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Solubility enhancement of diacerein by spray drying technique, formulation development, and *in vivo* study

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ABSTRACT

Purpose: The aim of the present research was solubility and dissolution rate enhancement of diacerein by spray drying technique. Materials and Methods: Solid dispersions of diacerein with PEG-600, guar gum, poloxamer-188 and aerosil were prepared in different ratios using 32 factorial designs. FTIR, DSC, XRD and SEM were performed to study the interaction between drug and polymers and evaluated for in-vitro drug release study. Results and Discussion:In spray dried solid dispersion formulations there was decrease in crystallinity of diacerein, which leads to increase in dissolution of diacerein from solid dispersions. Diacerein 50 mg immediate release tablets formulated (using spray dried solid dispersion formulation which shows maximum drug release) by 32 factorial design to study effect of binder (PVPK-30) and disintegrant (Crospovidone) concentration on disintegration and dissolution of diacerein. Formulation showed better analgesic and anti-inflammatory activity and increase in bioavailability. Conclusion: Spray drying found to be effective technique to improve solubility, dissolution and bioavailability of poorly water-soluble drugs.

Keywords: Solubility enhancement, Solid dispersion, Spray drying, Pharmacokinetic, Pharmacodynamic

INTRODUCTION

Poorly water-soluble drug candidates often emerge from contemporary discovery programs and present formulation scientists with considerable technical challenges. With the advent of combinatorial chemistry and high throughput screening, the number of poorly water-soluble compounds has dramatically.[1] In pharmaceutical industry recently developed more than 40% new chemical entities are practically insoluble in water. These poorly water soluble drugs are allied with slow drug absorption leading to inadequate and variable bioavailability and gastrointestinal mucosal toxicity.[2] Solubility and dissolution rate, together with the intestinal absorptive potential of a drug are of major importance for bioavailability of drug.[3] Oral drug delivery is the simplest and easiest way of administering drugs is due to ease of administration, patient compliance, flexibility in formulation, etc.[4,5] Oral bioavailability of drugs depends on its solubility and/or dissolution rate, therefore major problems associated with drugs is its very low solubility in biological fluids, which results into poor bioavailability after oral administration.[6] Drugs with low solubility and high permeability, that is, BCS Class II, and low solubility and low permeability, that is, BCS Class IV having the problem of solubility and bioavailability. Improvement in the release profile of such drugs and thereby the bioavailability is possible through solubility enhancement of the drug. So that potential therapeutic benefits of these active molecules can be realized.[7] The ability to increase aqueous solubility can thus be a valuable aid to increasing efficiency and/or reducing side effects for certain drugs. Various techniques are used to increase the solubility of poorly soluble drugs are chemical modifications, physical modifications techniques like media milling/nanocrystal technology, cryogenic technology, supercritical fluid process, modification of the crystal habit, complexation, micellar technologies, other techniques such as co-crystallization, co-solvency, hydrotrophy, and solid dispersion.[8]

Diacerein is 9, 10-dihydro-4, 5-dihydroxy-9, 10-dioxo-anthetharnoic acid diacetate, is NSAID used for the treatment of osteoarthritis.[6,8] It belongs to BCS class-II i.e. low solubility and high permeability. The solubility of diacerein,

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retards dissolution and results in poor bioavailability. Hence, enhancement of solubility useful to improve dissolution and ultimately bioavailability.

Manufacture of milk powder was one of the first applications of spray drying when the method was developed in 1920.[9] In spray drying drug and polymer dissolved or suspended in a common solvent or solvent mixture and then drying it into a stream of heated air flow to remove the solvent.[10] Today, spray drying is important technique in pharmaceutical industry because of the rapid drying and specific characteristics such as particle size and shape of the final product. The aim of the present study was improvement of solubility with solid dispersion using spray drying technique. Solid dispersions of diacerein were prepared with hydrophilic carries as PEG-6000, poloxamer-188, and guar gum separately. Aerosil was used as adsorbent and solubility enhancer. Further from these solid dispersions that showed maximum drug release used in formulation of immediate release tablet dosage form by 3^2 factorial design. The tablet formulations were evaluated for weight variation, hardness, thickness, friability, disintegration, and dissolution test. From tablet formulations that showed maximum drug release used for *in vivo* bioavailability study.

MATERIALS AND METHODS

Materials

The following materials were used in this study. Diacerein (Elder Pharmaceuticals, Mumbai, Maharashtra, India), PEG-6000, Poloxamer-188, Aerosil (Shreya Laboratories, Aurangabad, Maharashtra, India), and Guar gum (Research lab fine chem. Industries, Mumbai, Maharashtra). All other chemicals/solvents used were of analytical grade.

Methods

Preparation of spray dried solid dispersions

Solid dispersions of diacerein and polymers were prepared by spray drying method as: 20 g of diacerein was dissolved in 30 mL DMSO, and then 70 mL acetonitrile was added to it and mix the solution. In another beaker solution of polymer (60 g) was prepared in 100 mL of acetonitrile. Then Aerosil (30 g) added into solution of polymer and 100 mL acetonitrile was added to dilute the solution and mixed it with stirring. Then this solution added to diacerein solution and homogenous solution was prepared by magnetic stirring and spray dried using spray dryer (LU-222 - Advance spray dryer, Labultima). The varying ratio of diacerein, PEG-6000, guar gum, poloxamer-188, and Aerosil (1:1:0.5–1:3:1.5) was used to prepare solid dispersions.

Table 1: Independent variables and levels of factorial design

Independent variable	Levels		
	Low	Medium	High
X1 Amount of polymer (gm)	1	2	3
X2 Amount of Aerosil (gm)	0.5	1	1.5

Independent Variables: Amount of Hydrophilic Carrier (g) = X1, Amount of Aerosil (g) = X2
Dependent Variables: % Drug dissolved (Y)

The processing parameters were inlet temp: 90°C, outlet temperature: 70°C, plate temperature: 30°C, tube temperature: 20°C, inlet high temperature: 120°C, cool temperature: 50°C, outlet high temperature: 90°C, and aspiration speed was kept at 35%, whereas feed pump speed was 3 mL/min.

Experimental design
Spray dried solid dispersions of diacerein and polymers were prepared by 3^2 factorial design as follows [Table1].

Characterization of spray dried solid dispersions
Prepared solid dispersions were evaluated for.

Drug content
An accurately weighed quantity of solid dispersions equivalent to 50 mg of diacerein was taken into 100 mL volumetric flask, dissolved in 10 mL acetonitrile and diluted up to 100 mL using citrate buffer pH6, from this 5 mL solution taken and diluted to 50 mL with citrate buffer pH6 and then solution was filtered using Whatman filter paper no. 41. Absorbance of filtrate was measured spectrophotometrically at 340 nm.

Fourier-transform infrared spectroscopy (FTIR) analysis[11]
Samples were prepared by grinding drug, excipients, and solid dispersions with KBr and then, pressing powder in the sample holder and placed in IR chamber (Shimadzu-8400 S) and spectra of individual drug, excipients, and solid dispersions were obtained.

Differential scanning calorimetry (DSC) study[12]
Samples (3 mg) were sealed in flat bottomed aluminum pans and heated over a temperature range of 50–300°C at a rate of 10°C/min in a nitrogen atmosphere using Shimadzu-60 DSC.

X-ray powder diffraction (XRPD)[13]
Polymorphic nature of drug and solid dispersion was determined by XRPD technique. XRD patterns were obtained using Brukar D-8X-ray diffractometer at voltage of 40 kV and current of 40 mA and scanning rate of 1°/min at diffraction angle of 2 theta degree using Cu (as anode) and radiation of wavelength 1.540600 A°.

Scanning electron microscopy (SEM)[13]
SEM photographs of drug and spray dried solid dispersion were obtained using Zeiss EVO LS10 scanning electron microscope to identify surface morphology. Samples were mounted on a metal stub with an adhesive for 5–6 min under vacuum with magnification 1.58KX (Diacerein), 400–450 KX (Solid dispersions).

In vitro drug release study of solid dispersion formulations[14]
An accurately weighed quantity of solid dispersions equivalent to 50 mg of diacerein was filled in hard gelatin capsule shell. The dissolution studies of capsule were conducted by using type I, IP (paddle method) dissolution test apparatus (Labindia). The dissolution test was performed using 900 mL, Citrate Buffer pH6 as dissolution medium at temperature 37°C±0.5°C and paddle speed 75 rpm. Capsules were placed in sinker and when temperature of dissolution medium reached, put it in to dissolution medium. Aliquots of 5 mL were withdrawn at 5, 10, 15, 20, 25, 30, 35, 40, and 45 min time intervals, filtered using Whatman filter paper no. 41 and samples were replaced with fresh dissolution medium of same quantity to maintain volume of dissolution medium after each sampling and analyzed spectrophotometrically at 340 nm.

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Formulation of Diacerein 50 mg immediate release tablet by 3^2 factorial design

A 9 runs of 3^2 factorial design, Consisting of 2 factors at three levels was set up to investigate effect of different amount of binder and disintegrant on tablet disintegration and dissolution [Tables 2 and 3].

Best solid dispersion that gives maximum drug release was diacerein: poloxamer-188: Aerosil (1:3:1.5 ratio) by spray drying method. From this equivalent to 50 mg diacerein mixture taken to formulate immediate release tablet using 3^2 factorial designs. Direct compression method was used to prepare diacerein 50 mg immediate release tablets. Ingredients (2–6) were sifted through sieve no. 40. Dry mix was prepared by mixing spray dried solid dispersion, Super Tab 11SD, Crospovidone, Aspartame and PVP K-30, and blended for 30 min in octagonal blender. Dry mix finally lubricated with magnesium stearate for 5 min. Lubricated blend compressed using 8.0 mm round beveled edge flat faced punches (Composition of formulations shown in Table 4). Formulation were evaluated for precompression parameters such as angle of repose, bulk density, tap density, carr's index, Hausner's ratio, and post compression parameters as weight variation, hardness, thickness friability, disintegration, and dissolution test.

Table 2: Independent variables and levels of factorial design

Independent variable	Levels		
	Low (−1)	Medium (0)	High (1)
Amount of Binder (%) X1	0.5	1	1.5
Amount of Disintegrant (%) X2	2	4	6

Table 3: Factorial design layout formulation of Diacerein 50 mg immediate release tablet by 3^2 factorial design

Batch code	Independent variables	
	Binder (%) X1	Disintegrant (%) X2
A	0.5 (−1)	2 (−1)
B	1.0 (0)	2 (−1)
C	1.5 (1)	2 (−1)
D	0.5 (−1)	4 (0)
E	1.0 (0)	4 (0)
F	1.5 (1)	4 (0)
G	0.5 (−1)	6 (1)
H	1.0 (0)	6 (1)
I	1.5 (1)	6 (1)

Independent Variables: Amount of Binder (%) = X1, Amount of Disintegrant (%) = X2
Dependent Variables: Disintegration time, % Drug release at 30 min

In vivo pharmacodynamic and pharmacokinetic study[15-20]

The tablets of optimized batch (Formulation G) used for *in vivo* pharmacodynamic and pharmacokinetic study. The animal experiment was carried out in compliance with the protocol of Institutional animal ethical committee (Registration No: 212/PO/Re/S/2000/CPCSEA).

In vivo pharmacodynamic study

Analgesic activity (acetic acid induced writing method)

For the evaluation of analgesic activity abdominal writhing assay was used. Animals (Swiss albino mice) were divided into three groups comprising six animals in each group. Each group received respective formulations as follows:

- Group I: Acetic acid (Control)
- Group II: Diacerein (Standard)
- Group III: Formulation (Diacerein 50 mg Immediate Release Tablet)

Writhing response was elicited by intraperitoneal (i.p.) injection of freshly prepared acetic acid solution (0.6%, 10 mL/kg, i.p.). The number of writhes (constriction of abdomen, turning of trunk, and extension of hind limbs) due to acetic acid was expressed as a nociceptive response. The number of writhes per animal was counted during a 30 min period, beginning 3 min after the injection of acetic acid.

Anti-inflammatory activity (carrageenan induced rat-paw edema)

For the evaluation of anti-inflammatory activity carrageenan induced rat-paw edema was used. Animals (Wister rat) were divided into three groups comprising six animals in each

Table 4: Composition of diacerein 50 mg immediate release tablets

Ingredients	Formulations (Qty in mg)								
	A	B	C	D	E	F	G	H	I
Dry mix									
Solid Disp.≈50 mg Diacerein	275	275	275	275	275	275	275	275	275
SuperTab 11SD	63.5	61.75	60	56.5	54.75	53	49.5	47.75	46
Crospovidone*	7	7	7	14	14	14	21	21	21
Aspartame	1	1	1	1	1	1	1	1	1
Binder									
PVPK-30**	1.75	3.5	5.25	1.75	3.5	5.25	1.75	3.5	5.25
Extragranular									
Mg. Stearate¥	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75
Total weight (mg)	350	350	350	350	350	350	350	350	350

*Disintegrant Conc.: 2%, 4%, 6%, **Binder Conc.: 0.5%, 1%, 1.5 ¥Magnesium stearate: 0.5%

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group. Each group received respective formulation orally as follows:

- Group I: Carrageenan (Control)
- Group II: Diacerein (Standard)
- Group III: Formulation (Diacerein 50 mg Immediate Release Tablet)

After 30 min, 0.1 mL of 1% w/v carrageenan was injected in the plantar region of the left paw of standard group as well as all the groups. The right paw served as a reference non inflamed paw for comparison. Paw volume of both the legs was measured using plethysmometer at 1 h, 2 h, 3 h, 4 h, and 6 h after carrageenan challenge. Percent edema inhibition by the test substance was determined as follows:

$$\% \text{ Inhibition of inflammation} = (\text{Std–Test/Test}) \times 100 \quad (1)$$

Anti-ulcer activity (Ethanol-induced ulceration)

The antiulcer activity of the formulations was evaluated by testing the formulations for their therapeutic uclerogenic index in Wistar rat. Animals (Wister rat) were divided into three groups comprising six animals in each group. Each group received respective formulation orally as follows:

- Group I: Ethanol (Control)
- Group II: Diacerein (Standard)
- Group III: Formulation (Diacerein 50 mg Immediate Release Tablet)

Control group received 0.5% ethanol while, other groups received the respective formulation orally. Animals were sacrificed 7 h after the treatment. The stomach was removed, opened along the greater curvature, washed with saline, and observed for hemorrhagic lesions, produced in dissection microscope.

In vivo pharmacokinetic study

Selection of animals and housing

Albino rats of either sex weighing 175–200 g were used for pharmacokinetic study. Animals were housed under standard environmental conditions of temperature (24±1°C) and relative humidity of 30–70%. A 12:12 h light dark cycle was followed. All animals were free access to water and standard pelleted laboratory animal diet. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee constituted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA), Government of India.

Collection and separation of blood plasma

Blood was collected from retro-orbital plexus of Albino rats. After the centrifugation (at 10000 rpm, 4° C, for 10 min), clear supernatant plasma was separated from blood. Samples were kept at −20°C till further analysis. This plasma used for development and validation of HPLC method.

Study design

Animals were divided into two groups comprising six animals in each group (n = 6). The two groups were treated orally with pure drug and prepared formulation (both dispersed in Tween-80), respectively, before the commencement of study. Pure drug and formulation were administered orally to the male Albino rats (n = 6) weighing between 175 and 200 g. The animals were housed in a temperature controlled environment prior the experiment. Blood samples were drawn from retro-orbital plexus of Albino rats as per scheduled time intervals, namely, 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, and 12 h post dose into evacuated heparinized glass tubes. Blood samples were centrifuged at 4500 rpm for 10 min at 4°C; plasma was transferred directly into 5 mL plastic tubes and stored frozen at −20°C till drug analysis. Samples from each group (n = 6) were processed according to the procedure described earlier and analyzed using the validated HPLC method.

RESULTS AND DISCUSSION

Drug Content

Drug content in solid dispersions of diacerein and polymers (PEG-6000, guar gum, and ploxamer-188) was found between 98.90±0.015 and 100.50±0.454% w/w (Limit: 98–101% w/w).

Fourier-Transform Infrared Spectroscopy (FT-IR)

In FT-IR studies, the characteristic peak of diacerein at 3340.82 cm^{-1} (O-H stretch, aromatic), 1103.32 cm^{-1} (ester shows characteristic -C-O-stretching bond), 1766.85 cm^{-1} (C=O stretch, ester), and 1681.98 cm^{-1} (C=O, stretch, COOH) shows shifting of frequencies in spray dried formulations of diacerein with PEG-6000, guar gum, and poloxamer-188. Furthermore, all these formulations show no appearance of new peaks indicating absence of chemical interaction between drug and these excipient [Figure 1].

DSC

DSC thermogram of diacerein [Figure 2a] shows a sharp endothermic peak at 261°C ascribed to melting of crystalline diacerein, with enthalpy of −304.57 mJ, peak height 19.28m/W, onset temperature at 259°C, and end set temperature at 252.94°C. DSC thermogram of diacerein, PEG-6000 and Aerosil spray dried formulation F-9 [Figure 2b] shows a sharp melting endothermic peak at 57.28°C. DSC

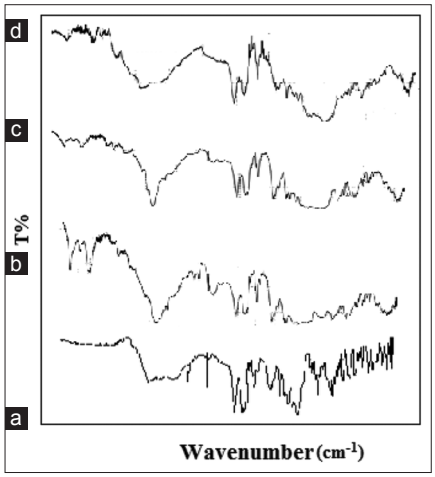

Figure 1: FTIR spectra of (a) Diacerein and its spray dried formulation (b) F-9, (c) F-18,F-(d)F-27

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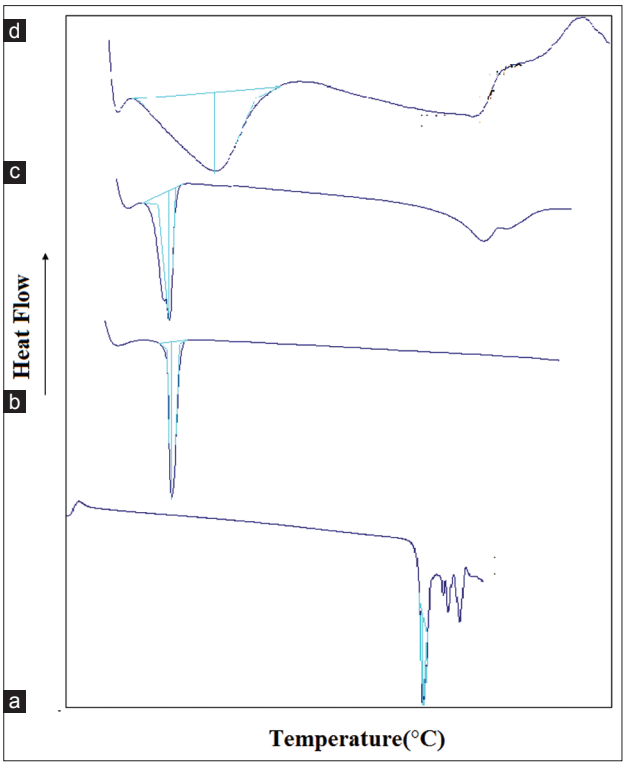

Figure 2: DSC spectra of (a) Diacerein and its spray dried formulation (b) F-9, (c) F-18,F-(d)F-27

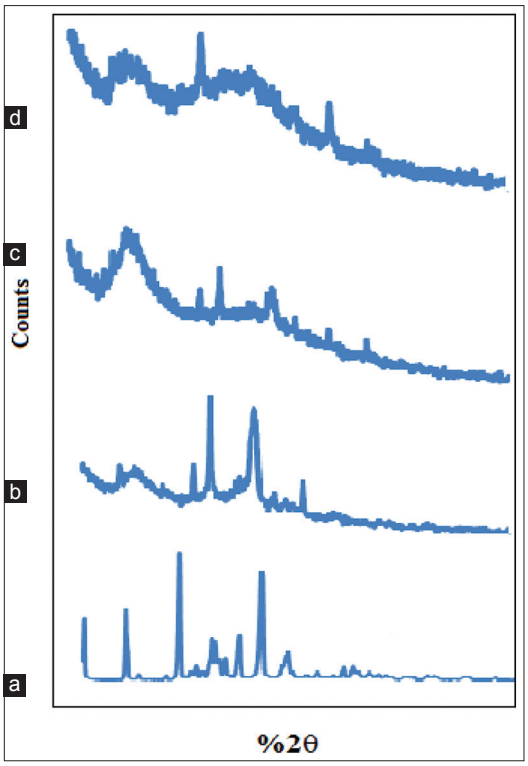

Figure 3: XRD spectra of Diacerein (a) and its spray dried formulation (b) F-9, (c) F-18,F-(d)F-27

thermogram of diacerein, poloxamer-188 and Aerosil spray dried formulation F-18 [Figure 2c] indicates a sharp melting endothermic peak at 51.03°C corresponding with melting of poloxamer-188. DSC thermogram of diacerein, guar gum and Aerosil spray dried formulation F-27 [Figure 2d] shows a broad melting endothermic peak at 81.67°C, indicates presence of polymer in spray dried formulation. In all spray dried formulations complete disappearance of endothermic peak of diacerein at 261°C may be due to dissolution of drug in solution of carrier and formation of amorphous solid dispersion and indicates morphological conversion diacerein from crystalline to amorphous form.

XRPD

The XRD spectra of diacerein and its spray dried formulations are shown in Figure 3. XRD spectra of diacerein [Figure 3a], having major characteristic high intensity peaks at diffraction angle of 2θ at 5.1989°, 10.3698°, 17.3307°, 24.9876°, and 27.772° with peak intensity 24599, 27960, 48897, 18597, and 41543, respectively, indicating crystalline nature of diacerein. XRD pattern of diacerein, PEG-6000, Aerosil by spray drying method formulation F-9 [Figure 3b], characterized by peaks at diffraction angle of 2θ at 17.52°, 19.18°, 23.28°, and 27.98° with peak intensity 632.3, 1294.5, 1036.5, and 439, respectively. XRD pattern of diacerein poloxamer-188, Aerosil by spray drying method formulation F-18 [Figure 3c], characterized by peaks at diffraction angle of 2θ at 17.54°, 19.16°, 23.3°, 27.94°, and 30.94°, with peak intensity 464.3, 547, 468.8, 292, and 257, respectively. XRD pattern of diacerein, guar gum, Aerosil by spray drying method formulation F-27 [Figure 3d], characterized by peaks at diffraction angle of 2θ at 17.52°, 21.6°, and 28° with peak intensity 573.8, 479.9, and 364.4, respectively. Spray drying technique found to be more significantly useful in reduction of crystalline characteristics of diacerein. Spray dried composition showed no sharp peaks, indicating its amorphous nature, that is, conversion of crystalline diacerein in to amorphous form. Also no new peaks are observed, indicates absence of interaction between drug and the carrier. Polymorphic transformation of diacerein from crystalline to amorphous form leads to improvement in dissolution rate due to higher internal energy as compare to pure diacerein.

SEM

Figure 4 shows SEM photograph of diacerein and its spray dried formulations. Diacerein consists of some large irregular crystals with fine particles. Spray dried products showed marked loss of crystalline and irregular shape with a change in form, from crystalline to amorphous nature and appeared as irregular particles in which the regular morphological features of both the drug and polymer disappeared and tiny aggregates of amorphous pieces of irregular size were present. Modification in the shape of drug particles is indicative of new solid state. Therefore, reduced particle size, increased surface area and close contact between hydrophilic carrier and drug might responsible for the improved dissolution rate. Thus change in morphology of spray dried formulation compared to pure drug showed interaction between drug and polymers.

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In Vitro Drug Release Study of Solid Dispersion Formulations

Diacerein shows 11.834±0.647% drug release at 45 min. The slowest dissolution rate of diacerein is due to its hydrophobicity that leads to floating of powder on the surface of dissolution medium and prevents its surface contacting the medium. It clears that drug having poor dissolution and needs to further dissolution enhancement. Solid dispersions of diacerein with PEG-6000 and Aerosil (F-9) show improvement in dissolution profile of drug as compared to pure drug. Formulation containing highest concentration of polymers (1:3:1.5) shows 89.110±0.460% drug release at 45 min [Table 5]. A PEG-6000 lead to improvement is dissolution of diacerein. Since PEG-6000 dissolve more of the drug leading to a greater percentage drug in the molecularly dispersed form and that the higher viscosity of the PEG-6000 hindered precipitation of the drug following dissolution of carrier. Also in this molecular weight (6000) the water solubility of PEG-6000 is still very high but hygroscopy is not problem. If low molecular weight PEG used leads to formation of sticky product. Drug release of diacerein, poloxamer-188 and Aerosil solid dispersion prepared by spray during method are shown in Table 6. It was found that spray drying is more effective method for improvement in drug dissolution, best releases shown by diacerein, poloxamer-188 and Aerosil, F-18 (1:3:1.5 ratio) spray dried solid dispersion, that is, 96.110±0.647 % at 45 min. Poloxamer-188 shows better drug release as compare to other polymers, that is, PEG-6000 and guar gum. It exists in solution as unimers but self-assemble into micelles. At concentration above CMC, a hydrophilic propylene oxide core can incorporate water insoluble molecules resulting in increased solubility of the drug molecules. In addition, greater hydrophilicities and surfactant properties of poloxamer result in reduction interfacial tension between drug and dissolution medium leading to increased wettability of drug, dispersibility and reduced particle size of the drug might contribute to dissolution diacerein. In diacerein, guar gum and Aerosil spray dried solid dispersions, formulation containing highest concentration of polymers F-27 (1:3:1.5) shows 93.210±0.818% drug release at 45 min [Table 7]. Guar gum is used in solid dosage form as binder and disintegrant. However, due to swelling ability of the carrier profound influence on the improvement in dissolution of poorly water soluble drugs. Hydrophilic nature of the guar gum leads to change in hydrodynamic microenvironment around the particles. At time of dissolution, drug and carrier from mixtures comes in contact with dissolution fluid, passing of dissolution medium into drug carrier particles takes place, which initiates the formation of stagnant gel layer of carrier around the particles. The drug particles that are separate entities but disperse rapidly throughout dissolution medium and expose a greater surface area, resulting in rapid drug release.

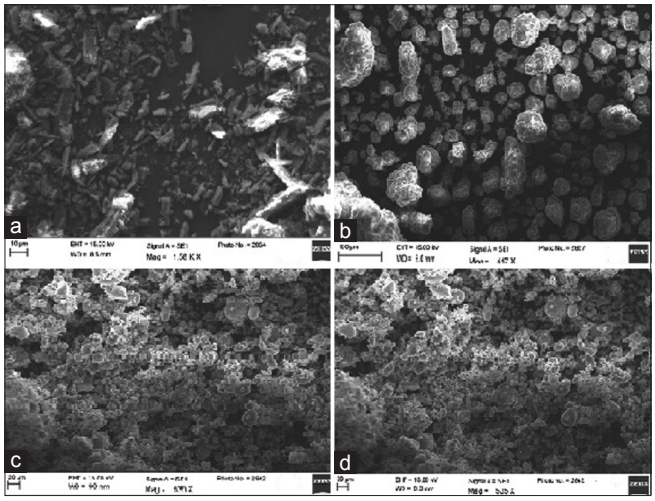

Figure 4: SEM photograph of (a) Diacerein its spray dried formulation (b) F-9, (c) F-18,F-(d) F-27

Spray drying has been found to faster release of drug due to reduced aggregation tendencies of particles and it leads to improved wetting and thereby increased dissolution rate. Another reason is conversion of crystalline drug into amorphous state, which enhances drug release because no energy is required to break up the crystal lattice during dissolution process.

Spray Dried Solid Dispersion 3^2 Factorial Design

All formulations were subjected for dissolution studies to find out optimized batch from every design for each hydrophilic

Table 5: The predicted and observed response variables for spray dried solid dispersions of Diacerein + PEG-6000 + Aerosil

Batch code	Diacerein (g)	PEG -6000 (g)	Aerosil (g)	%Drug release		
				Predicted value	Observed value	Prediction error (%)
F-1	1	1	0.5	81.28	80.99	−0.36
F-2	1	2	0.5	83.89	83.19	−0.84
F-3	1	3	0.5	86.50	86.99	0.56
F-4	1	1	1	83.16	82.68	−0.58
F-5	1	2	1	85.77	86.90	1.30
F-6	1	3	1	88.38	88.73	0.39
F-7	1	1	1.5	85.03	85.50	0.54
F-8	1	2	1.5	87.64	87.81	0.19
F-9	1	3	1.5	90.25	89.11	−1.26

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Table 6: The predicted and observed response variables for spray dried solid dispersions of Diacerein + Poloxamer-188 + Aerosil

Batch code	Diacerein (g)	Poloxamer-188 (g)	Aerosil (g)	% Drug release		
				Predicted value	Observed value	Prediction error (%)
F-10	1	1	0.5	90.53	90.99	0.50
F-11	1	2	0.5	91.78	91.90	0.13
F-12	1	3	0.5	93.02	92.25	−0.83
F-13	1	1	1	91.70	91.89	0.20
F-14	1	2	1	92.94	92.51	−0.46
F-15	1	3	1	94.19	94.81	0.65
F-16	1	1	1.5	92.86	92.83	−0.03
F-17	1	2	1.5	94.11	93.19	−0.98
F-18	1	3	1.5	95.35	96.11	0.79

Table 7: The predicted and observed response variables for spray dried solid dispersions of diacerein + guar gum + Aerosil

Batch code	Diacerein (g)	Guar Gum (g)	Aerosil (g)	% Drug release		
				Predicted value	Observed value	Prediction error (%)
F-19	1	1	0.5	80.64	80.13	−0.63
F-20	1	2	0.5	84.13	85.03	1.05
F-21	1	3	0.5	87.62	87.98	0.40
F-22	1	1	1	83.49	83.16	−0.39
F-23	1	2	1	86.89	86.20	−0.80
F-24	1	3	1	90.47	90.10	−0.41
F-25	1	1	1.5	86.34	87.05	0.81
F-26	1	2	1.5	89.83	89.99	0.17
F-27	1	3	1.5	93.33	93.21	−0.12

polymers and each method. It was found that the batches containing highest concentration of polymer and Aerosil showed better drug release i.e. formulations F-9, F-18, and F-27 are considered as optimized batches and out of these solid dispersion formulation F-18 containing Diacerein, poloxamer-188, and Aerosil shows better release than others and used for further studies and characterization. The results are shown in Tables 5-7.

Fitting of data to the model

The two factors with lower, middle and upper levels are shown in Tables 5-7. All responses observed for nine formulations of design were fitted into models using design expert 9 software along with regression equation generated for each batch.

The results of ANOVA shown in Table 8 for the dependent variable demonstrate that model is significant for the response variable.

For solid dispersion of Diacerein + PEG-6000 + Aerosil, Diacerein + Poloxamer-188 + Aerosil, Diacerein + Guar Gum + Aerosil, the model F-values are 46.30, 18.34, and 140.94, respectively, implies that model is significant and there is only a 0.02%, 0.28%, and 0.01% chance that an F-value this large could occur due to noise, respectively. It is observed that dependent variables X_1, (Concentration of hydrophilic polymer) and X_2 (Concentration of Aerosil) have positive effect on response Y (i.e. percent drug release).

Table 8: Results of analysis of variance of solid dispersions

	DF	SS	MS	F	Significance p
PEG-6000					
Model	2	62.00	31.00	46.30	<0.0002 Significant
Residual	6	4.02	0.67	-	-
Total	8	66.02	31.67	-	-
PXM-188					
Model	2	17.42	8.71	18.34	<0.0028 Significant
Residual	6	2.85	0.47	-	-
Total	8	20.27	9.18	-	-
Guar Gum					
Model	2	121.94	60.97	140.94	<0.0001 Significant
Residual	6	2.60	0.43	-	-
Total	8	124.54	61.4	-	-

DF: Degrees of freedom, SS: Sum of Square, MS: Mean Sum of Square, F: Fischer's ratio

Regression equation of fitted linear model

For

PEG-6000:

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$$Y = 76.7922+2.61000X_1+3.75333X_2 \quad (2)$$

PXM-188:

$$Y = 88.12556+1.24333\ X_1+2.33000\ X_2 \quad (3)$$

Guar Gum:

$$Y = 74.29667+3.49167\ X_1+5.70333\ X_2 \quad (4)$$

The coefficient with more than one factor term in the regression equation represents interaction terms. It also shows that the relationship between factors and responses is not always linear. When more than one factor are changed simultaneously and used at different levels in the formulation, a factor can produce different degrees of responses. The interaction effect of X_1 and X_2 are favorable (+ve) for response Y.

Response surface plot analysis

Contour plot and three dimensional plots generated by Design Expert 9, software are shown in Figure 5, for studied response, that is, percent drug release.

Figure 5a and b shows response surface plot of PEG-6000 concentration (X_1) and Aerosil concentration (X_2) on percent drug release. This shows that X_1 and X_2 shows linear effect i.e. when increased from low to high concentration.

Figure 5c and d shows response surface plot of poloxamer-188 concentration (X_1) and Aerosil concentration (X_2) on percent drug release. This shows that X_1 and X_2 show linear effect, that is, when increased from low to high concentration.

Figure 5e and f shows response surface plot of Guar gum concentration (X_1) and Aerosil concentration(X_2) on percent drug release. This shows that X_1 and X_2 show linear effect, that is, when increased from low to high concentration.

The percent drug release increased due increase in surface area for drug adsorption during spray drying.

Evaluation of Diacerein 50 mg Immediate Release Tablet

Precompression parameters

Powder blend of all formulations was evaluated for angle of repose, bulk density, tap density, Carr's index and found to be in the range of 25.12±0.020–29.03±0.739, 0.458±0.008–0.505±0.009, 0.542±0.009–0.600±0.025, and 15.41–15.84%, respectively. The results of angle of repose <30° indicate good flowability of powder blend. This is supported by lower compressibility index value up to 15.84% indicated good flow properties.[21]

Post-compression parameters (Evaluation of Diacerein 50 mg immediate release tablet)

Average weight, thickness, hardness, % friability, disintegration time, and % drug content

The Diacerein 50 mg tablet formulations were evaluated for uniformity of weight, thickness, hardness, friability, disintegration time, and drug content (assay). In weight variation test, the average weight of all formulations found between 350.4±0.80 mg and 352.2±0.95 mg and percent weight deviation of all tablet formulations was found within limits (IP Limits: Percent deviation in average weight of 20 tablets not more than 5%) and hence all formulations pass the test for uniformity of weight as per pharmacopoeial specifications. Thickness, Hardness was found to be in the range of 4.23±0.0051–4.28±0.0075 mm and 6.2±0.063–8.4±0.057 kp, respectively. Friability of all formulations was NMT 1%, which is within limits, that is, a maximum loss of weight not more than 1% and disintegration time was found to be 155, 166, 180, 143, 140, 171, 130, 144, and 159 s for formulations A, B,

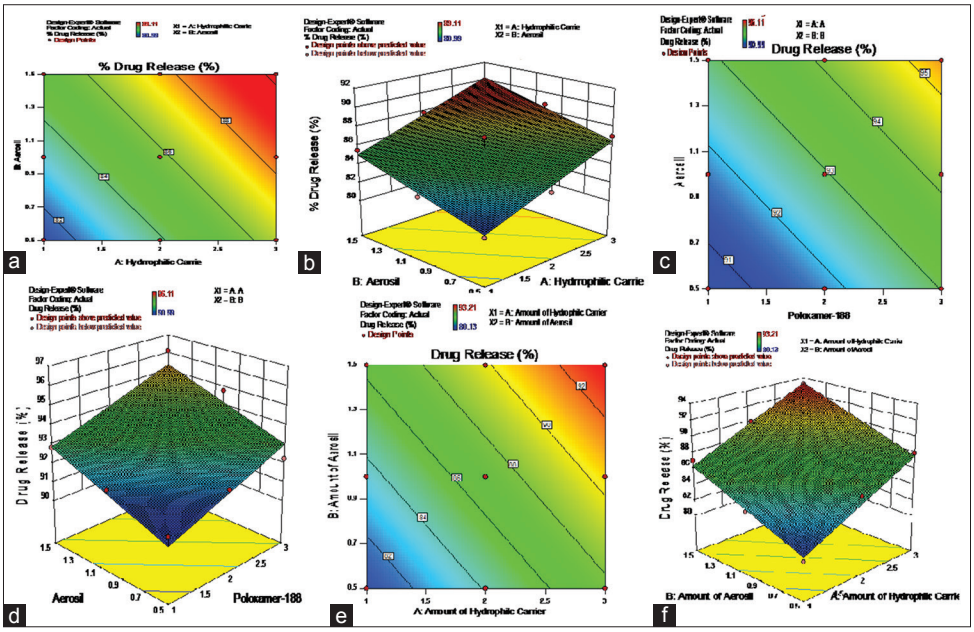

Figure 5: (a) Contour plot of formulation F-9, (b) 3D surface plot of formulation F-9, (c) contour plot of formulation F-18, (d) 3D surface plot formulation of F-18, (e) contour plot of formulation F-27, (f) 3 D surface plot of formulation F-27

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Table 9: *In vitro* drug release of Diacerein 50 mg immediate release tablet (formulations A to I)

Time (min)	Formulations/Drug Release (%)								
	A	B	C	D	E	F	G	H	I
5	16.127±0.569	10.530±0.842	6.025±0.643	20.210±0.870	17.556±0.859	11.930±0.760	**25.175±0.449**	19.520±0.577	15.320±0.830
10	55.185±0.638	50.060±0.481	47.985±0.423	57.580±0.532	55.737±0.774	51.775±0.871	**62.462±0.670**	58.645±0.660	54.540±0.304
15	74.150±0.732	70.200±0.681	65.376±0.429	76.535±0.783	72.531±0.507	70.625±0.512	**78.908±0.633**	75.552±0.562	73.595±0.523
30	82.166±0.649	79.135±0.431	75.796±0.478	85.967±0.736	83.595±0.619	79.090±0.459	**90.075±0.805**	86.401±0.332	84.760±0.608
45	92.303±0.830	90.485±0.497	90.255±0.894	93.875±0.372	92.472±0.668	90.864±0.410	**95.195±0.580**	93.690±0.878	93.115±0.503

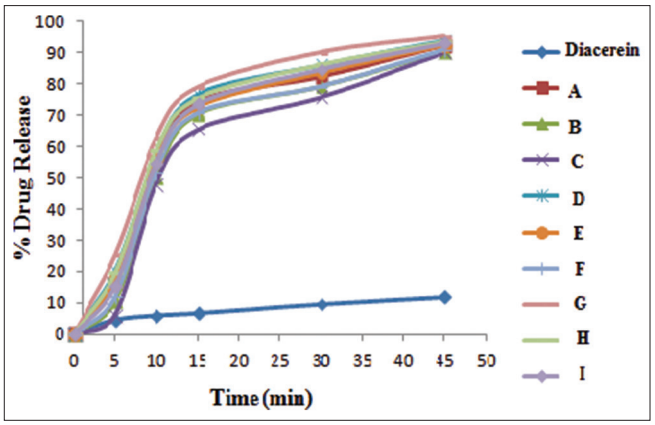

Figure 6: *In vitro* drug release of Diacerein 50 mg immediate release tablet formulations A to I and Diacerein API

C, D, E, F, G, H, and I, respectively. The results of disintegration test revealed that formulation G has faster disintegration and it disintegrate within 130 s, it might be due to higher concentration of disintegrant. Drug content of all formulations was found between 99.44±0.060 and 99.62 ± 0.050% w/w (complies with the limits, i.e., 98–101% w/w). From the physiochemical parameters, it is clear that all formulations fulfilled the official requirements of immediate release tablets.

In vitro drug release of Diacerein 50 mg immediate release tablets

From all formulations of diacerein immediate release tablet [Table 9 and Figure 6], formulation G has shown highest drug release i.e. 95.195±0.580% as compare to other formulations, which may be due to faster disintegration, that is, 130 s (higher concentration of disintegrant and lower concentration of binder in formulation, facilate the breakup of tablet and faster drug release).

Factorial design (3^2) for Diacerein 50 mg immediate release tablets

The two factors and responses with lower, middle, and upper levels are shown in Table 10, All responses observed for nine formulations of design were fitted into models design expert 9 software along with regression equation generated for each batch.

Factorial equations

For disintegration time

By fitting Quadratic model to disintegration time, regression equation 5, for response variable determined by multiple regression analysis is:

$$\text{Disintegration time: } Y = 142.44+28.66X_1-1.33X_2+0.25X_1X_2-0.66X_1^2-0.54X_2^2 \quad (5)$$

In above equation coefficient X_1 bear positive sign and coefficient X_2 bear negative sign. This shows that as the concentration of binder increases it is expected to increase in disintegration time and increasing concentration of superdisintegrant is expected to decrease disintegration time. It is observed that increase in concentration of binder leads to increase in disintegration time may be due to binding effect between molecules leads to slow down the water uptake by tablets and disintegrant do not get sufficient water to swell and facility breakup of tablet.

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Table 10: Concentration of independent variables and response factors of Diacerein 50 mg immediate release tablets

Batch Code	Factors		Response	
	Binder (%) X_1	Disintegrant (%) X_2	Disintegration Time (Sec.)	% Drug Release at 30 min
A	0.5	2	152	82.166
B	1.0	2	166	79.135
C	1.5	2	180	75.796
D	0.5	4	143	85.967
E	1.0	4	158	83.595
F	1.5	4	171	79.090
G	0.5	6	130	90.075
H	1.0	6	144	86.401
I	1.5	6	159	84.760

Table 11: Results of analysis of variance for response surface quadratic model of Diacerein 50 mg immediate release tablets

Source of variance	Sum of squares (SS)	Degree of freedom (DF)	Mean square (MS)	F-value	*P*-value
Model	1918.03	5	383.61	1336.43	<0.0001
A (Amount of Binder %)	1204.17	1	1204.17	4195.16	<0.0001
B (Amount of Disintegrant %)	704.17	1	704.17	2453.23	<0.0001
AB	0.25	1	0.25	0.87	0.4195
A^2	0.056	1	0.056	0.19	0.6897
B^2	9.39	1	9.39	32.71	0.0106
Residual	0.86	3	-	-	-
Cor. Total	1918.89	8	-	-	-

Table 12: Results of analysis of variance for response surface linear model of Diacerein 50 mg immediate release tablets

Source of variance	Sum of squares (SS)	Degree of freedom (DF)	Mean square (MS)	F-value	*P*-value
Model	154.54	2	77.27	214.96	<0.0001
A (Amount of Binder %)	57.42	1	57.42	159.75	<0.0001
B (Amount of Disintegrant %)	97.12	1	97.12	270.17	<0.0001
Residual	2.16	6	0.36	-	-
Cor. Total	156.70	8	-	-	-

For drug release at 30 min

By fitting linear model to drug release at 30 min, regression equation 6, for response variable determined by multiple regression analysis is:

Drug release at 30 min: $Q_{30} = 81.13\text{-}6.18X_1 + 2.01X_2$ (6)

The results of multiple regression analysis shows that both coefficient X_1 and X_2 posses opposite signs, indicates that increase in concentration of disintegrant leads to increase in dissolution time due to faster disintegration of tablet and negative sign of coefficient X_1 suggest that increase in concentration of binder leads to increase in disintegration time and slows down the drug release.

Fitting of data to the model

The results of ANOVA are shown in Tables 11 and 12 for the dependent variable demonstrate that model was significant for the response variable.

For disintegration time (ANOVA for response surface quadratic model)

Results of ANOVA analysis using design expert 9 are shown in Table 11 indicates that quadratic model is significant ($P < 0.0001$) with good regression value $R^2 = 0.9996$. The value ($R^2 = 0.9996$ acceptable) indicates that there are effects on responses and p-value suggests the terms of significance.

For drug release at 30 min, Q_{30} (ANOVA for response surface linear model)

Results of ANOVA analysis using design expert is shown in Table 12 indicates that surface linear model is significant ($P < 0.0001$) with good regression value $R^2 = 0.9862$. The value ($R^2 = 0.9862$ acceptable) indicates that there are effects on responses and *P*-value suggests the terms of significance. The results of *P*-values of A (Binder) and B (Disintegrant) are significant, that is, <0.001. Above result indicate that both the

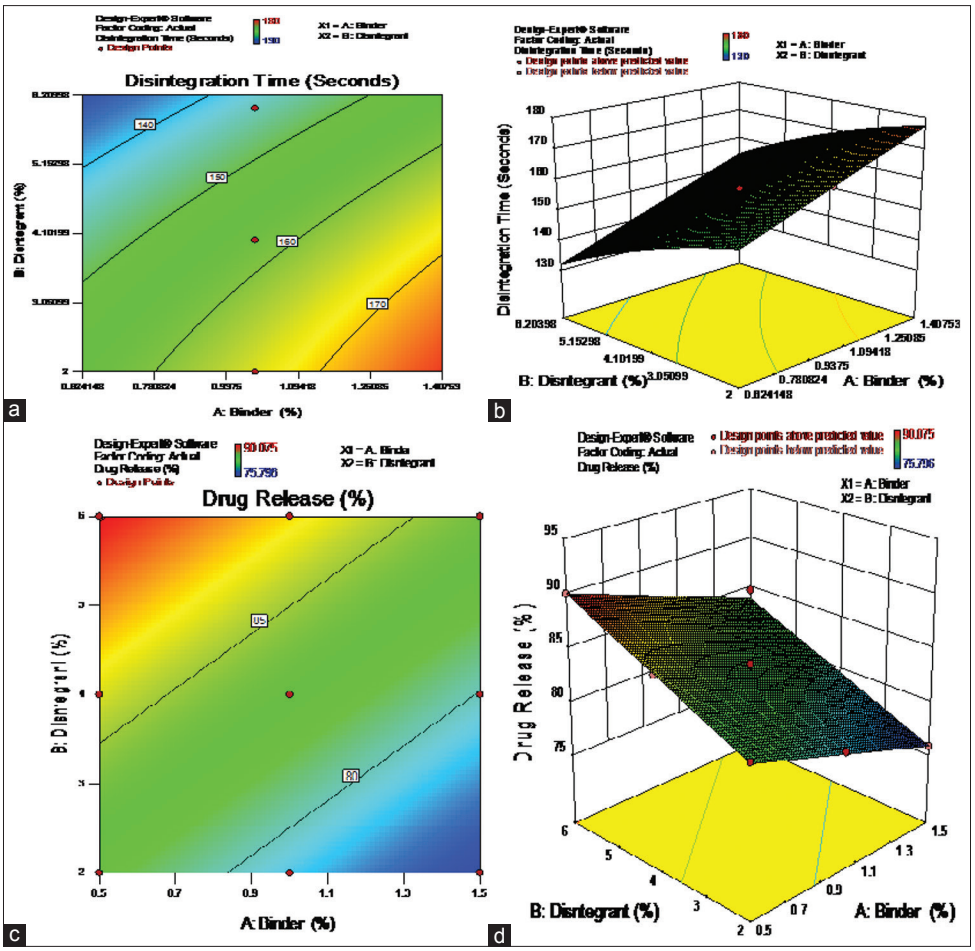

Figure 7: (a) contour plot of disintegration time, (b) 3D Surface plot of disintegration time, (c) contour plot of drug release at 30 min, (d) 3D surface plot of drug release at 30 min for Diacerein 50 mg immediate release tablets

factors play an important role in the formulation of Diacerein tablet.

Three dimensional and contour plots for measured responses formed based on the model to assess changes of response surface. Furthermore, relationship between dependent and independent variables can further understood by these plots. From graphs, it is found that concentration of both factors having impact on disintegration and drug release of diacerein tablets [Figure 7].

In Vivo Pharmacodynamic and Pharmacokinetic Study

In vivo pharmacodynamic study

Analgesic activity (acetic acid induced writhing method)

Formulated Diacerein tablet treated groups shows significant decrease in number of writhes. Formulation treated group showed better analgesic activity in terms of decrease in number writhes induced by acetic acid than Diacerein (std) [Table 13].

Anti-inflammatory activity (Carrageenan induced rat-paw edema)

Both, that is, standard (Diacerein) and formulation (diacerein tablet) treated groups show significant benefits in terms of decrease in paw edema when compared against control group at all-time intervals. Interestingly, formulation diacerein tablet treated group shows benefits better than control and standard treated groups at all-time intervals [Table 14].

Table 13: Analgesic activity (acetic acid induced writhing method) of Diacerein 50 mg immediate release tablet

S. No.	Treatment (15 mg/kg)	Number of writhes
1.	Control (Acetic acid)	34.5±3.69
2.	Standard (Diacerein)	26.75±1.89
3.	Formulation Diacerein Tablet	18.75±3.40

Anti-ulcer activity

The antiulcer activity of the formulations was evaluated by testing the formulations for their therapeutic uclerogenic index in Wistar rat.

Comparison against ethanol induced ulcer group (control) showed significant decrease in volume of content in stomach in both standard (diacerein) and formulated diacerein tablet treated groups. Furthermore, the decrease in volume of content was more with formulation diacerein tablet treated group when compared to standard diacerein treated group [Table 15].

Comparison against ethanol induced ulcer group (control) showed significant increase in pH of content in stomach in both standard (diacerein) and formulated diacerein tablet

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Table 14: Anti-inflammatory activity (Carrageenan induced rat-paw edema) of Diacerein 50 mg immediate release tablet

S. No.	Treatment (15 mg/kg)	% increase in paw thickness				
		0 h	1 h	2 h	3 h	4 h
1.	Control (Carrageenan)	0	21.13±1.13	41.02±2.26	60.12±3.24	68.91±3.13
2.	Standard (Diacerein)	0	14.39±1.17**	26.25±1.06**	31.21±1.19**	40.83±2.21**
3.	Formulation Diacerein Tablet	0	08.27±1.06***,##	12.33±1.14***,##	18.49±1.22***,###	22.53±1.12***,###

Each value is expressed as Mean±S.E.M., n=6; Significant difference is denoted by **P<0.01, ***P<0.001 as compared against the control group. ##P<0.01 - as compared against the standard group. Groups were compared using One-way ANOVA followed by Tukey's honest test

Table 15: Anti-ulcer activity (ethanol-induced ulceration) of Diacerein 50 mg immediate release tablet

S. No.	Treatment (15 mg/kg)	Volume of content in stomach (mL)	pH	Ulcer index
1.	Control (Ethanol)	2.49±0.21	2.4±0.24	**8.25±1.1**
2.	Standard (Diacerein)	1.46±0.23	5.2±0.37	**4.18±0.24**
3.	Formulation Diacerein Tablet	1.23±0.15	5.6±0.4	**2.29±0.15**

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Table 16: Diacerein concentration in plasma at different time interval for pure drug

Time (h)	Concentration (mcg/mL)						Avg
	1	2	3	4	5	6	
0	0	0	0	0	0	0	0
0.5	1.245	1.225	1.170	1.275	1.135	1.135	1.198±0.059
1	2.008	2.148	2.030	1.983	2.150	2.150	2.078±0.079
2	2.649	2.626	2.566	2.680	2.611	2.671	2.634±0.042
3	1.270	1.259	1.278	1.281	1.282	1.312	1.280±0.017
4	0.854	0.818	0.846	0.805	0.873	0.873	0.845±0.149
5	0.596	0.649	0.643	0.629	0.660	0.660	0.640±0.024
6	0.510	0.480	0.450	0.475	0.490	0.490	0.483±0.019
8	0.338	0.368	0.382	0.374	0.403	0.403	0.378±0.024
10	0.235	0.263	0.265	0.255	0.283	0.283	0.264±0.018
12	0.175	0.122	0.180	0.188	0.175	0.173	0.169±0.023

Table 17: Diacerein concentration in plasma at different time interval for Diacerein 50 mg immediate release formulation

Time(h)	Concentration (mcg/mL)						Avg
	1	2	3	4	5	6	
0	0	0	0	0	0	0	0
0.5	2.075	2.042	1.950	2.125	1.892	1.892	1.996±0.098
1	4.724	5.053	4.776	4.665	5.059	5.059	4.889±0.187
2	3.197	3.169	3.097	3.234	3.152	3.224	3.179±0.050
3	2.117	2.098	2.130	2.135	2.137	2.187	2.134±0.029
4	1.367	1.309	1.354	1.288	1.396	1.396	1.352±0.044
5	0.690	0.649	0.643	0.629	0.660	0.660	0.655±0.020
6	0.535	0.503	0.471	0.498	0.513	0.513	0.505±0.021
8	0.423	0.460	0.478	0.467	0.504	0.504	0.472±0.030
10	0.302	0.339	0.340	0.328	0.364	0.364	0.340±0.023
12	0.217	0.203	0.222	0.233	0.217	0.215	0.218±0.009

treated groups. Furthermore, the increase in pH of content was more with formulation diacerein tablet treated group when compared to standard diacerein treated group [Table 15].

Comparison against ethanol induced ulcer group (control) showed significant decrease in ulcer index in both standard (diacerein) and formulated diacerein tablet treated groups.

Table 18: Pharmacokinetic parameters of Diacerein and its formulation following the oral administration

Formulations	T_{max} (h)	C_{max} (µg/mL)	AUC_t (µg.h/mL)	K_{el} (h^{-1})	$t_{½}$ (h)
Diacerein (Pure Drug)	2	2.634	10.586	0.201	3.44
Diacerein 50 mg immediate release tablet	1	4.889	15.735	0.193	3.58

Furthermore, the decrease in ulcer index was more with formulation diacerein tablet treated group when compared to standard diacerein treated group [Table 15].

In vivo pharmacokinetic study

Pharmacokinetic evaluation of diacerein and its immediate release tablet formulation [Tables 16-18] was carried out in albino rats for 12 h. From the data [Table 18] of plasma concentration, the maximum plasma concentration was found to be 2.634 μg/mL (Cmax) and the corresponding time for the maximum plasma concentration (t_{max}) was 2 h for pure drug suspension. While for immediate release tablet the C_{max} was found to be 4.889 μg/mL with t_{max} of 1 h. AUC_t (extent of absorption) of the pure diacerein was found to be 10.586 μg.h/mL and for formulation 15.735 μg.h/mL, which revealed greater absorption of diacerein immediate release tablet than pure drug. Furthermore, the elimination rate constant (K_{el}) for diacerein was found to be 0.201 h^{-1} and for diacerein tablet it was 0.193 h^{-1}. As compare to pure drug there was decrease in elimination rate constant (K_{el}) of diacerein immediate release tablet indicates that drug was available longer time for absorption. From all pharmacokinetic parameters it can be concluded that diacerein 50 mg immediate release formulation (containing solid dispersion) showed an enhanced absorption and higher bioavailability as compare to pure diacerein.

CONCLUSION

Solid dispersion of diacerein prepared with PEG-6000, poloxamer-188, guar gum, and Aerosil by spray drying technique showed physiochemical modifications, that is, to amorphism of diacerein. Spray dried solid dispersions leads to improvement in dissolution of diacerein as compare to pure drug. Diacerein immediate release tablets were formulated using solid dispersion by 3^2 factorial design to study effect of binder (PVPK-30) and disintegrant (Crospovidone) concentration on disintegration and dissolution of diacerein. Factorial equations show that increase in concentration of binder (PVPK-30) increase disintegration time and slows down drug release. Also increase in concentration of disintegrant (Crospovidone) decrease disintegration time and increase drug release. ANOVA study shows that both factors plays important role in formulation of diacerein immediate release tablet and are significant. Response surface plot and contour plot show that concentration of both factors having impact on disintegration and drug release of diacerein. Formulations showed better analgesic activity in terms of decrease in number writhes induced by acetic acid than pure diacerein. Anti-inflammatory response of the formulations was studied using carrageenan induced rat-paw edema. Diacerein tablet formulations showed decrease in paw edema when compared against control and standard group at all-time intervals. Pharmacokinetic evaluations of diacerein tablet formulations were carried out in rats showed an increase in Cmax, AUC, $t_{½}$ values indicating enhanced absorption and higher bioavailability as compare to pure drugs. From the results, it is concluded that spray dried solid dispersions of diacerein alone and when used in tablet formulations leads to an improvement of drug solubility, dissolution rate, and bioavailability.

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