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Preparation and characterization of Diacerein microcrystals

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ABSTRACT

Diacerin (DCN) is a novel nonsteroidal anti-inflammatory drug having pharmacological properties different than classical NSAIDs. It exhibits poor aqueous solubility and slower dissolution rate. These properties were modified by novel microcrystallization technique in an attempt to enhance dissolution behaviour. Acetone and water were used as solvent and antisolvent system respectively while Poly-vinyl pyrollidone (PVPK30), Poly-ethyleneglycol 6000 (PEG 6000) and PXMR 188 (PXMR 188) were used as polymers in crystallization process. The microcrystals were characterized by XRPD (X-ray Powder Diffractometry), SEM (Scanning Electron Microscopy), FTIR (Fourier Transformation Infra-red Spectroscopy) and dissolution test. It was found that dissolution characteristics of microcrystals were significantly improved than that of pure diacerein. Also XRPD, FTIR reflected altered molecular arrangement in their structure. Thus microcrystals could be a suitable technique to improve physicochemical characteristics of diacerein.

Keywords: Diacerin, Poly-vinyl pyrollidone, Poly-ethyleneglycol 6000, Poloxamer 188, microcrystals, physicochemical characteristics.

INTRODUCTION

Poor aqueous solubility of the drugs is one of the severe problems in the development of drug formulations [1, 2, 3]. Poor aqueous solubility in turn is responsible for poor dissolution of the drug. According to Noyes–Whitney equation, bioavailability depends upon dissolution of drugs [4, 5]. The bioavalability could be enhanced by improving its dissolution rate [1, 6]. Therefore improvement in dissolution has been tried by various methods like formulation of the amorphous solid form [7], inclusion complexation [8], use of surfactants [9], solid dispersion [10], spherical crystallization [11], microparticles [12], microcrystals [13] and nanoparticles [14]. Among the methods mentioned above, preparation of microcrystals is usually carried out to improve its physicochemical characters. It involves reduction in particle size in microns which could be responsible for better dissolution characteristics and thereby bioavailability.

Diacerein (DCN) is 4,5- diacetoxy-9,10-dioxo-9,10- dihydroanthracene-2-carboxylic acid (Figure 1) and mainly used for the treatment of osteoarthritis [15, 16]. DCN is the di-acetylated derivative of rhein and lacks

cyclooxygenase inhibitory activity therefore having no effect on prostaglandin synthesis [17, 18, 19]. It is a selective inhibitor of interleukin-1 having protective effect on granuloma-induced cartilage breakdown by a reduction in the concentrations of proinflammatory cytokines [20, 21]. It is slightly yellowish crystalline powder and has limited solubility. Due to this it suffers from limited dissolution problems [22] and less amount of drug reaches systemic circulation [23]. Therefore it is necessary to improve its physicochemical properties by means of novel microcrystallisation technique for better therapeutic effect.

In the present study, an attempt was made to improve physicochemical properties by preparing microcrystals of diacerein using an antisolvent precipitation technique in the presence of polymer for the enhancement of physicochemical properties. The physicochemical properties of raw DCN and DCN microcrystals were characterized in the solid state using several techniques such as Scanning electronic microscopy (SEM), Fourier transformation-infrared spectroscopy (FTIR), X-ray powder diffraction analysis (XPRD) and dissolution test.

MATERIALS AND METHODS

Material

Diacerein was obtained as gift sample from Glenmark Pharmaceuticals Ltd., Mumbai, India. PVP K30, PXMR 188 and PEG 6000 were gift sample from Indoco Remedies, Mumbai, India. All solvents were pure analytical grade purchased from Loba Chemie, Mumbai, India. Double distilled water was used throughout the experiment.

Preparation of Microcrystals

Diacerein was dissolved in 10 mL good solvent acetone and was added to a solution of PXMR188 (1 gm) in water. The same procedure was repeated using PVPK30 and PEG6000. Drug was crystallized by adding drug solution to 500 mL glass vessel containing 100 mL distilled water containing polymer. The mixture was stirred continuously using controlled speed stirrer to obtain microcrystals. The speed of agitation was kept at 1000 rpm. The crystals obtained were separated by filtrations and dried at room temperature.

Evaluation of Microcrystals

Drug Content

Microcrystals (5mg) were triturated and dissolved in 5mL dimethyl formamide (DMF) and volume was made upto 50 mL with water by sonicating for 15 minutes. The solution was filtered through Whatmann filter paper no.41. After appropriate dilutions with water it was analyzed spectrophotometrically at 258nm (Shimadzu 1800, Japan). Drug content was calculated from the calibration curve of diacerein in mixture of DMF and distilled water.

Dissolution Studies

Dissolution studies were carried out using Phosphate buffer (pH6.8) in a Type II (paddle) dissolution apparatus (Model Disso 2000 tablet dissolution test apparatus, Lab India, India) using paddle method. The stirring speed used was 50 rpm and the temperature of the dissolution medium was maintained at 37± 0.5˚C. The drug concentration in the dissolution medium was assayed spectrophotometrically at 258 nm. The results of dissolution studies were statistically analyzed.

Fourier Transformation Infrared Spectroscopy (FTIR)

FTIR spectra were obtained using a Shimadzu FTIR spectrometer (IR Affinity 1 Model, Japan) spectrometer. The samples were prepared into KBr disks. The scanning range was kept from 4000 to 500 cm^{-1}.

X-Ray Powder Diffractometry (XRPD)

The XRPD data of pure DCN and microcrystals were recorded on a Philips Analytical X-ray-PW 3710 (Phillips, Almedo, The Netherlands) diffractometer with tube anode Cr over the interval 10-70°/2Θ under following set of conditions: The generator tension (voltage): 40 kV and generator current: 25 mA.

Scanning Electron Microscopy (SEM) and Particle Size measurement

The surface morphological properties of pure drug and microcrystals were investigated by scanning electron microscopy (SEM-Jeol Instruments, JSM-6360, Japan). Samples were mounted on a double-faced adhesive tape, sputtered with gold. Scanning electron photographs were taken at an accelerating voltage of 15 kV and obtained micrographs were examined at X2000, X5000, X10000 and X15000 magnifications. The particle size measurement was carried out using compound microscope (Micron OPTIK) on stage micrometer.

RESULTS AND DISCUSSION

Determination of drug content

Percentage drug contents of prepared microcrystals were found to be in the range of 98.05 ± 0.95 w/w to 99.09 ± 1.01 w/w.

In vitro dissolution studies

The dissolution curves of diacerein alone, and microcrystals with PEG 6000, PVPK30, PXMR 188 in phosphate buffer (pH 6.8) are shown in Figure 2. Table 1 shows % drug dissolved in 5 minutes (DP_5) and 30 minutes (DP_{30}) and dissolution efficiency values (DE_5) at 5 and (DE_{30}) min for all formulations.

When DE_{30} values of all formulations were statistically analyzed, it was observed that all microcrystals have significantly improved dissolution rate in comparison to pure drug alone ($p < 0.001$). The DCN microcrystals with PXMR188 and PVPK30 gave 100% drug release at 15 min and PEG6000 microcrystals in 20 min. However, the release of pure drug was incomplete even in 90 min. In case of PVPK30, increase in dissolution might be due to reduction in contact angle, as it is adsorbed on the surface [24]. In PXMR microcrystals, greater hydrophilicity and surface property of PXMR, increased wettability and dispersibility and particle size reduction of drug might be the reasons for improvement in dissolution profile of diacerein [25]. In PEG6000, increased weight fraction of polymer might have contributed to increased solubility and ultimately dissolution [26].

Fourier Transformation Infrared Spectroscopy (FTIR)

The possible interaction between the drug and the carrier was studied by FTIR spectroscopy.

The FTIR patterns of pure diacerein, and formulations are shown in (Figure 3). The principal absorption peaks of DCN were observed at 3300 cm^{-1} (O-H, stretch, broad, COOH), 3069 cm^{-1} (C-H, stretch, aromatic), 2935 cm^{-1} (C-H, stretch, aliphatic, *sym*), 1770 cm^{-1} (C=O, stretch, ester), 1679 cm^{-1} (C=O, stretch, COOH), 1693 cm^{-1} (C=O, stretch, ketone), 1593 cm^{-1} (C=C, stretch, aromatic), 1450 cm^{-1} (C-O, stretch, COOH), 1026 cm^{-1} (C-O, stretch, ester), 760 cm^{-1} (*m* substituted benzene), 704 cm^{-1} (benzene) [27].

The FTIR spectrum of PXMR188 was characterized by principal absorption peaks at 3485 cm^{-1} (O-H, stretch, broad), 2884 cm^{-1} (C-H stretch aliphatic), 1343 cm^{-1} (in plane O-H bend) and 1111 cm^{-1} (C–O stretch) [27], which were consistently appeared in microcrystals of DCN. PVPK30 showed characteristic broad band at 3454cm^{-1}(O-H, stretch, broad), 1666 cm^{-1} (C=O) indicating oxygen functionalities. PEG6000 displayed their characteristic bands assignable to C-O-C and OH groups and C-H at 1108 cm^{-1}, 3475.17 cm^{-1} and 2886 cm^{-1} respectively.

All microcrystals showed remarkable attenuation of IR bands of both drug as well as polymer with significant reduction in the intensity of the peaks. Moreover, most of the peaks were found to be diffused indicating formation of microcrystals. However, no formation of any new peak was detected indicating the absence of chemical interaction [28, 29]. Further the smoothening of the peaks in formulation depicted presence of strong physical interaction between drug and polymer in the presence of acetone and water [28, 29].

X-ray Powder Diffractometry (XRPD)

Pure diacerein exhibited crystalline nature as shown in (Figure 4). Diacerein showed diffraction peaks at 15.045°, 25.375°, 32.27°, 37.09° and 41.45° (2θ) with peak intensities of 117, 117, 139, 104 and 306 respectively. The polymers, PXMR188 and PEG6000 displayed characteristic diffraction peaks indicating their crystalline nature on the other hand PVPK30 showed characteristic hollow pattern due to its amorphous nature.

The microcrystals of diacerein with polymers showed entirely different diffraction pattern pointing out their formation with altered molecular arrangement in their structure. Figure 4 shows that all DCN samples had the similar peak positions in the XRPD patterns. However, there were some differences among formulations in the relative intensities of some peak. These differences could be explained by changed crystallinity due to use of polymers [30]. The difference might be due to the change in the relative intensities of the peak because planes exposed to the X-ray source might have changed in microcrystals with use of hydrophilic polymers [31, 32].

Table 1. Size measurement analysis of pure diacerein and microcrystals

System	Size Analysis
DCN	2.79±0.009
DP1	1.09±0.025[a]
DP2	1.02±0.012[a]
DP3	1.10±0.015[a]

[a] significant difference compared to pure drug and microcrystals; Diacerein- DCN, Diacerein+PVPK30 (1:2)- DP1, Diacerein+PXMR188 (1:2)- DP2, Diacerein+PEG6000 (1:2)- DP3

Table 2. Dissolution data of pure DCN and its microcrystals in phosphate buffer (pH 6.8) at 37 ± 0.5°C

System	DP5* ± S.D.	DP30* ± S.D.	DE5* ± S.D.	DE30* ± S.D.
DCN	20.68±1.54	60.71±2.51	71.71±1.41	72.09±1.35
DP1	94.04±1.26	100.0±0.52 [a]	100.0±0.78[a]	100.0±0.520[a]
DP2	54.15±2.01	100.0±0.32 [a]	100.0±0.67 [a]	100.0±0.92 [a]
DP3	58.92±1.76	100.0±0.54 [a]	100.0±0.89 [a]	100.0±0.82 [a]

S.D.: Standard deviation; DP: % drug dissolved; DE: dissolution efficiency; [a] significant difference compared to pure Diacerein i.e. significant (p <0.001);Diacerein- DCN, Diacerein+PVPK30 (1:2)- DP1, Diacerein+PXMR188 (1:2)- DP2, Diacerein+PEG6000 (1:2)- DP3

Figure 1. Chemical structure of DCN

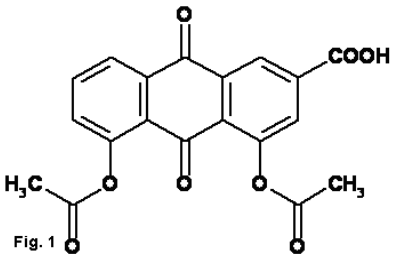
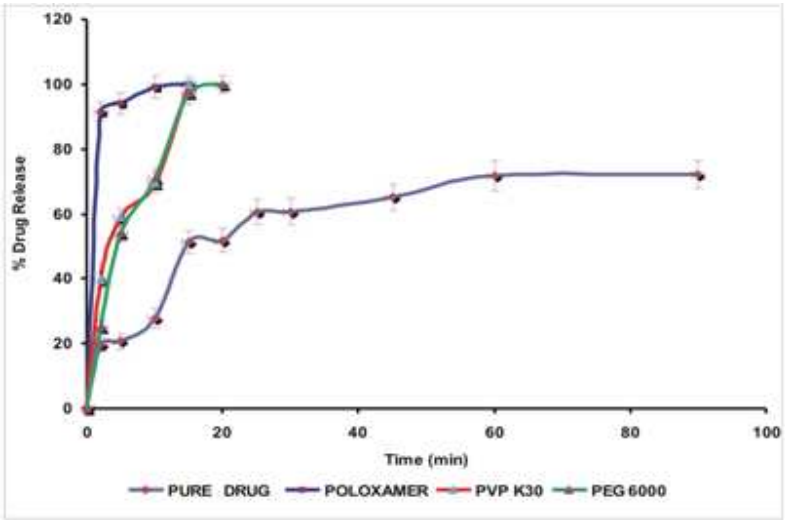


Figure 2. The dissolution curves of pure DCN and its microcrystals in phosphate buffer (pH 6.8) at 37 ± 0.5°C



Scanning Electron Microscopy (SEM) and Particle Size measurement

The SEM microphotographs of pure DCN and its SDs are shown in (Figure 4). Pure DCN consisted of some large irregular crystals with course particles. The SEM photomicrographs of microcrystals showed platy structure [33]. Further some microcrystals indicated needle like appearance [33]. However shape of the particles did not interfere in the dissolution of microcrystals. All the formulations exhibited better dissolution than that of the raw drug. The particle size was reduced upto 1.09 µm in PVPK30 microcrystals, 1.02 µm in PXMR188 formulations and in PEG6000 it was found to be 1.10 µm. The size measurement depicted significant reduction in size ($p < 0.001$) as the

raw drug has 2.79 µm size. Therefore reduction in size, greater surface area and use of polymers might have given enhanced dissolution.

Figure 3. FTIR spectra of (A) Diacerein; (B) PXMR 188; (C) PEG6000; (D) PVPK30; (E) DP1;(F) DP2;(G) DP3.

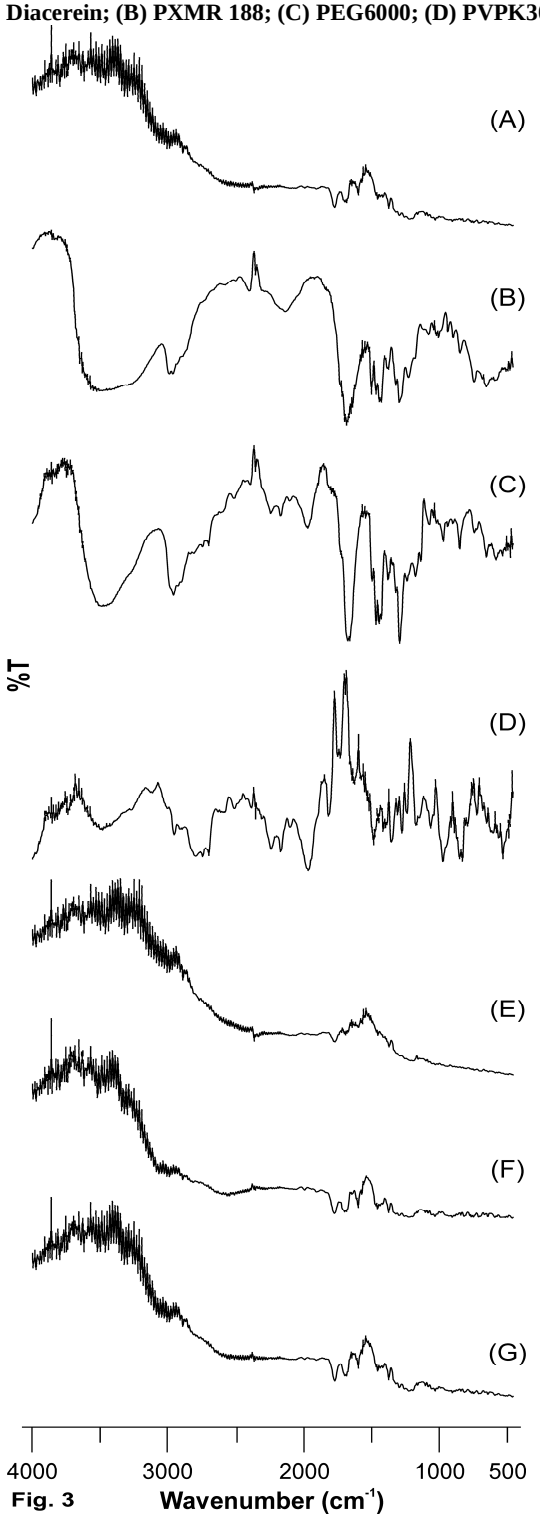


Fig. 3

Figure 4. XRPD patterns of (A) Diacerein; (B) PXMR 188; (C) PEG6000; (D) PVPK30; (E) DP1;(F) DP2;(G) DP3.

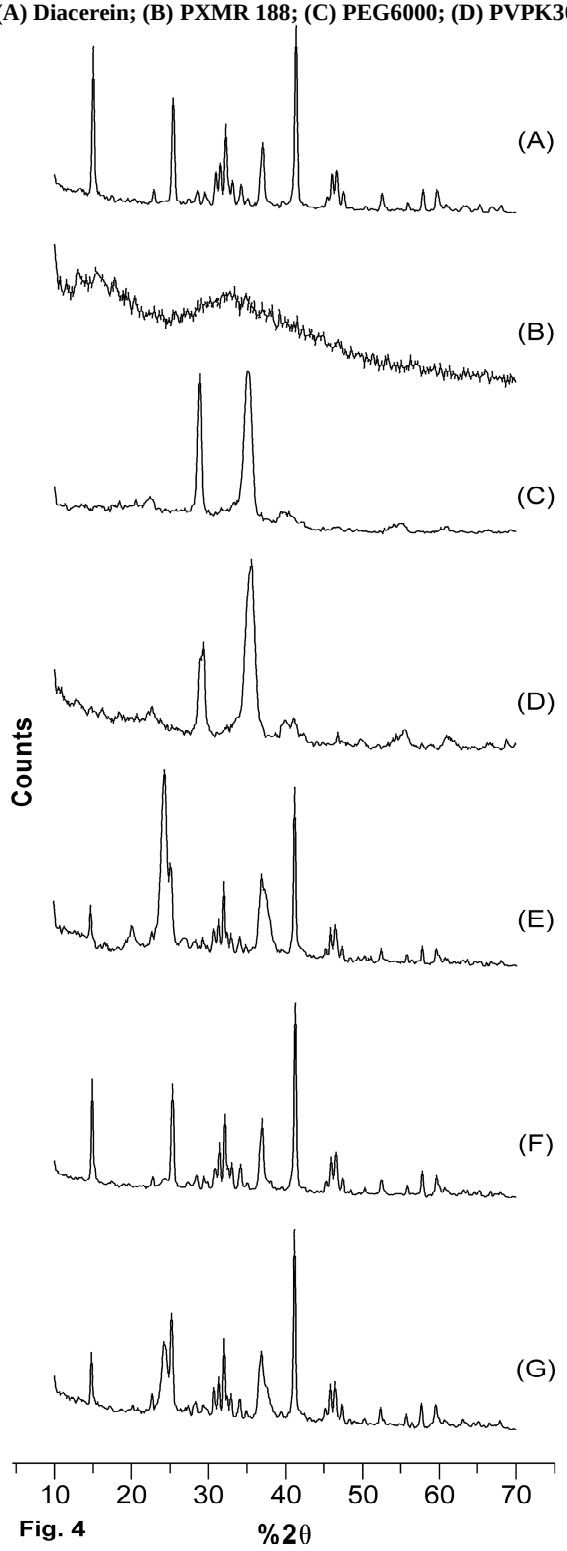


Fig. 4

Figure 5. Scanning Electron Microphotographs of (A) Diacerein; (B) DP1 ;(C) DP2 ;(D) DP3.

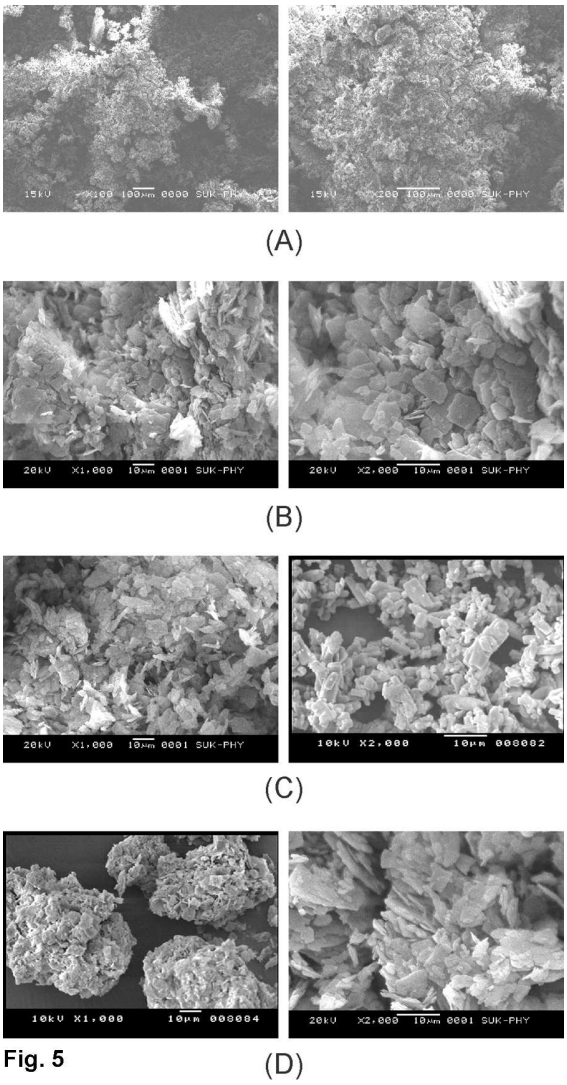


Fig. 5

CONCLUSION

The anti-solvent technique used in this study produced microcrystals that exhibited significantly faster dissolution rates than the pure DCN crystals. The improved solubility and dissolution are attributable to the modification of DCN by microcrystallisation due to specific interactions between the drug and the polymers. Therefore DCN microcrystals yielded better physicochemical properties and would be one of the better alternative to conventional methods of solubility enhancement.

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