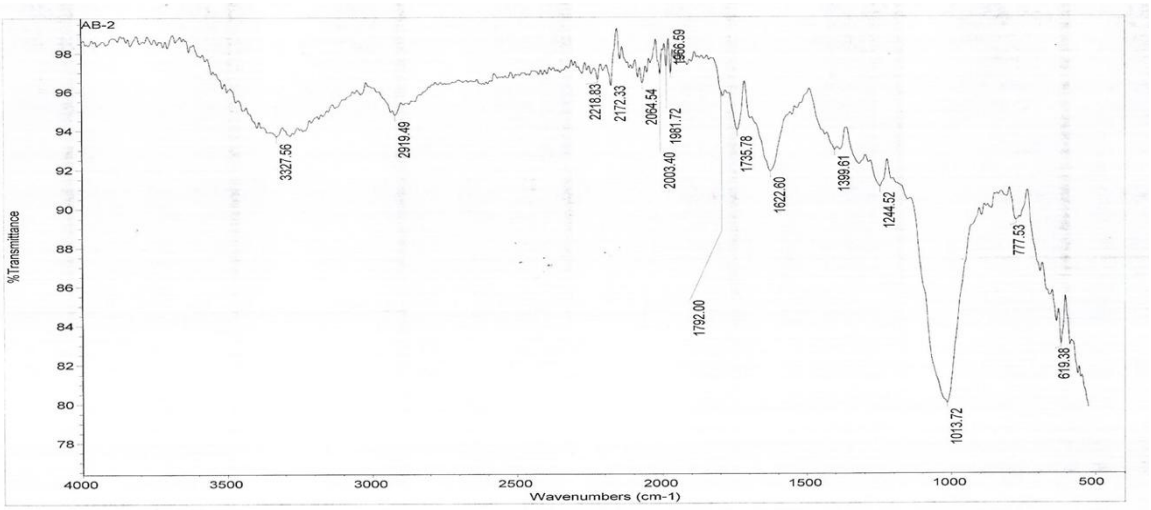

Figure 4 FTIR spectrum of diacerein with concinna

DSC was performed to investigate the possible drug excipient interaction. Figures 5 and 6 represent the DSC thermograms of pure diacerein and diacerein with acacia concinna. The thermograms show sharp endothermic peaks at 218.9 and 218.5 for diacerein corresponding to its melting point indicating there is no interaction among the drug and excipient.

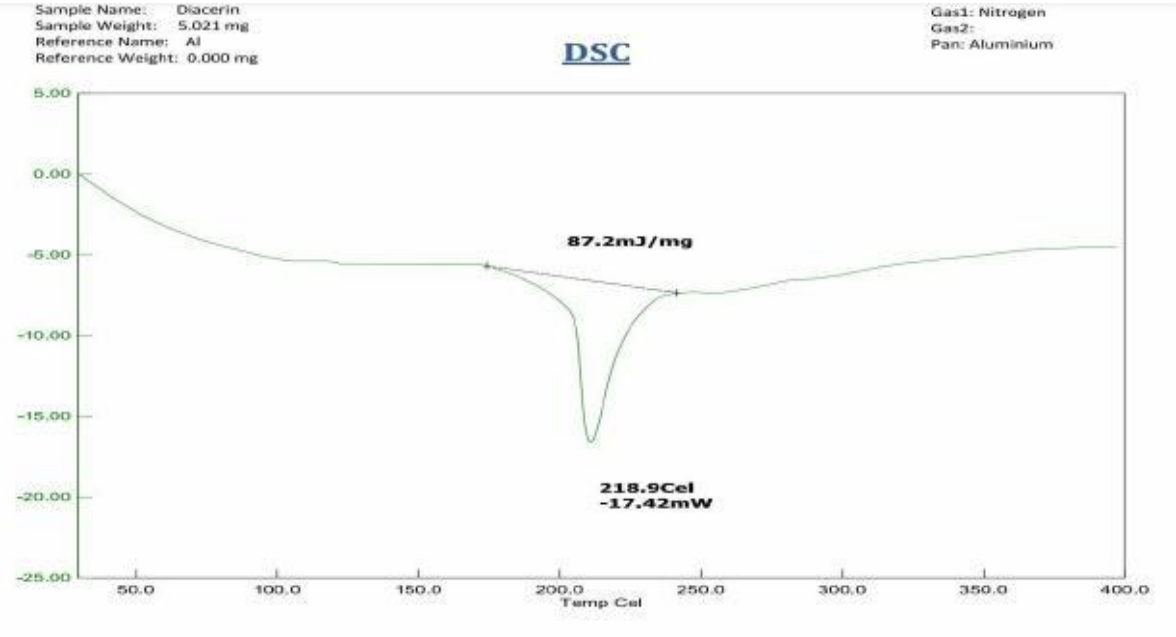

Figure 5 DSC scan of pure diacerein

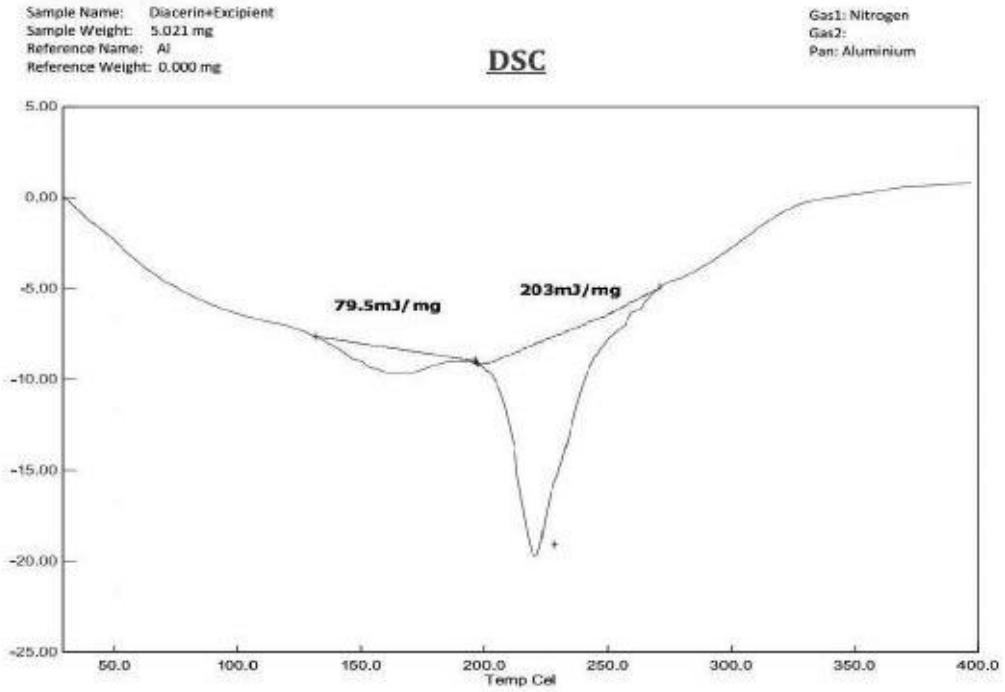

Figure 6 DSC scan of diacerein with acacia concinna

Experimental Design (ED)

Statistical optimization using 2^3 full factorial designs is an efficient way to evaluate the effect of independent variable. The concentration acacia concinna (A), tween 80 (B), and span 20 were selected at two levels considered as independent variables. The main and combined effects of

surfactant on formulation of transferosomes were studied at two levels (high and low). The model with higher R^2 value was taken into the consideration for suitability of factorial design. Vesicle size, % EE, and % DR were taken as the responses formula optimization. The obtained data were statistically explored and visualized. The vesicle size of TFs was found to be in the range of 250±1.8-480±1.5nm, % EE was found to be in the range of 480±1.5-79.33±0.28%w/w, and % DR was found to be in the range of 69.21-94.35% w/w. The table 4 presents full factorial design.

Formulation code	Independent variable			Dependent variable (responses)		
	AC (A)	Span 20 (B)	Tween 80 (C)	VS (nm)	%EE	.% DR
F1	-	-	-	480±1.5	58.04±0.52	73.05
F2	75	-	-	250±1.8	79.33±0.28	94.35
F3	-	75	-	400±1.7	35.02±0.23	72.19
F4	75	75	-	360±1.5	43.49±1.15	80.25
F5	-	-	75	430±1.3	54.89±1.21	70.09
F6	75		75	350±1.4	39.60±0.23	82.34
F7	-	75	75	370±1.7	40.89±0.90	69.21
F8	75	75	75	300±1.5	54.20±0.59	82.76

(Mean ± SE, n = 3)

Table: 4 Full factorial design with obtained results

Particle size, zeta potential, and PDI

The model equation for vesicle size was given by

Vesicle size = + 368.13 -51.88 A -10.63 B -5.63 C +24.38 AB +14.38 AC -16.87 BC-21.88 ABC

In the above equation the positive value favours the optimization and negative value has counter effect between factor and responses. The results indicates the vesicle size was profoundly affected by A and AB as independent factors. The formulation (F2) prepared using acacia concinna shown mean particle size 250±1.8 illustrated in figure 7 and with zeta potential -30.97±0.73 indicated in figure 8. Smaller particle size has better penetration cross SC with improved therapeutic effects compared with the formulations (F3 and F5) prepared using Span 20 and Tween 80. Such negative zeta potential value also helps the particles establish electrostatic repulsion and prevent aggregation. However the combined surfactant was seen to increase the particle size indicating significance of concentration of acacia concinna on transfersomes formation.

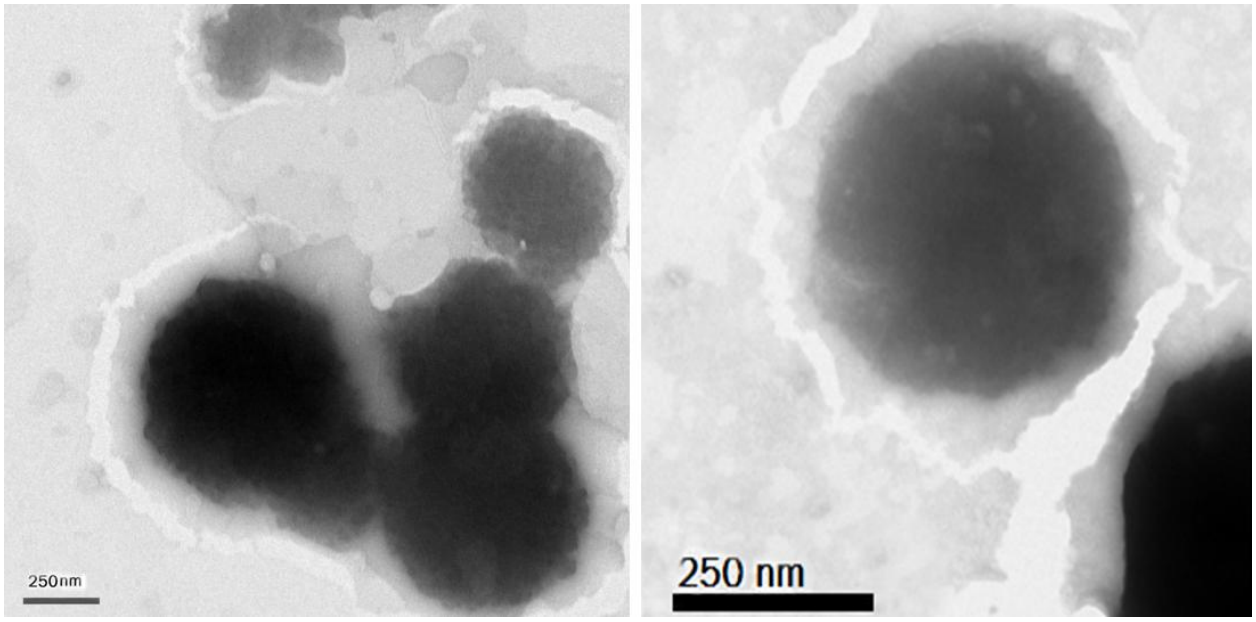

Figure: 7 TEM images of Diacerein transfersomes

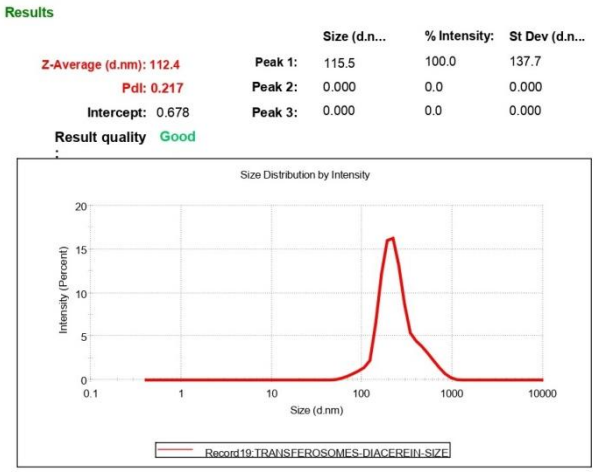

Figure: 8 Zeta potential of diacerein FTs

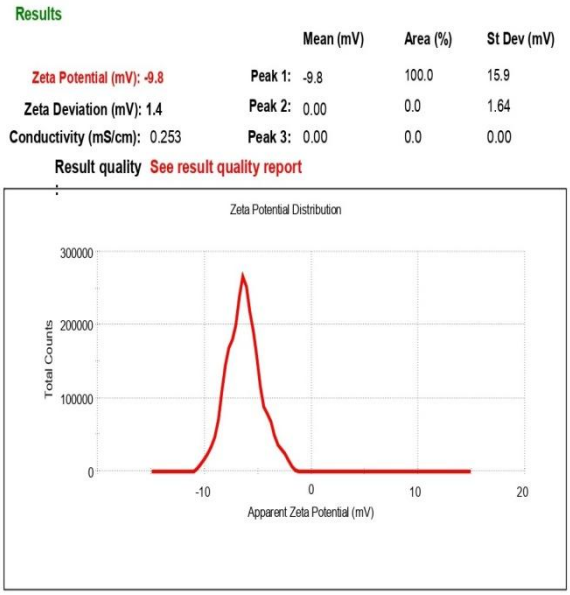

Figure: 9 zeta potential of diacerein FTs

The response surface plots helps to visualize the effect of surfactant on particle size. From the plot it can be seen increasing concentration of Acacia helps reduce particle size and combined effects of acacia and span 20 or Tween 80 increases particle size which is not desirable. The PDI value of F2 was found to be 0.17±0.01 indicating good homogeneity of vesicle in the preparation.

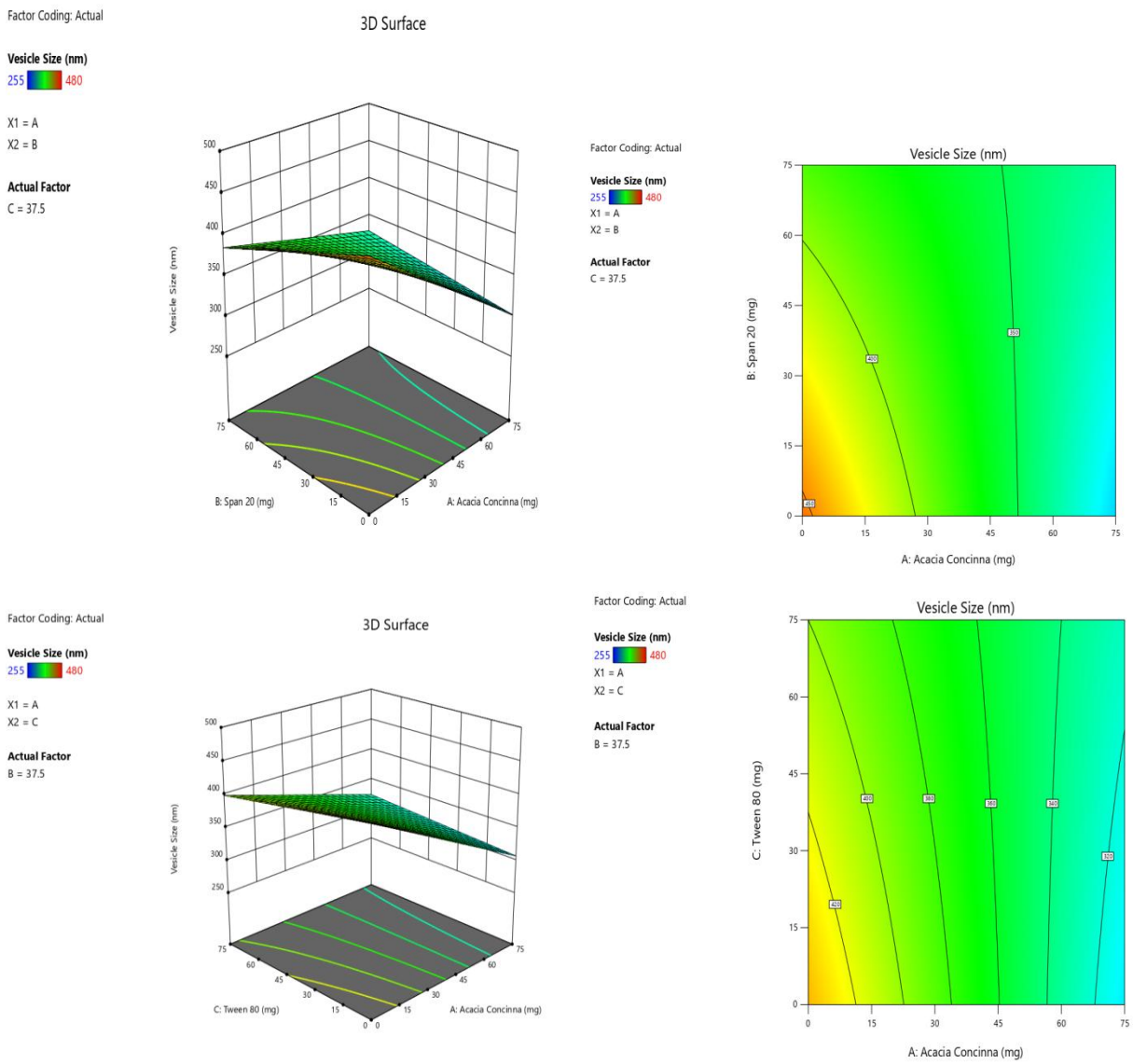

Figure: 10 Surface Plots indicating the effect of Acacia concinna on particle size.

Percent entrapment efficiency

The model equation for percent entrapment efficiency was given by

$$\text{Entrapment Efficiency} = +50.68+3.47A-7.28B-3.29C+1.97AB-3.97AC+7.43BC+5.18ABC$$

The equation indicates the EE was affected by concentration of all three surfactants as independent factors. The formulation (F2) prepared using acacia concinna alone was found to have higher entrapment efficiency of 79.33±0.28% w/w. Data was further explored using

response surface plots. The plots illustrate concentration of surfactant has linear effect on EE. However formulations prepared using combination of surfactant decreases the EE

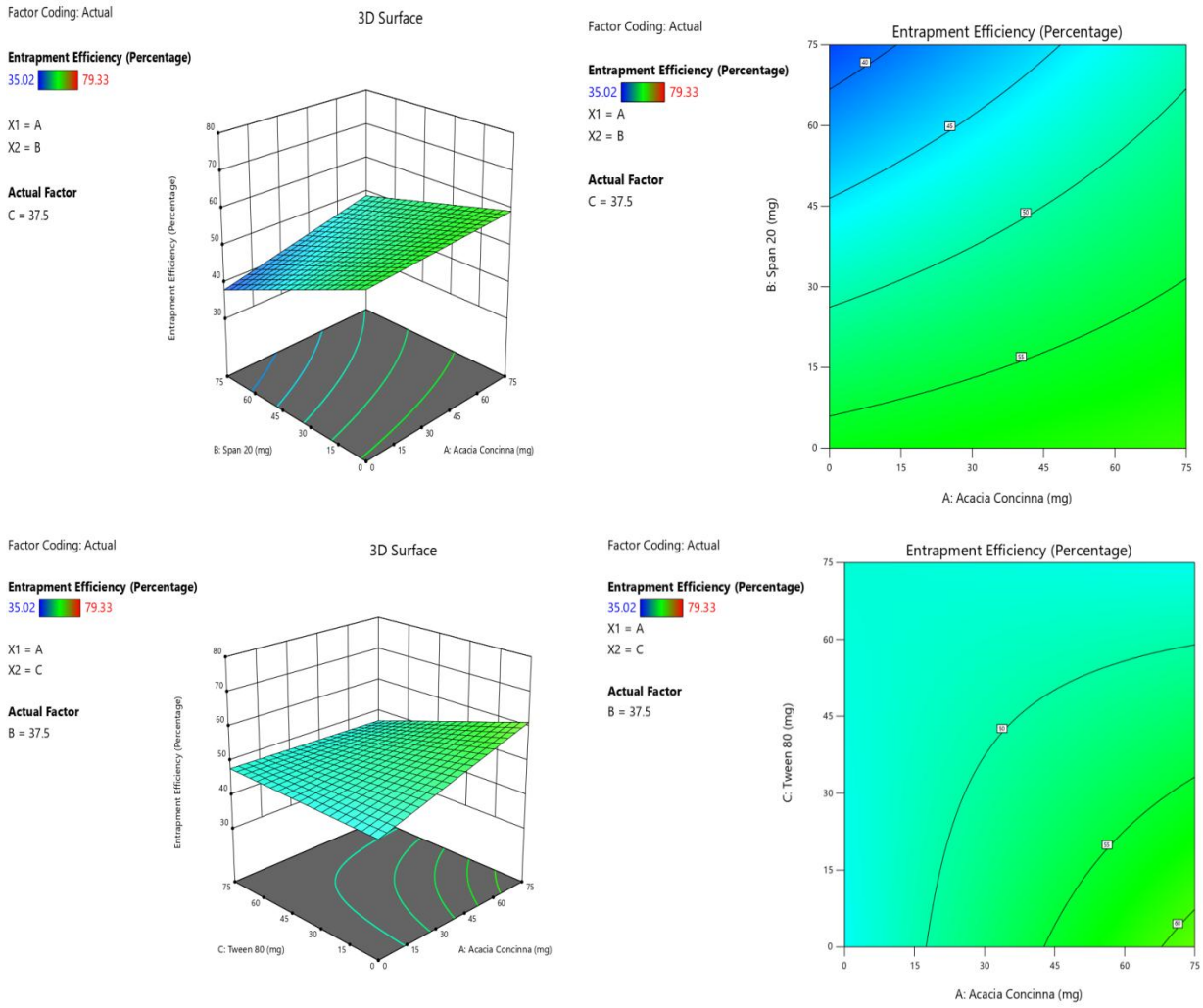

Figure: 11 Surface Plots indicating the effect of Acacia concinna on entrapment efficiency.

Percent drug release

The model equation for percent drug release was given by

$$\% \text{ drug release in 12 hours} = +78.03 + 6.89\,A - 1.93\,B - 1.93\,C - 1.49\,AB - 0.4450\,AC + 1.81\,BC + 1.82\,ABC$$

The equation indicates the %DR was increased by the concentration of acacia concinna. The cumulative %DR for formulations (F1-F8) was found to be 69.21 to 94.35 % w/w. The release of

diacerein FTs containing acacia concinna (F2) was found to be higher compared to the formulations (F3 and F5) containing Span 20 and Tween 80.

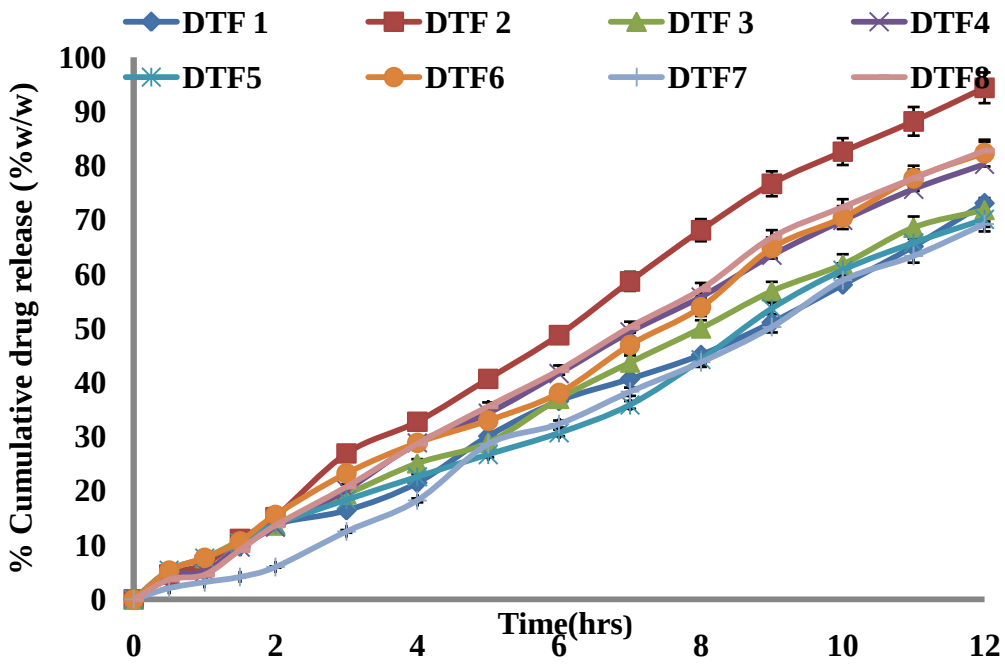

Figure: 12 % cumulative drug release from the formulations.

Formulation code	Zero order		First order		Higuchi	KM Peppas
	K_0 (h^{-1})	R^2	K (h^{-1})	R^2	R^2	R^2
DTF1	0.302	0.896	0.326	0.884	0.916	0.874
DTF2	0.256	0.984	0.354	0.715	0.877	0.875
DTF3	0.284	0.932	0.329	0.689	0.903	0.844
DTF4	0.279	0.866	0.314	0.662	0.878	0.898
DTF5	0.284	0.827	0.326	0.649	0.863	0.842
DTF6	0.279	0.844	0.295	0.656	0.871	0.854
DTF7	0.296	0.867	0.277	0.637	0.846	0.933
DTF8	0.285	0.852	0.284	0.641	0.839	0.899

Table: 5 *In vitro* kinetic model drug release

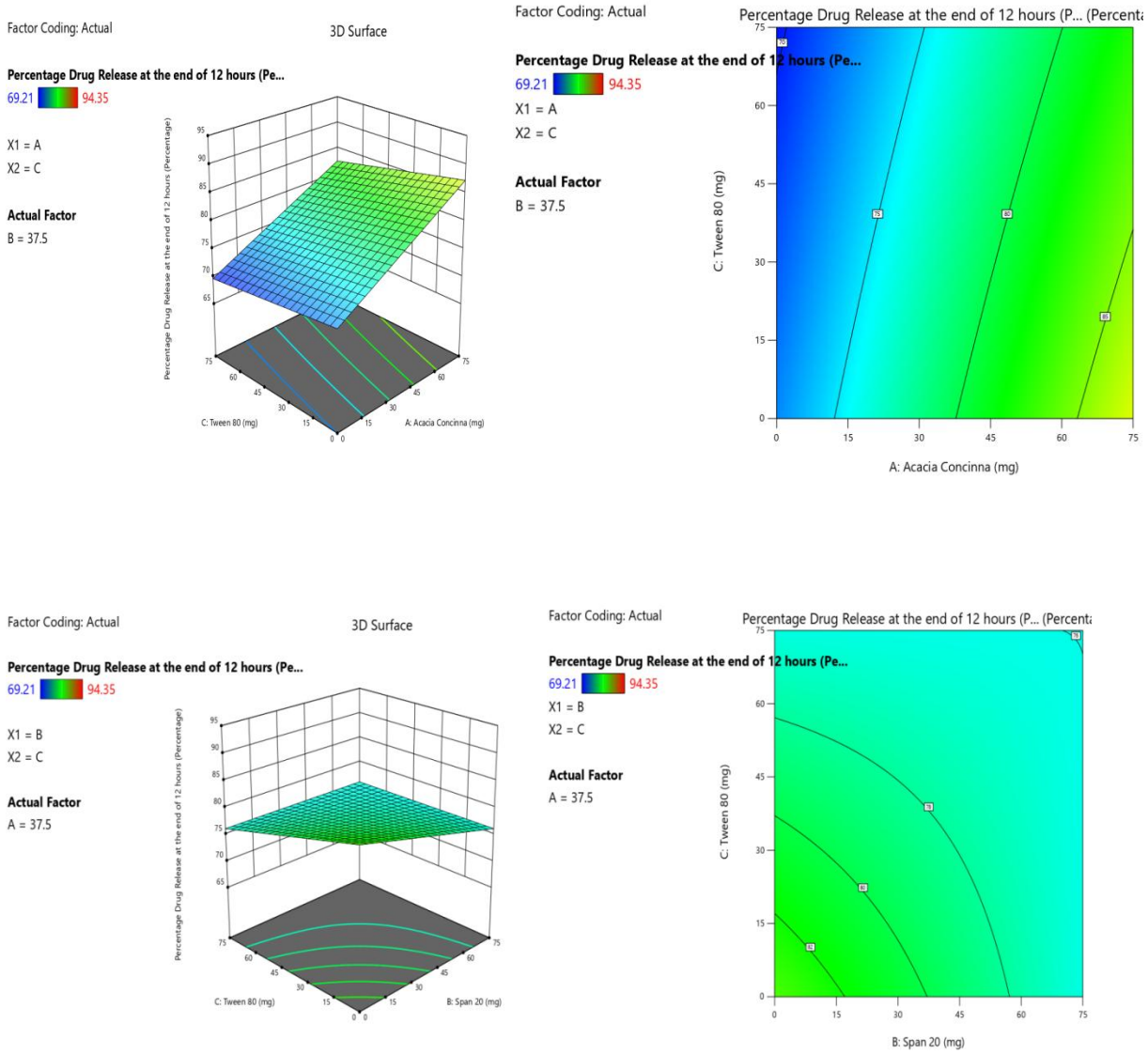

Figure: 13 Surface Plots indicating the effect of Acacia concinna on percent drug release at the end of 12 hours.

Evaluation of diacerein FTs-G

The viscosity of diacerein and spreadability FTs-G

The viscosity is an important factor to be considered for topical application. The viscosity of optimized formulation containing different concentrations of carbopol was determined and given in the table. The FTs-G containing 0.80s% carbopol 934 shows good viscosity. These values indicate ideal for application of gel on skin. The viscosity of preparation is inversely correlated

with spreadability. The spreadability of FTs-G was found to be in the range of 17-19 mm. this result suggests easy spreading of preparation.

Formulation	Viscosity(cP) (N=3)
DTFG1	2424±175
DTFG2	2651±225
DTFG3	2850±130

Table: 6 viscosity of transfersomal gels (Mean ± SE, n = 3)

Skin irritation of diacerein FTs-G

The FTs-G prepared using carbopol 934 does not show any sign of itching or irritation or erythema at the site of application. Moreover the histopathological evaluation indicates no morphological changes skin integrity indicating safe TD preparation.

Ex-vivo permeation of diacerein FTs-G

The amount of diacerein permeation studies were performed across the excised male wistar rat abdominal skin and the results were represented in table and Cumulative amount DTFG permeated Vs time profile, mean flux depicted in figure. The result showed that the formulation containing highest amount of Carbopol 934 Concentration able to cross the maximum amount of drug.

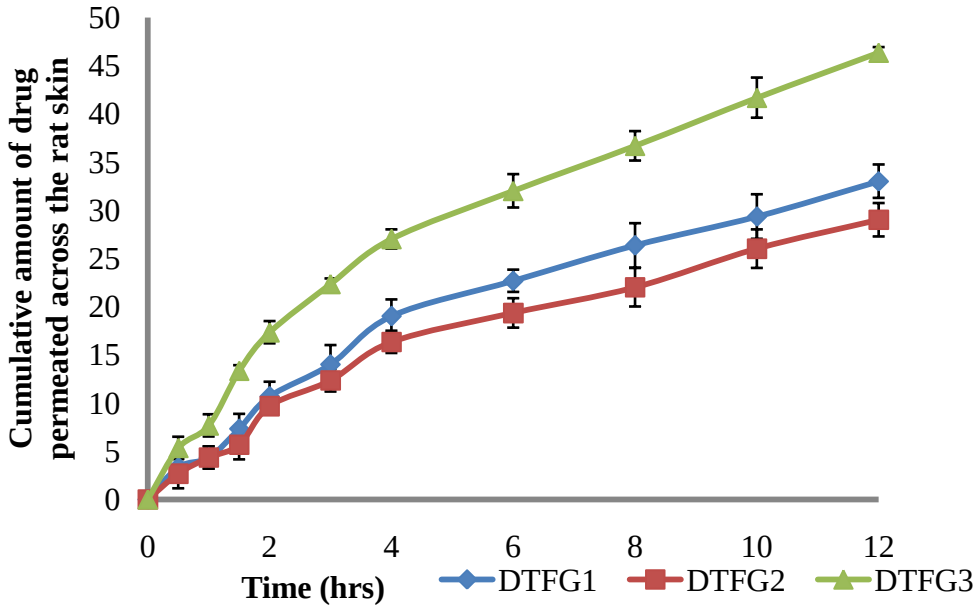

Figure: 14 Cumulative amount of DN permeated diacerein FTs-G through the rat skin

Permeation data analysis of diacerein FTs-G

The permeation data was given table 7. From the obtained results it can be inferredthat formulation DTFG 3 had amount of drug permeation across rat skin at the end of 12 hours46.33±3.8 (Q12 (mg)), flux 3.22±1.02 (Jss (mg/cm2/h)) and permeability coefficient 0.064(Kp (cm/h)x10-3).

Formulation code	Q_{12} (mg)	Jss (mg/cm^2/h)	Kp (cm/h)x10^{-3}
DTFG1	33.33±0.6	2.26±0.84	0.045
DTFG 2	29.67±1.2	4.32±1.25	0.086
DTFG 3	46.33±3.8	3.22±1.02	0.064

(Mean ± SE, n = 3)

Table 7: Amount of drug permeated across rat skin form diacerein FTs-G

Q12 (amount of drug permeated in 12 hrs), Jss(steady state flux), Kp(permeability coefficient).

Stability study of diacereinFTs-G

The sample was tested after six months the mean size of formed diacerein FTs-G were measured by diluting each sample and analysed change in particle size by optical microscope. Viscosity and *ex-vivo* permeation. The corresponding data was given in the below table 8. There are no significant changes for selected parameter for stability analysis.

Formulation (DTFG3)	**Viscosity (CPs)**	**Particle size (nm)**	**cumulative drug permeation across skin (mg)**
Before 6 months	2541.10±46	340±23	46±2.3
After 6 months	2988.33±53	360±45	43±3.5

(Mean ± SE, n = 3) **Table 8: stability studies of optimized formulation**

Conclusion:

Diacerein loaded FTs formulation containing acacia concinna a natural surfactant were successfully prepared and optimized for transdermal application. Thin film hydration method was employed to prepare the FTs. A factorial design statistical method helped to investigate the optimized formulation. The results of particle size, EE and % DR were considered as evaluation parameter for optimization. The resulting diacerein FTs were converted in to gel preparation for topical application. Viscosity, spreadability and skin irritation tests confirmed the preparation alternate delivery approach through skin.

Abbreviations

OA= Osteo arthritis

TD= Transdermal

SC= Stratum corneum

SFT= Surface tension

FTIR= Fourier transform infrared spectroscopy

DSC= Differential Scanning calorimeter

CA= Acacia Concinna

FTs= Transfersomes

RBF= Round bottomed flask

RE= Rota evaporation

ED= Experimental design

% EE= Percent entrapment efficiency

FTs-G= Transfersomal gels

CMC= Critical micelle concentration

PDI= Poly dispersity index

DN= Diacerein

VS= Vesicle size

Authors have no conflict of interest

References

1. Zhang, Y, Jordan, J. M. (2010). Epidemiology of osteoarthritis. Clinics in geriatric medicine, 26:355–369. https://doi.org/10.1016/j.cger.2010.03.001
2. Felson DT, Lawrence RC, Dieppe PA, et al.(2000) Osteoarthritis: new insights. Part 1: the disease and its risk factors. Ann Intern Med. 133:635-646. https://doi.org/10.7326/0003-4819-133-8-200010170-00016
3. Lawrence RC, Felson DT, Helmick CG, et al.(2008). Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. Arthritis Rheum. 58:26-35. https://doi.org/10.7326/0003-4819-133-8-200010170-00016
4. Singh I, Morris AP. (2011).Performance of transdermal therapeutic systems: Effects of biological factors. Int J Pharm Investig.1:4-9. https://doi.org/10.4103/2230-973X.76721
5. Rajan R, Jose S, Mukund VP, Vasudevan DT. (2011). Transferosomes - A vesicular transdermal delivery system for enhanced drug permeation. J Adv Pharm Technol Res. 2:138-143. https://doi.org/10.4103/2231-4040.85524
6. Martorell M, Castro N, Victoriano M, et al. (2021). An Update of Anthraquinone Derivatives Emodin, Diacerein, and Catenarin in Diabetes. Evid Based Complement Alternat Med. 2021:3313419. https://doi.org/10.1155/2021/3313419
7. Tamura T, Ohmori K.(2001) Rhein, an active metabolite of diacerein, suppresses the interleukin-1alpha-induced proteoglycan degradation in cultured rabbit articular chondrocytes. Jpn J Pharmacol.85:101-104. https://doi.org/10.1254/jjp.85.101
8. Jain, A., Singh, S. K., Singh, Y., & Singh, S. (2013). Development of lipid nanoparticles of diacerein, an antiosteoarthritic drug for enhancement in bioavailability and reduction in its side effects. J Biomed Nanotechnol, 9:891–900. https://doi.org/10.1166/jbn.2013.1580
9. Li, N., Qin, Y., Dai, D., Wang, P., Shi, M., Gao, J., Yang, J., Xiao, W., Song, P., & Xu, R. (2022). Transdermal Delivery of Therapeutic Compounds With Nanotechnological Approaches in Psoriasis. Front Bioeng Biotechnol, 9, 804415. https://doi.org/10.3389/fbioe.2021.804415
10. Shaikh IA, Muddapur UM, Bagewadi ZK, et al.(2022) Characterization of Bioactive Compounds from Acacia concinna and Citrus limon, Silver Nanoparticles' Production by

A. concinna Extract, and Their Biological Properties. Molecules.27:2715. https://doi:10.3390/molecules27092715

11. Muhammad, M. T., & Khan, M. N. (2018). Eco-friendly, biodegradable natural surfactant (Acacia Concinna): An alternative to the synthetic surfactants. Journal of Cleaner Production, 188:678-685. https://doi.org/10.1016/j.jclepro.2018.04.016
12. Ghagi R, Satpute SK, Chopade BA, Banpurkar AG (2011). Study of functional properties of Sapindus mukorossi as a potential bio-surfactant. Indian Journal of Science and Technology, 4:530-3.
13. Sarkar, p., & Biswas majee, S. (2022). Formulation development and in vitro characterization of ternary hydrotropic solid dispersions of aceclofenac. Asian J Pharm Clin Res 15:174-9 https://doi.org/10.22159/ajpcr.2022.v15i9.45158
14. Fouad, S. A., Malaak, F. A., El-Nabarawi, M. A., Abu Zeid, K., & Ghoneim, A. M. (2021). Preparation of solid dispersion systems for enhanced dissolution of poorly water soluble diacerein:In-vitro evaluation, optimization and physiologically based pharmacokineticmodeling. PLoS One, 16(1), e0245482. https://doi:10.1371/journal.pone.0245482
15. Marwah H, Garg T, Rath G, Goyal AK.(2016)Development of transferosomal gel for trans-dermal delivery of insulin using iodine complex. Drug Deliv.23:1636-1644. https://doi:10.3109/10717544.2016.1155243
16. Bnyan, R., Khan, I., Ehtezazi, T., Saleem, I., Gordon, S., O'Neill, F., & Roberts, M. (2019). Formulation and optimisation of novel transfersomes for sustained release of local anaesthetic. J Pharm Pharmacol, 71:1508–1519. https://doi.org/10.1111/jphp.13149
17. Patel P, Patel P, Mahajan A, Koradia S, Saluja A, Shah K. (2022). Formulation, Evaluation, and Optimization of Diacerein Loaded Transferosomal Gel for Arthritis. Pharmacophore.13:7-16 https://doi.org/10.51847/b55j6uv4KX
18. El-Say, K. M., Abd-Allah, F. I., Lila, A. E., Hassan, A.el-S., & Kassem, A. E. (2016). Diacerein niosomal gel for topical delivery: development, in vitro and in vivo assessment. J Liposome Res 26:57-68 https://doi.org/10.3109/08982104.2015.1029495
19. Rehman, M., Madni, A., Ihsan, A., Khan, W. S., Khan, M. I., Mahmood, M. A., Ashfaq, M., Bajwa, S. Z., & Shakir, I. (2015). Solid and liquid lipid-based binary solid lipid nanoparticles of diacerein: in vitro evaluation of sustained release, simultaneous loading

of gold nanoparticles, and potential thermoresponsive behavior. nt J Nanomedicine. 10:2805-2814 https://doi.org/10.2147/IJN.S67147

20. Ashok, M., Habibuddin, M., & Raju, J. (2021). Provesicular Based Colloidal Carriers for Transdermal Drug Delivery: Formulation Aspects and Bioavailability Enhancement of Acyclovir Proliposomal Gels. Int. J. Pharm. Investigation, 11:195-203