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FORMULATION AND EVALUATION OF NANOSUSPENSION TO IMPROVE SOLUBILITY AND DISSOLUTION OF DIACEREIN

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ABSTRACT: Diacerein is an Osteoarthritic drug that exhibited suboptimal oral bioavailability upon oral administration of conventional dosage form. Nanosuspension gaining more attention for improving oral bioavailability of such drugs. This study was aimed to enhance the solubility and dissolution of diacerein by preparing Nanosuspension. Diacerein Nanosuspension was prepared using combination of High Speed Homogenization (HSH) and Media Milling (MM), Poloxamer 407 (stabilizer) and ZrO_2 beads (milling media). Various formulation and processing parameters were optimized in preliminary studies. Concentration of stabilizer and milling media were optimized for Cumulative percentage release (%CPR), saturation solubility (SS) and mean particle size (MPS) using 3^2full factorial design. Optimized batch was derived statistically using desirability function of Minitab17. Model was validated by formulating checkpoint batch. Accelerated stability study was carried out for optimized batch. Identification and compatibility study of drug and stabilizers were carried out using FTIR and DSC studies. Preliminary parameters were optimized by varying one parameter at a time, while keeping other constant by trial and error method. Optimized batch has 100%w/v milling media and 1%w/v of Poloxamer407. %CPR, SS and MPS were found to be 97.74%, 1.245mg/ml and 221.5nm respectively. Four hundred times enhancement in SS that of bulk drug and 97.74%CPR at 2min was observed after nanonization. It was concluded that Combination of HSH and MM technique can be successfully used for preparation of Diacerein Nanosuspension. Prepared nanosuspension significantly enhances solubility and *in-vitro* dissolution of Diacerein.

INTRODUCTION: Most of active pharmaceutical ingredients identified and screened through discovery screening programs are poorly water soluble [1]. These molecules/drugs are often difficult to formulate using conventional approaches and are associated with numerous formulation-related performance issues such as poor bioavailability, lack of dose proportionality, slow onset of drug action and some other attributes resulting into poor patient compliance [2].

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Most of the synthetic molecules available in the market are either weakly acidic or basic in nature which shows narrow absorption window, when given orally for systemic therapies. The poor absorption of these molecules is intimately related with different physiological pH of gastrointestinal tract. Poor aqueous solubility leads to poor bioavailability [3]. If drug solubility is not improved it will not be absorbed through gastrointestinal tract and will not exert its pharmacological action [4].

To resolve these problems many conventional approaches were adopted like micronization, use of co-solvents, salt formation, dispersion systems, precipitation, etc., but these methods having limited use in solubility enhancement. Some novel approaches like liposome, dispersion of solids,

micro-emulsion methods and inclusion complexes with β-cyclodextrine shows beneficial effect as drug delivery systems but major hurdle of these techniques are lack of universal applicability to all drugs [5]. Nanotechnology gaining more attention in pharmaceutical industries due to their many uses in oral, parenteral, transdermal, transmucosal, ocular and pulmonary drug delivery routes [6]. Over last decade, nanoparticle engineering has been developed and reported for pharmaceutical applications. There are many advantages of nanosuspension such as increased rate of absorption, increased oral bioavailability, rapid onset of action, reduction in required dose, reduction in fed/fasted variability, high drug loading capacity, suitability for hydrophilic drugs, dose reduction is possible, enhanced the physical and chemical stability of drugs [7–9].

Nanosuspension is one of the most important strategies to enhance the oral bioavailability of poorly water soluble drugs [10]. Preparing nanosuspension can be a challenging technique. Various methods which are generally used to prepare nanosuspension are bottom-up including precipitation, and top-down including media milling, emulsion solvent diffusion method, supercritical fluid method, dry co-grinding, high-pressure homogenization, Nano edge [6, 11]. Nanosuspension have two particular advantageous properties, firstly increased saturation solubility and secondly the enlarged surface. Both properties results in an increase in the dissolution rate according to the Noyes-Whitney Law [12]. In general it is advantageous to use nanoparticles that are as small as possible to achieve a maximum improvement in the oral bioavailability or a very rapid dissolution rate [13, 14].

To produce such nanosuspension top-down approaches comprising high-pressure homogenization and media milling are used in which size of the particle is reduced by repeatedly forcing a suspension through a very thin gap at extremely high velocity, the later comprises mechanical attrition of suspended drug particles using milling media such as pearls or balls consist of ceramics (cerium- or yttrium-stabilized zirconium dioxide), stainless steel, glass, or highly cross-linked polystyrene resin-coated beads.[15] Here major limitations for media milling is that usually it takes long time for conversion of drug particles in the nano stage when the speed is average and high pressure homogenization requires very costly instrumentation. Another method is to use high speed homogenizer instead to high pressure because it is less costly and easier one but it can hardly give the particle size up to 600 nm, which will be sufficient to take full advantage of nano particles [16–18]. To overcome this limitation combination method (High Speed Homogenization and Media Mill) was used in this research work to prepare Diacerein nanosuspension[19].

Diacerein (DCN) is chemically 4,5-diacetoxy-9,10-dioxy-9,10-dihydroanthracene-2-carboxylic acid which is a newer anti-inflammatory agent and chondro-protective agent used in the treatment of osteoarthritis [20, 21] and it metabolized to active constituent rhein. Rhein is thought to act via inhibition of interleukin-1β and proteolytic enzymes along with which it stimulates the synthesis of cartilage components and modifies the understanding pathological conditions. As it does not inhibit the synthesis of prostaglandins is emerging as better and safe therapeutic agent as compared to NSAIDs [22, 23]. It belongs to BCS class II with poor aqueous solubility (3.197mg/ml) [24].

Diacerein lacks cyclo-oxygenase inhibitory activity and hence has no effect on prostaglandin synthesis. It is a selective Interleukin-1 inhibitor with protective effect on granuloma induced cartilage breakdown by its reduction of the concentration of pro inflammatory cytokines [25, 26]. However, the poor aqueous solubility and hence limited dissolution of Diacerein means that only 35-56% of the drug reaches the systemic circulation [24, 27]. Poor bioavailability of a drug often results in limited therapeutic response. So, the aim of the current research work is to improve the aqueous solubility and dissolution rate of Diacerein via nanosuspension preparation.

MATERIALS AND METHODS:

Materials: Diacerein was received as a gratis sample from Ami life Science, Mumbai. The Poloxamer 407, Poloxamer 188 and PVP K30 were obtained from SD fine chemicals, Mumbai. HPMC K15 and TWEEN 80 were collected from ACS chemicals Ahmadabad and Loba chemicals Pvt.

Ltd Mumbai respectively. Other chemicals used for the study were of analytical grade.

Preparation of nanosuspension: Aqueous Nanosuspension of Diacerein was prepared using combination of High Speed Homogenization (HSH) and Media Milling (MM) technique [28, 29]. Poloxamer 407 with the concentration of 1% of the drug amount was used as stabilizer. The mixture of drug and stabilizer was added in homogenizer vessel containing 20ml distilled water and it was subjected to high speed homogenizer at specific speed for specific period of time. After completion of homogenization step suspension was transferred in a glass vial containing weighed quantity of zirconium oxide beads (ZrO_2) and this suspension was stirred on magnetic stirrer using magnetic bead for a specific period. The concentration of prepared nanosuspension was 10mg/ml. Nanosuspension was evaluated for various parameters and results of design of experiment were analyzed to reduce any effect of systematic errors all the experiments were randomized. Finally prepared nanosuspension was stored at 2-8°C

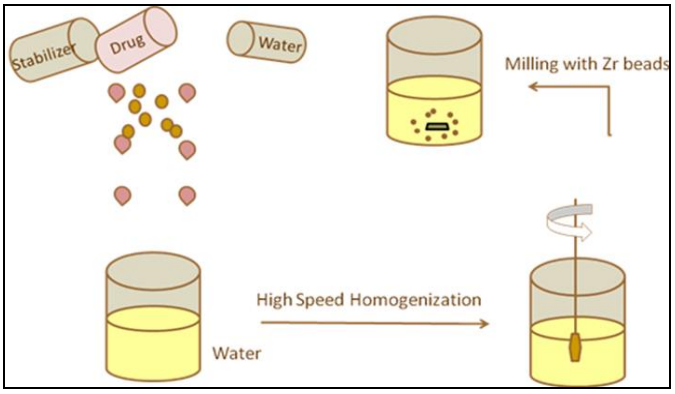

FIG. 1: PREPARATION OF NANOSUSPENSION

Experimental design: Initial screening trials were carried out for evaluating the formulation and processing aspects of nanosuspension. Various factors like type of stabilizer, concentration of stabilizer, homogenization speed, homogenization time, media milling time and concentration of milling media (beads) were identified as critical to give product in nano range. The results from the initial trial suggested that concentration of stabilizer and concentration of media milling are the main factors which affect the mean particle size (MPS) and percentage cumulative drug release of nanosuspension. Effect of particle size on oral absorption

A randomized 3^2 full factorial design with two factors at three levels was adopted to study the formulation and preparation parameters of Diacerein nanosuspension. The two independent factors identified for this study were concentration of stabilizer and concentration of milling media. Both factors were operated at three levels (+1, 0, -1). The type of stabilizer, homogenization speed, homogenization time, media milling time and aqueous media i.e. purified water were kept same for all the experimental runs. Minitab 17 software was used to conduct the study. Total 9 experimental trials were designed by the software. Experiments were run in random order to increase the predictability of the model. The dependent variables, percentage cumulative drug release at 2min (Y_1), saturation solubility (Y_2) and Mean particle size (Y_3) were selected on the basis of trials taken during preliminary batches. **Table 1** list out the formula composition of nanosuspension with respect to percentage as mentioned in experimental design.[30, 31]

TABLE 1: GENERAL FORMULA COMPOSITION OF NANOSUSPENSION BATCHES

Batch code	Drug Concentration	Stabilizer concentration	HSH speed (rpm)	HSH time (Hr)	MM time (Hr)	Bead ratio (L:S)	
						L	S
DCN	1%	1%	6000	2	20	100%	0
Aqueous media- Purified water							

HSH=High Speed Homogenization, MM=Media Milling, L= Large, S=Small

Evaluation of nanosuspension:

FTIR Spectroscopy: [32] FTIR spectroscopy studies were carried out for identification of pure drug and compatibility study of physical mixture of nanosuspention. FTIR spectroscopy was conducted using a shimadzu FTIR 8400 spectrophotometer (shimadzu, Japan) and spectrum was recorded in the wavelength region of 4000-400cm^{-1}. The procedure consisted of dispersing sample in KBr and compressing into discs by applying pressure. The pellet was placed in the light path and spectrum was recorded.

Differential Scanning Calorimetry (DSC): [32]
DSC studies were carried out for identification of pure drug and compatibility study of physical mixture of nanosuspention. DSC study was performed using DSC-60 C (Shimadzu, Tokyo, Japan) calorimeter to study the thermal behaviour of drug. The instrument comprised of calorimeter (DSC-60), flow controller (FCL60), thermal analyser (TA 60WS) and operating software (TA 60). The Diacerein drug sample was heated in hermetically sealed aluminium pans under air atmosphere at a scanning rate of 10°C/min from 30 to 300°C in an air atmosphere. Empty aluminium pan was used as a reference.

Particle Size Distribution and Zeta potential Analysis: [33] Particle size, size distribution and zeta potential of diacerein nanosuspension were determined using Zetatrac (Microtrac Inc., USA). Zetatrac utilizes a high-frequency AC electric field to oscillate the charged particles. The Brownian motion power spectrum was analyzed with modulated power spectrum technique, a component of power spectrum resulting from oscillating particles. Nanocrystals equivalent to 100 mg of sample were suspended with sufficient water, samples were directly placed into cuvette and particle size as well as zeta potential were measured.

Poly despersity index (PDI): [34] Mean particle size and Polydispersity index (PDI) of prepared Nanosuspension were obtained using Zetatrac. After suitable dilution, prepared nanosuspension was added to the sample cell and determination was carried out. PDI values give idea about uniformity of size distribution.

Saturation solubility studies: [35] The saturation solubility studies were carried out for both unprocessed pure drug and different batches of Nanosuspension. Nanosuspension and suspension of bulk drug was stirred for 24 hours on magnetic stirrer at 100rpm and room temperature to have saturation. Then the sample was filled in centrifugation tube and centrifuge for 10 min at 10,000 rpm in Cooling Centrifuge. Clear supernant was collected using 0.22µm syringe filter and analysed using UV spectrophotometer at 258 nm. The results were analysed and noted.

***In-vitro* Dissolution Studies:** [21, 36, 37] *In-vitro* dissolution of Diacerein nanosuspension was studied for 60 min using USP apparatus type 2 (paddle type) at 75 rpm (Electrolab Dissolution Tester TDT-06P, USP) using 900 ml citrate buffer pH 6.0 as dissolution medium maintained at 37°±5°C. Diacerein nanosuspension (5ml) was accurately inserted in the medium and sample aliquots of 5ml sample was withdrawn from the vessel and filtered through 0.22µm syringe filter at predetermined time intervals (2, 4, 6, 8, 10,15, 20, 30, 45 and 60 min) and replaced with 5ml fresh dissolution media. The withdrawn samples were analysed using UV visible spectrophotometer at 258nm by the regression equation of standard curve developed in the same range in the linearity range of 2–8 µg/ml. The experimental data obtained were validated by ANOVA combined with the F test. The determination coefficient (R^2, agreement between the experimental results and predicted values obtained from the model) and the model F value (Fisher variation ratio, the ratio of mean square for regression to mean square for residual) were applied for statistical evaluation.

Short term accelerated Stability study: [24] Optimized batch of Diacerein nanosuspension was subjected to short term stability study for a period of 1 month as per ICH guidelines. In the present study, stability study was carried out at 40 °C ± 2°C and 75% ± 5% relative humidity (RH). Nanosuspension was evaluated for particle size, viscosity, sedimentation rate and cumulative percentage drug release.

RESULT AND DISCUSSION:

FT-IR and Differential Scanning Calorimetry [32]

FT-IR study: FTIR spectroscopy was done with drug, Poloxamer 407 and physical mixture of both. Comparative spectra of them are presented in **Fig. 2** and comparative bands are tabulated in **Table 2** showing Drug and Polymer's compatibility.

DSC Study: DSC thermogram corresponding to Diacerein, Poloxamer 407 and Physical mixture of both are shown in **Fig. 3**. Diacerein exhibits a characteristic endothermic peak at 258.55°C corresponding to its melting point. A sharp endothermic peak at 61.37°C was observed for Poloxamer 407.

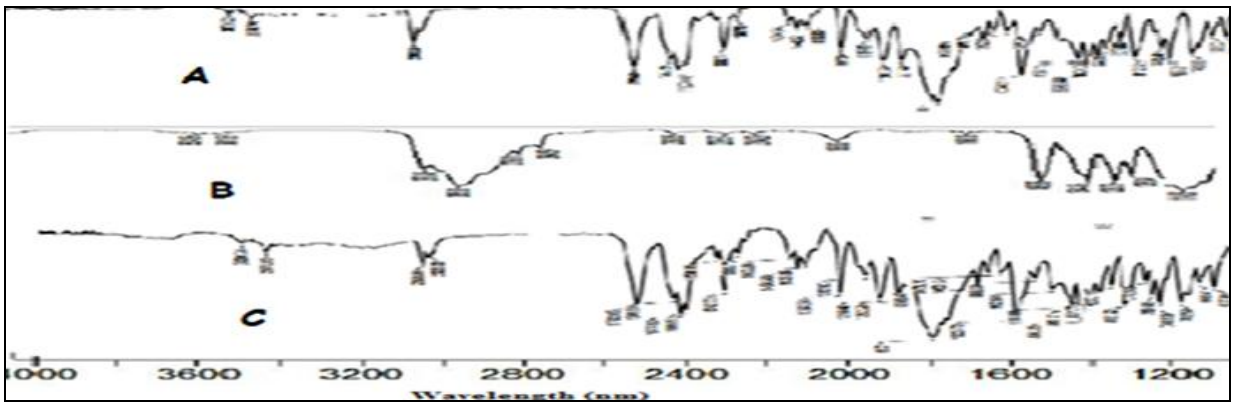

FIG. 2: COMPARISON OF FTIR STUDY OF DIACEREIN (A), POLOXAMER 407(B) AND PHYSICAL MIXTURE (C)

TABLE 2: COMPARATIVE FTIR INTERPRETATION

Characteristic bands	Physical mixture	Diacerein	Poloxamer 407
-C-H stretching aromatic	3070.22	3069.74	3481
-C-H stretching aliphatic	2974.74	2971.85	2970
-C = O stretching	1764.40	1762.95	-
-COOH Functional group	1692.55	1691.59	1649
-C = C stretch, aromatic	1592.73	1592.73	-
-C-O stretch of COOH	1450	1450	1465.95
-C-H bending, COOH	1298	1298	1280
-C-O stretch	1026	1026	1114.89
Benzene ring	704	704	-

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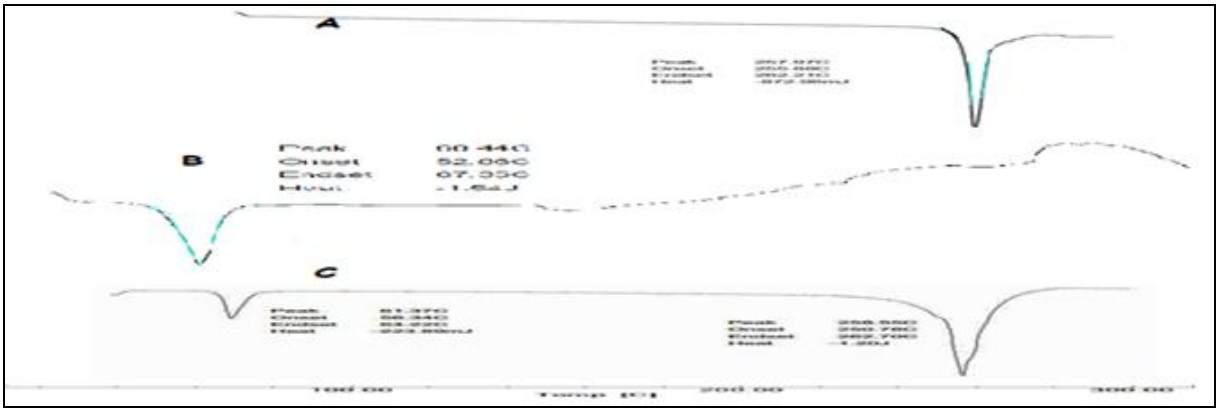

FIG. 3: COMPARISON OF DSC THERMOGRAM OF DIACEREIN (A), POLOXAMER 407 (B) AND PHYSICAL MIXTURE OF BOTH (C)

Experimental Design:

Effect of concentration of stabilizer: Various formulations were prepared varying the percentage w/v ratio of Poloxamer 407 to nanosuspension. As shown in **Table 3,** a 3^2 full factorial design was applied to evaluate the effect stabilizer's concentration on the predetermined dependent variables viz., cumulative percentage drug release at 2 min, saturation solubility and mean particle size. The coefficients in **Table 4** represent the respective quantitative effect of independent variables (X_1 and X_2) and their interactions on the various responses. It was seen that all the independent variables had a significant effect on the response ($p<0.05$). The negative sign (-) of the coefficient indicated that increase in the value of

independent variable decreases the value of response and vice versa. The absolute value of the coefficient indicates the magnitude of effect of the independent variable on the response; the higher the value, the higher the magnitude. The relationship between the dependent and independent variables was further elucidated by response surface plots. All the determination coefficients R^2 are larger than 0.9, indicating that over 90 % of the variation in the response could be explained by the model and the integrity of the model fitting was confirmed. The F_{ratio} was found to be much higher than that of theoretical value ($F_{significance}$) with very low probability for each regression model, shows that the regression model was significant with 95% confidence level. The observed responses showed a wide variation suggesting that the concentration stabilizer had a significant effect on resultant, cumulative percentage drug release at 2 min, saturation solubility and mean particle size.

Effect of milling media concentration: Secondly 3^2 full factorial design was used to optimize and evaluate the main effects, interaction effects and quadratic effects of milling media concentration. Cumulative percentage drug released at 2 min (Y_1), saturation solubility (Y_2) and mean particle size (Y_3), were selected as the response variables; as shown in **Table 3**, the observed responses showed a huge variation, suggesting that the independent variables had a significant effect ($p<0.05$) on the selected dependent variables.

TABLE 3: LAYOUT AND OBSERVED RESPONSES OF 3^2 FACTORIAL DESIGN FOR PROCESS VARIABLES

Batch No.	X_1 (Concentration of Poloxamer 407(%w/v))	X_2 (Concentration of milling media (%w/v))	%CPR* (at 2min) (Mean ±SD)	SS* (Mean ±SD)	MPS* (Mean ±SD)
1	-1	-1	74.16±1.30	0.54±0.03	364±57
2	0	-1	83.88±2.07	0.88±0.03	313±10
3	1	-1	77.49±0.90	0.66±0.15	339±43
4	-1	0	91.98±1.51	0.92±0.02	235±71
5	0	0	97.74±3.70	1.24±0.02	221.5±15
6	1	0	82.62±3.21	0.98±0.27	306±39
7	-1	1	92.88±0.86	0.66±0.09	271.1±83
8	0	1	93.24±1.96	0.95±0.03	273.6±52
9	1	1	77.4±1.57	0.77±0.09	377±45

Translation of coded levels in actual unit

Variable level	Low (-1)	Medium (0)	High (+1)
X_1	0.5%	1%	1.5%
X_2	50%	100%	150%

*Mean of three readings
%CPR = Cumulative Percentage Release, SS = Saturation solubility (mg/ml) and
MPS = Mean Particle size (nm)

TABLE 4: STATISTICAL ANALYSIS OF 3^2 FACTORIAL DESIGN FOR X_1

	Co-efficient			P-value		
	Y_1	Y_2	Y_3	Y_1	Y_2	Y_3
b_0	96.69	1.235	223.5111	2.91E-06	1.57E-05	0.00011
b_1	-3.585	0.043333	25.31667	0.008573	0.044833	0.011247
b_2	4.665	0.053	-15.7167	0.004036	0.026785	0.040146
b_2^2	-8.865	-0.266	45.98333	0.003084	0.001312	0.009847
b_1^2	-7.605	-0.315	68.78333	0.004807	0.000796	0.003107
b_{12}	-4.7025	-0.0045	32.725	0.007058	0.796235	0.009671
F_{ratio}	56.16526	72.32289	38.01385			
R^2	0.984462	0.991772	0.984462			

Process Optimization: The optimum formulation was selected based on the criteria of attaining the minimum MPS (Y_3) and the maximum %CPR (Y_1) and SS (Y_2). An overall desirability function dependent on all investigated formulation variables was used to predict the ranges of variable where the optimum formulation might occur. The desirable ranges are from zero to one (least to most desirable,

respectively). From optimized batch, an optimum D of 1.000 was obtained at respective levels of X_1(1%) and X_2 (100%). A final formulation was prepared as per level obtained in optimization and as shown in Fig. 4.7; the optimized formulation had a particle size of 296.75 nm. Suggesting that significant particle size reduction at nano level took place which could be responsible for significantly better drug release ($p<0.05$) of the optimized formulation as compared to pure diacerein. **Table 5** shows evaluation parameters which confirmed that there was a close agreement between predicted and observed values of responses.

TABLE 5: COMPARISON OF CALCULATED DATA WITH EXPERIMENTAL DATA

Response	Predicted	Observed	% Bias
%CPR(at 2 min) (Y_1)	96.47%	95.22%	-0.0131
SS (Y_2)	1.16mg/ml	1.355mg/ml	0.144
MPS (Y3)	296.75nm	271.1nm	-0.348

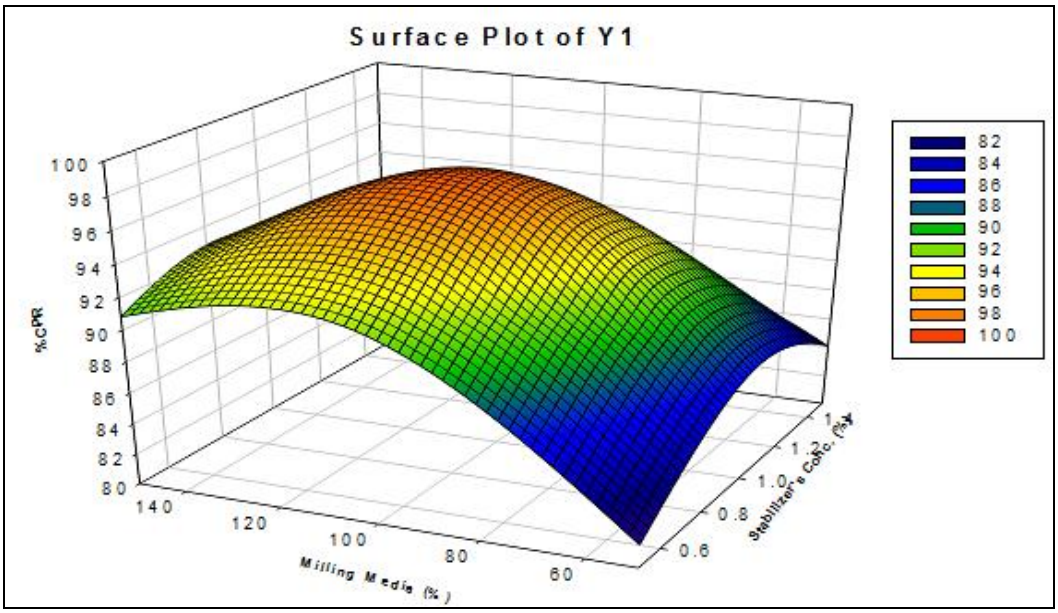

FIG. 4: RESPONSE SURFACE MODEL (RSM) SHOWING THE INFLUENCE OF THE INDEPENDENT VARIABLES ON THE QUALITY ATTRIBUTE Z-AVERAGE CUMULATIVE PERCENTAGE DRUG RELEASE (AT 2 MIN) (%CPR)

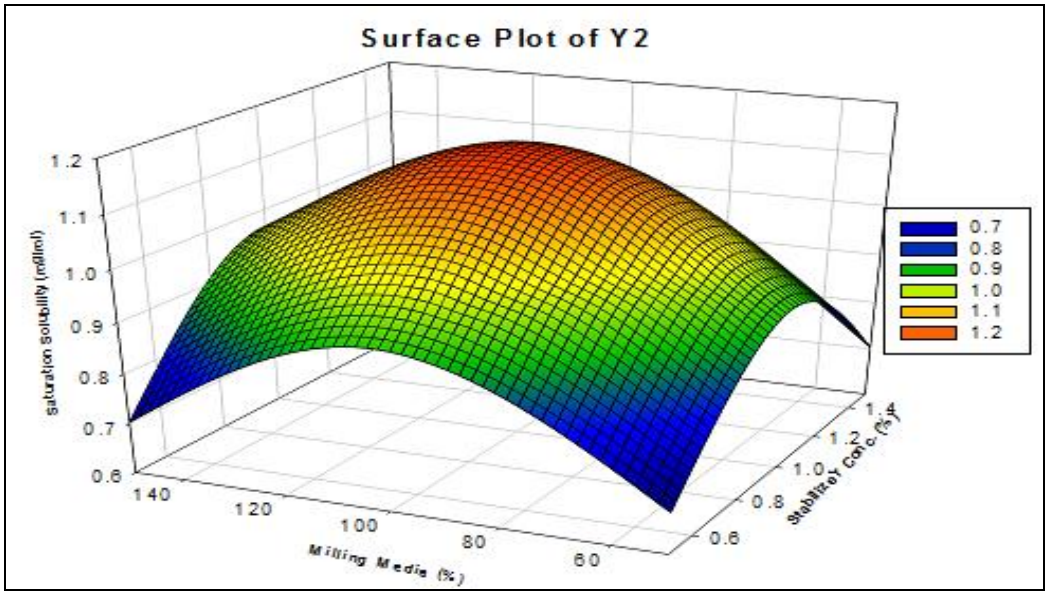

FIG. 5: RESPONSE SURFACE MODEL (RSM) SHOWING THE INFLUENCE OF THE INDEPENDENT VARIABLES ON THE QUALITY ATTRIBUTE Z-AVERAGE SATURATION SOLUBILITY (mg/mL)

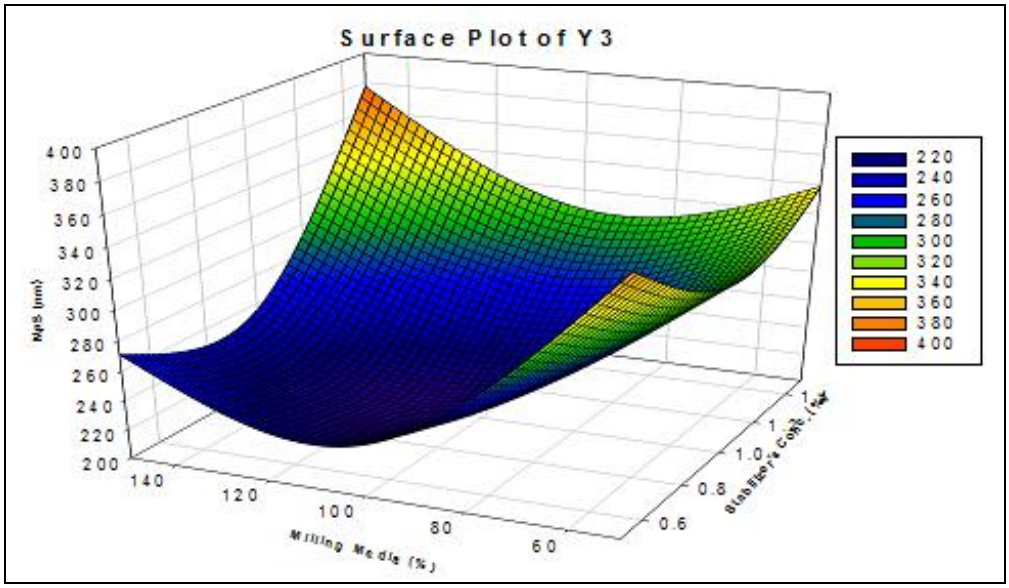

FIG. 6: RESPONSE SURFACE MODEL (RSM) SHOWING THE INFLUENCE OF THE INDEPENDENT VARIABLES ON THE QUALITY ATTRIBUTE Z-AVERAGE MEAN PARTICLE SIZE (NM)

Mean Particle Size: Particle size distribution of the optimized batch (i.e. DCN at 0 day) is shown in fig. 4.7 and fig. 4.8 Mean Particle size of optimized batch was found to be 221.5±10nm. Diacerein nanosuspension based final formulation was prepared for oral administration for which PDI and particle size above 5µm is not critical. The particle size of a nanosuspension for oral use is considered to be around 200-1000 nm [38]. From the fig. 4.7, it was found that mean particle size of optimized formulations were in the nanometer rage. It showed that all formulations fulfilled the requirements of a nanosuspension.

Zeta potential: Poloxamer 407 a non-ionic surfactant is used as stabilizer which provides steric stabilization. So, both negative Zeta potential is attributed to drug Nanosuspension. In general, zeta potential value of ±30mV is sufficient for stability of nanosuspension [39]**.** Preferably it should be between -10mV to -30mV. Zeta potential of our optimized formulation was observed 24.04±11 which complies with requirement of zeta potential.

Saturation solubility studies: Saturation solubility of optimized batch of Nanosuspension and pure drug was found to be 1.245±85mg/ml and 0.003±0.07mg/ml respectively it indicates that saturation solubility of nanosuspension was 400 times enhanced than that of pure drug. This improvement in saturation solubility is due to reduction in particle size and subsequent increase in surface area. So, it can be assumed that this increase in saturation solubility may improve bioavailability.

***In- vitro* Dissolution Studies:** The dissolution profile of Nanosuspension, Un-milled (pure drug) suspension and marketed formulations (DIATAL® Tablet, ORCERIN® Capsule) are presented in below fig. 4.6 and table 4.5. In Nanosuspension more than 95 % drug was released in 2 min, while maximum cumulative percentage drug release of un-milled suspension, tablet and capsule (marketed formulations) was found to be 46.05% at 20 min, 47.88% at 60 min and 93.86% at 45 min respectively. So, Nanosuspension enhanced rate of *in-vitro* dissolution of Diacerein to great extent.

TABLE 6: COMPARISON OF *IN-VITRO* DISSOLUTION OF NANOSUSPENSION WITH MARKETED FORMULATION

Time (min)	Pure drug* Mean±SD*	Marketed Tablet* Mean±SD*	Marketed Capsule* Mean±SD*	Nanosuspension* Mean±SD*
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
2	27.72±4.71	17.64±1.57	35.10±3.10	97.74±2.30
4	27.33±2.04	21.25±0.93	47.35±2.91	84.63±1.57
6	31.18±1.58	23.53±1.87	55.98±2.71	83.78±0.98
8	32.27±2.79	26.63±0.95	62.08±0.89	83.82±1.00
10	31.47±2.15	27.40±2.30	62.56±1.07	78.30±0.93

15	39.18±1.98	37.29±1.87	63.66±0.87	77.78±1.62
20	46.05±0.91	36.59±1.20	72.59±1.57	73.57±1.87
30	44.44±2.46	37.81±0.98	76.78±1.67	76.84±1.20
45	38.75±3.12	37.52±0.89	93.86±3.51	73.53±0.95
60	26.1215±3.57	47.888±1.62	65.654±2.48	71.99±0.89

* Mean of three values

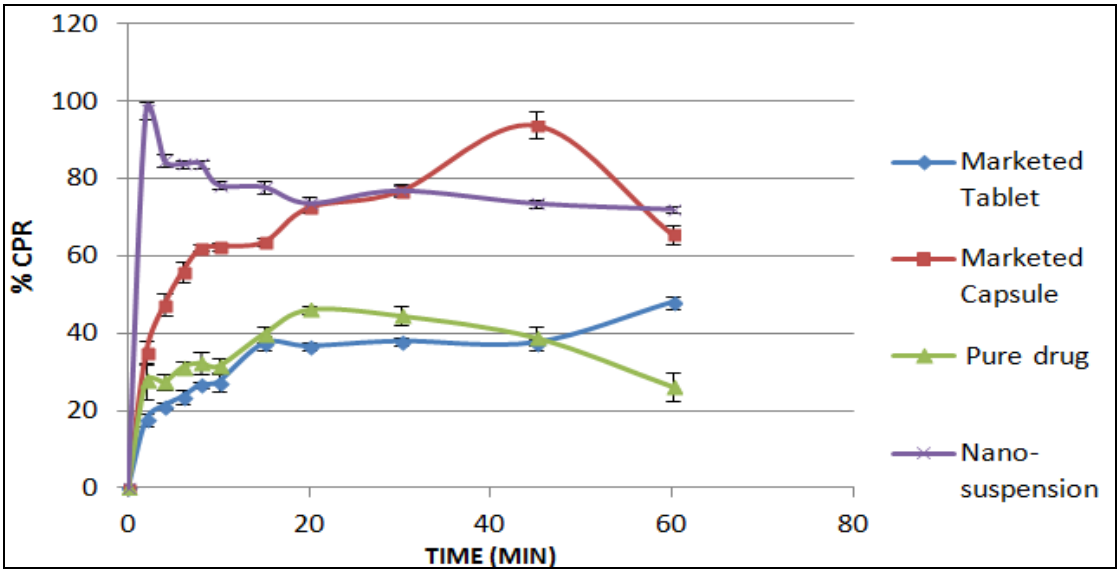

FIG. 7: COMPARISON OF *IN-VITRO* DISSOLUTION PROFILE OF NANOSUSPENSION WITH MARKETED FORMULATIONS

TABLE 7: CHARACTERIZATION DATA OF OPTIMIZED BATCH OF NANOSUSPENSION

Sr. No	Parameters	Results
1	Cumulative % Drug Release (at 2 min)	97.74%
2	Saturation Solubility	1.245mg/ml
3	Mean Particle Size	221.5nm
4	Viscosity	0.811 cp
5	Drug content	97.80%w/w

Short term accelerated Stability study: Stability study indicated that the formulation was physically and chemically stable when stored at the 40 ± 2°C and 75 ± 5 % RH for a period of one month. It was observed that there was a slight change in all optimization parameters which have less than ±5% bias which was insignificance. Negligible difference was observed in results obtained from optimized batch before and after stability study.

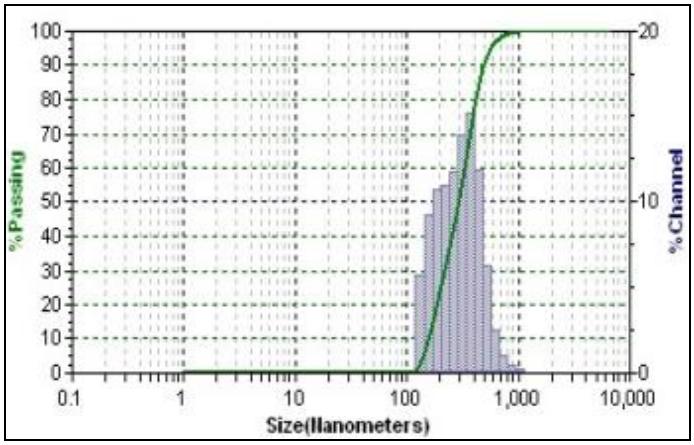

FIG. 8: DCN PARTICLE SIZE GRAPH
(At 0 day i.e. before stability)

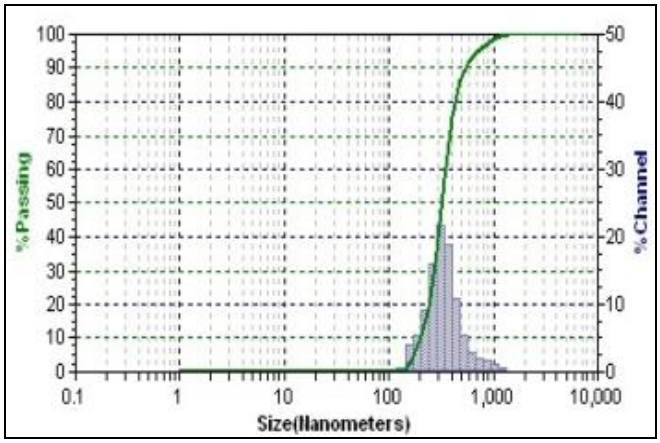

FIG. 9: DCN PARTICLE SIZE GRAPH
(At 30 day i.e. after stability)

TABLE 8: EVALUATION PARAMETERS OF STABILITY STUDY

Sr. No	Parameters	40° C ± 2°C and 75% RH ± 5% RH		% Bias
		0 Day	30 Day	
1	Cumulative % Drug Release (at 2 min)	97.74%	95.22%	-0.0168
2	Saturation Solubility	1.245 mg/ml	1.038mg/ml	-0.199
3	Mean Particle Size	221.5 ± 10nm	220.2 ± 15nm	0.2165
4	Viscosity	0.811 ± 0.017cp	0.78 ± 0.009cp	-0.039
5	Drug content	97.80%w/w	95.4%w/w	-0.0715

From the obtained stability study data, f_1 and f_2 were found to be 1.19 and 87.52 respectively which indicated that the dissolution data of before and after stability study was equivalent. Thus, from the f_1 and f_2 data it could be concluded that both dissolution profile were similar and no significant variation was observed so, nanosuspension of Diacerein was stable.

CONCLUSION: Diacerein nanosuspension was prepared successfully by combination of high speed homogenization and media milling technique. Preliminary and compatibility study were carried out using FTIR and DSC studies and showed drug-excipients compatibility. After preliminary optimization 3^2 full factorial design was applied for optimization of formulation. The optimized batch had 100% w/v of milling media and 1% concentration of Poloxamer 407 from the desirability results of Minitab 17. Cumulative percentage release (at 2 min), saturation solubility and mean particle size were found to be 97.74%, 1.245 mg/ml and 221.5nm respectively. Tremendous enhancement in saturation solubility as 400 times that of bulk drug and cumulative percentage drug release as 97.74 % at 2 min than that of the un-milled drug results as 43.42% at 20 min was observed after nanosizing of drug. Thus, from the results obtained it was concluded that *In-vitro* dissolution of prepared nanosuspension in dissolution media was much faster than that of un-milled suspension of drug. As nanosizing enhances the surface area which ultimately improves dissolution rate. This indicates that Diacerein nanosuspension improves dissolution as well as saturation solubility which may improve oral bioavailability of drug.

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