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# Biopharmaceutical Process of Diclofenac Multiparticulate Systems for Chronotherapy of Rheumatoid Arthritis

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## ABSTRACT

**Objective:** Diclofenac exhibits limited solubility, low bioabsorption and gastric toxicity. The objective is to address above limitations and to design multiparticulate formulation for chronotherapy of Rheumatoid arthritis (RA).

**Materials and methods:** Solid dispersions of diclofenac (DC) with sodium starch glycolate (SSG) and guar gum (GG) were prepared. Uniform sized (~400 μm) non-pariel seeds were coated with solid dispersions to produce immediate release pellets (DMP-1 and DMP-2) and controlled release pellets (DMP-3 and DMP-4). The resultant controlled release pellets were further layered with methaacrylate polymers to obtain pulsatile release pellets (DMPP). Solubility, FTIR, DSC, micromeritics, SEM, drug content, drug release, pharmacokinetics and stability studies were performed for DMPP.

**Results:** Solubility of DC was improved by 164-folds due to presence of hydrophilic carries in the solid dispersions. No chemical and physical interactions were noticed in FTIR spectra and also in thermograms. Fluidized bed processor facilitated the production of high-quality, circular and regular pellets with angle of repose less than 19.5º and DC content in between 95.18-98.87%. Maximum drug was released from DMPP at the end of 12 hours. DMP-1 and DMP-2 pellets had 2 hrs of drug release and pulsatile controlled release pellets had 6 hrs lag phase followed by 12 hrs controlled release. Both DMP-1 and DMP-2 immediate had shown first-order release followed by Hixson-Crowell kinetics whereas DMPP pellets followed zero

order release with Higuchi's kinetics. Maximum concentration of DC in plasma was recognized as 400.8 ng/ml at 5 hr for DMP-2 and 381.1 ng/ml at 14 hr for DMPP-5. The solubility of DC was increased with the application of solid dispersion technique and in turn increased the pharmacokinetics. The pellets were plausibly stable above a time period. Thus, multiparticulate pulsatile systems of DC were as effective as chronotherapeutics in the treatment of circadian rhythm based ailments like RA.

**Key words:** Chronomodulation, Circadian, Fluidized-bed, Hydrophilic carrier, NSAID, Wurster process.

## INTRODUCTION

Rheumatoid arthritis (RA) is a chronic progressive autoimmune disorder due to dense innervation in joint capsule and synovium and results in peripheral inflammation. Chronic inflammation and pain in fingers, wrists, elbows, shoulders, knees, feet and ankles will occur in RA[1-3]. A conference in Paris, in the year 2010, proposed "pain modifying analgesic drugs"[4] in management of RA. Clinical treatment options for RA are NSAID's, opioid analgesics, tricyclic antidepressants, corticosteroids, anticonvulsants, topical agents and serotonin norepinephrine reuptake inhibitors[5]. But still, each category of drug holds its own limitations. Disease modifying antirheumatic drugs (DMARDs) is currently recommended for the treatment of arthritis. Triple therapy with other drugs such as methotrexate, sulfasalazine, and hydroxychloroquine is the flourishing combination with DMARDs. High dosing, long term usage of above mentioned medications develops gastric mucosal damage, tolerance and accumulation of drug metabolites in kidney, liver and causes nephro and hepato toxicity[6].

However, NSAIDs are most commonly prescribed therapeutic agents in the management of RA like other pain cases. Among the notable NSAIDs, diclofenac (DC), a fenamic acid derivative is very usually prescribed agent due its potentiality against pain and inflammation in RA patients. DC is believed to be inhibit the synthesis of substance P, a pro-inflammatory neuropeptide and nociceptive prostaglandins in synovial tissue as well as in blood and thus useful in RA treatment[7]. DC being an insoluble BCS class II drug shows t½ of 2 hr and low bioavailability[8]. Nevertheless the conventional oral medication of DC was resulted with severe gastric mucosal damage and other side-effects of NSAID's [9]. As severity of joint pain, surrounding muscle stiffness and fatigue in RA patients depends on circadian rhythm and mostly observed at early hours, a dosage form that releases the drug according to time (circadian rhythm) would be a promising system. Now a days, Pulsatile® and Diffucap® systems are gaining much research interest in chronotherapy of circadian rhythm based ailments so as to release the drug after a predetermined lag phase. Multiparticulate formulations are composed of immediate and controlled release particulates and superior in presenting diverse drug delivery patterns in circadian fashion[10,11].

Diclofenac, a regular medicament used in the management of RA was chosen as a model drug in this study[12]. It was aimed to develop multiparticulate systems of DC containing immediate and pulsatile controlled release pellets using non-pariel sugar seeds to avoid gastric mucosal damage and enable pulsatile release in the small intestine.

## EXPERIMENTAL

### Materials

Diclofenac (DC) and non-pariel seeds were gifted from M/s. Lee Pharma, Visakhapatnam, India. Guar gum (GG), sodium starch glycolate (SSG), eudragit RS100 and eudragit L100 were purchased from S.D. Fine Chem., Mumbai, India. All other chemicals and reagents used in the study were of analytical grade.

### Preparation of solid dispersions of diclofenac

Solid dispersions of DC were prepared using GG and SSG in 1:1 and 1:2 weight ratios as mentioned **Table 1**. The ingredients were kneaded thoroughly in a glass mortar until uniform mass is formed for about 20 min. The resultant mass was screened through #80 mesh and kept in a vacuum desiccator for further evaluation and formulation of pellets[13].

### Solubility study

An excess of solid dispersion was taken into calibrated glass vials containing 10 ml of 0.1 N HCl, pH 6.8 and 7.4 phosphate buffer solutions. The resultant solutions were equilibrated at 37ºC for 72 hr using rotary shaker. After equilibration, the solutions were filtered through 0.45 µm nylon filter and assayed using UV-visible spectrophotometer *(Analytical Technologies, T 70)* at 276 nm[14].

### Preparation of multiparticulate pellets

**Step–1:** A known quantity (a batch) of non-pariel seeds were coated with DC: SSG and DC: GG solid dispersions in 1:1 and 1:2 proportions. This results in the production of immediate and controlled release pellets.

#### *Non- Pariel seeds coating with solid dispersion using Solution layering technique*

About 150 g of non-pariel seeds (sugar spheres) were transferred into vertical chamber of Fluidized bed processor *(GPCG 1.1.Glatt, D-Binzen)* (Wurster Process). Solution layering process was initiated with inlet temperature of 45-50°C by layering the solid dispersions (300 g) of step 1, on pellets using spray guns, atomized at 2-4.5 barr and operated at 60-120 g/min spray rate until bed was wet and tacky. The process was continued for 45 min to produce the desired size of immediate release pellets (DMP-1 and DMP-2) and controlled release pellets (DMP-3 and DMP-4). The pellets obtained were sifted through #14 and #18 sieves and the controlled release pellets retained over #18 were selected for step - 2[15].

***Step–2: Preparation of pulsatile- controlled release pellets***

In this step, DMP-3 and DMP-4 pellets (controlled release pellets) were coated with the solutions of eudragit RS100 and eudragit L100 prepared in the solvent mixture of Triethylamine (plasticizer) and isopropyl alcohol. The compositions of Eudragits are mentioned in **Table 2.** The process parameters employed in this step were similar to that of step-1. Step-2 resulted in the formation of pulsatile release pellets (DMPP-1 to DMPP-5). Pulsatile pellets sifted and retained over #20 mesh were selected for further characterized and evaluation studies[16,17].

***ATR-FTIR spectroscopy***

The chemical compatibility of DC and polymer mixtures was examined using ATR (Attenuated total resonance) FTIR spectrophotometer (Agilent CARY 630 ATR-FTIR). The sample to be tested was cited on a diamond ATR crystal and analyzed by means of Agilent resolutions pro software. Each spectrum was checked at 32 single average scans at 4 $cm^{-1}$ resolution in 400-4000 $cm^{-1}$ absorption region.

**Differential Scanning Calorimetry**

Thermal analysis of DC and polymer mixtures was carried out using Differential Scanning Calorimeter *(Shimadzu DSC50)* to check the physical compatibility. During each scan, about 1 to 2 mg of the sample was positioned in sealed aluminum sample pans at a rate of 10°C/min under nitrogen atmosphere between temperature range of 25 °C and 350°C. An empty aluminium pan was used as reference.

**Scanning Electron Microscopy (SEM)**

The surface morphology and cross section of the prepared pellets was determined using Scanning Electron Microscope (Jeol, JSM-1600, Tokyo, Japan). The pellets were coated with fine gold at 10 mA ion current for 5 min under 0.1 Torr pressure using ion sputter and laden on studs for examination.

**Drug content**

A batch of pellets was transferred into a calibrated volumetric flask and dissolved in 10 ml of methanol by ultra-sonication for 10 sec. The volume of the preparation was made up to 100 ml with the same, filtered and assayed.

**Entrapment efficiency and drug loading**

Batch of pellets (DMP-1 to 4 and DMPP-1 to 5) equivalent to 100 mg of diclofenac were transferred into a standard Vensil® flask containing 100 ml of phosphate buffer (pH 6.8) and stored overnight. The sample solution was filtered through 0.44 µm nylon filter to remove undissolved portions of pellets and analyzed at 276 nm. An average of three estimations was considered for each batch of pellets formulated in the study. The entrapment efficiency and drug loading of pellets were calculated from the equations (1) and (2)[18,19].

$$\% \text{ Entrapment efficiency} = \frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} \text{ X } 100 \qquad (1)$$

$$\% \text{ Drug loading} = \frac{\text{Mass of drug in pellets}}{Mass\ of\ pellets} \text{ X } 100 \qquad (2)$$

***In-vitro* dissolution study**

Pellets of DMP-1to DMP-4 and DMPP-1 to DMPP-5 equivalent to 100 mg of drug was subjected to i*n-vitro* drug release studies using USP type I dissolution test apparatus at 37ºC and 50 rpm in 0.1 N HCl, pH 6.8 and pH 7.4 as dissolution media for first 2 hrs, next 3 hrs followed by next 13 hrs respectively. Aliquot of 5 ml samples were withdrawn at various time intervals by maintaining sink condition, filtered and analysed. The study was repeated for six independent observations and the results were built-in to diverse kinetic models.

***In-vivo* study**

Male rabbits of 10-14 weeks old, weighing for about 2-3 kg was preferred for the study. The animals were housed under 12-12 hr light-dark cycle and fed with standard diet and water. The study process was approved by institutional animal ethical committee (CPCSEA/1657/IAEC/CMRCP/PhD-15/43). Total of 12 rabbits were equally divided into three groups (Group-1, Group-2 and Group-3) and were depilated on the dorsal surface of ear pinna. Animals were fasted for 24 hrs before the study. Dose of the drug to be administered was calculated based on rabbit body weight using the formula $Rabbit\ dose = \frac{\text{Total Dose (in humans) X 0.07 (for 1.5 kg rabbit)}}{1.5}$. Group 1, 2 and 3 were administered with DC, DMP2 and DMPP-5 samples respectively. About 1 ml of blood sample was collected at regular time intervals into heparinized Eppendrof tubes. The samples were shaken thoroughly, centrifuged at 1500 rpm and plasma was collected. A previously developed and validated HPLC method was employed[20] to determine the plasma drug concentrations as function of time. The C18 (250 mm × 4.6 mm, 5 m) column, mixture of acetonitrile and methanol (70:30, v/v) as mobile phase and UV detector (276 nm) were adopted and Empower software version-2 was used in assaying DC concentrations in plasma against DC as internal

standard. Sample about 20 µl was injected into the HPLC system (Waters, 2695) after filtering the plasma through 0.2 µm membrane filter. Mean concentrations of diclofenac in plasma samples as function of time were calculated and demonstrated in **Figure 7**. The pharmacokinetic parameters such as $t_{1/2}$, $K_e$ and $V_d$ were characterized and compared statistically using analysis of variance (ANOVA) and Tukey-Krammer test applied for the columns comparison. $P < 0.05$ was considered statistically significant correlation.

**Stability study**

Accelerated stability testing of pellets was carried out at 25±2° C/ 60±5% RH and 40±2° C/ 75±5% RH conditions of ICH guidelines for a period of 120 days. The pellets were then tested for their flow properties; drug content and *in-vitro* drug release [21,22].

**RESULTS**

Pulsatile drug delivery as multiparticulate systems are demonstrated to be the successful technologies to overcome the limitations such as low solubility and less bioabsorption faced by BCS class II and IV drugs as single unit drug delivery systems. This solubility study was resulted with increased solubility of DC by164-folds as solid dispersion technique was employed along with hydrophilic polymers. Resultant amorphous solid dispersions of DC displayed with good micromeritics.

The FTIR spectra (**Figure 1**) of diclofenac confirmed the existence of peaks at 3317, 1677, 1120 and 745 $cm^{-1}$ due to NH stretching, C=O stretching, C-O bending and C-Cl bending as characteristic functional groups of DC. Similar functional groups indicating peaks were observed without significant shifting of DC in DMPP-5 mixture. As shown in **Figure 2,** DSC thermograms had displayed endothermic peaks of DC at 276ºC and DMPP-5 at 275ºC respectively.

The SEM image (**Figure 3**) of pellets demonstrated the smooth, well alienated, spherical, and uniformly distributed and micron sized particles with 400-500 µm range. The pellets (both types) prepare by Wurster process had displayed substantial entrapment efficiencies and drug loading capabilities as mentioned in **Table 3**.

*In-vitro* drug release profiles of the diclofenac pellets are depicted in **Figure 4** and **5**. Sodium starch glycolate, a hydrophilic carrier composed DMP-1 and DMP-2 pellets had demonstrated the burst drug release as 94.2 and 99.5% at end of 2nd hr. Controlled release pellets, DMP-3 and BMP-4 had 98.9 and 99.1% at end 10th hr due to the composition of hydrocolloidal polymer guar gum. While the pulsatile release pellets, DMPP-1-DMPP-5 had

shown 96.1, 96.3, 97.4, 99.1 and 99.5% respectively at end of 18$^{th}$ hr (with 6 hrs lag phase). Release of DC from immediate release pellets followed First-order release with Hixson-Crowell cube root model kinetics whereas pulsatile release pellets followed Zero-order with Higuchi kinetic model.

The developed HPLC method eluted the DC peak with a retention time of 2.337 min as shown in **Figure 6**. The limit of detection (LOD) and limit of quantification (LOQ) for the HPLC method employed was found to be 3 and 9 ng/ml respectively. Upon assaying the plasma samples by this validated HPLC method, pharmacokinetics of DMP-2 pellets such as $t_{1/2}$, $K_e$ and $V_d$ were found to be increased by 2.03, 1.733 and 1.55 folds respectively. Bioavailability parameters viz., $C_{max}$, $t_{max}$ and AUC were increased by 1.9, 1.72 and 1.25 times respectively when compared to DC. In case of pulsatile pellets, DMPP-5 formulation 5.78, 4.1 and 1.82 folds of increase in pharmacokinetics and about 1.82, 4.82 and 1.31 times increase in bioavailability parameters was observed respectively as given in **Table 4**.

The physicochemical properties for all the pellets were intact over a period of time due to eudragit coatings. There were no changes in evaluation parameters of pellets even after exposure to various temperature and relative humidity conditions.

**DISCUSSION**

Kneading technique employed in the formulation facilitated the entanglement of particles till molecular level as polymeric networks and therefore enhanced the solubility of diclofenac was achieved with solid dispersions. Moreover, the solid dispersions of DC had shown amorphous geometry with excellent micromeritic properties.

SSG and GG are well established as carriers in recent past and were compatible with diclofenac moiety as evidenced in the FT-IR spectra. There were no chemical bonds established between DC and carriers other than hydrogen bonding which were evidenced in the wave numbers of FTIR spectrum. Thus, spectra showed no chemical interactions between DC and carriers selected in the study. The same was attributed in thermograms of DMPP pellets as a minor shift in the endothermic peak of DC was identified in DMPP-5's thermogram as characteristic interaction between drug and selected carriers.

The defined surface morphology of the pellets was possible only due to the accurate layering of diclofenac on sugar spheres. The dimensions of pellets were linearly increased with the composition of eudragits as coating solutions and the same was resulted in SEM image. Pulsatile pellets were relatively larger than that of immediate release pellets as they

were undergone two steps of coating and also with methaacrylate polymer coating. The Wurster process by fluidization adopted here was successful in producing the non-agglomerated, free flowing and moderately high diclofenac content (95.18–98.87%) pellets. The pellets with good entrapment efficiency and

Eudragit RS100, a polymer of enteric grade and cationic polymer possess low permeability provides an additional sustaining activity of drug release along with the gelling properties of guar gum while Eudragit L100 being an anionic polymer with high permeability helps in maintaining lag phase for about 6 hrs. DMPP pellets were composed of swellable polymer, guar gum and enteric polymers (Eudragit L100 and Eudragit RS100). Optimized pulsatile release of DC was achieved with desired lag phase from DMPP pellets owing to typical composition of hydrocolloidal GG and pH dependent eudragits.

Enhanced oral absorption of diclofenac by multiparticulates was proved. Precise analysis of plasma samples using HPLC technique had proven with the quick onset of action due to DMP-2 formulation owing carrier effect of SSG, hydrophilic polysaccharide. Micron size pellets of immediate release had contributed for the loading dose while the pulsatile pellets have contributed for maintenance dose of drug in rabbits. Contribution of guar gum, a polycolloidal polymer resulted with controlled release of DC from DMPP to reach maximum concentration in plasma. Desired lag phase of 6 hrs was achieved with a typical composition of anionic and cationic methaacrylate polymers (Eudragits) at 40: 60 ratios. Increased bioabsorption and pharmacokinetics of DC by multiparticulate pulsatile formulation were evident and hence it was attributed to reach adequate concentration of DC at synovium to reduce the inflammation. Administration of DMP-2 and DMPP-5 pellets as multiparticulate formulations of diclofenac could produce both immediate and controlled release profiles of DC at predetermined time in small intestine so as to cater the requirements of chronotherapy of rheumatoid arthritis. The physical stability of the pellets was due to the application of superior technology (fluidized coating) and method (solution layering) in the study.

## CONCLUSION

Solid dispersions were prepared using different hydrophilic, polysaccharides and methacrylate polymers to address the solubility issues of diclofenac. A novel solution layering technique was employed in association with fluidized bed processor to obtain typically micron sized, smooth surfaced and spheronized pellets of immediate release and controlled release category. Further, controlled release pellets were coated with enteric

polymers to produce 6 hrs lag phase in drug release. Promised *in-vitro* drug release profiles were achieved by pulsatile technology based multiparticulates of diclofenac to cater the needs of chronotherapy of circadian rhythm based chronic diseases like rheumatoid arthritis. Thus, the suitability of applied technologies in the design of multiparticulate drug delivery systems in chronotherapy of rheumatoid arthritis using diclofenac was convincingly proved by *in-vitro* evaluation. The outcomes of *in-vitro* study were further fortified by *in-vivo* studies using rabbits. While characterizing the plasma drug concentrations using sensitive and precise HPLC methodology, plasma-drug parameters such as $t_{½}$, and $K_e$ were proved as suitable chronopharmacokinetic parameters. Characteristics and parameters of bioavailability were also contributed in this chronomodulated delivery of diclofenac. It was concluded that, the design of spheronized pulsatile pellets of diclofenac is useful in the chronotherapy of rheumatoid arthritis, but scale-up techniques are required for commercial viability.

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**DECLARATION OF INTEREST**

The article content has no declarations of interest.

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**Figure 1**

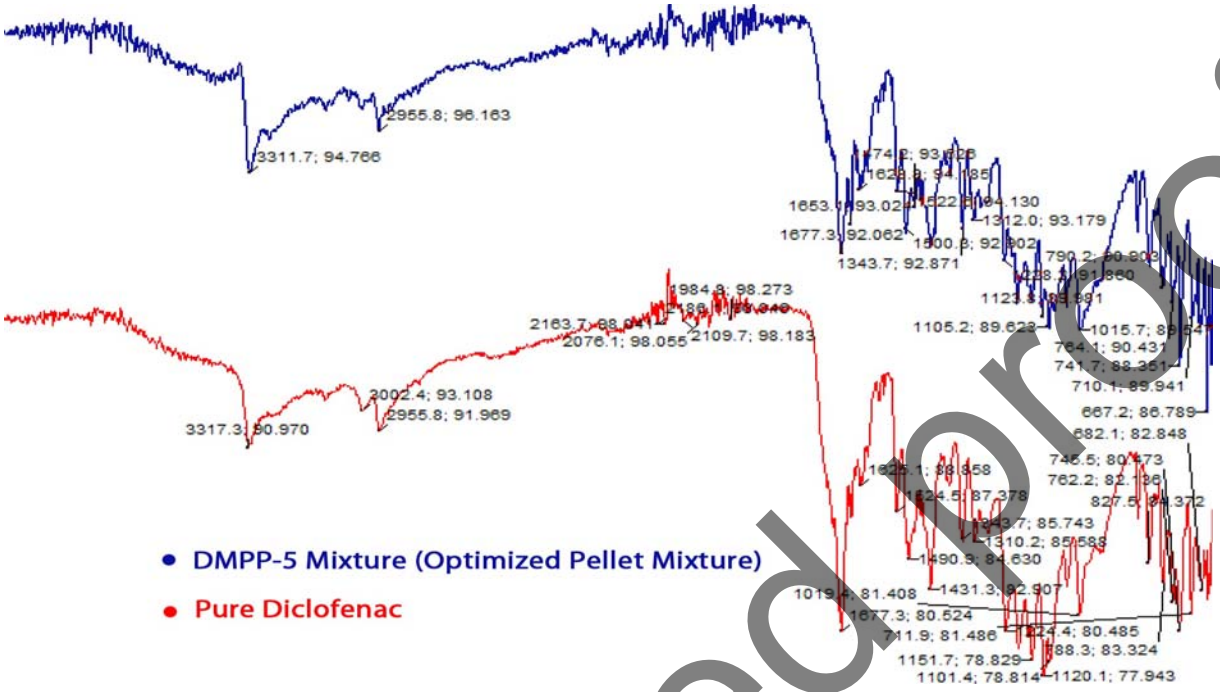


**Figure 2**

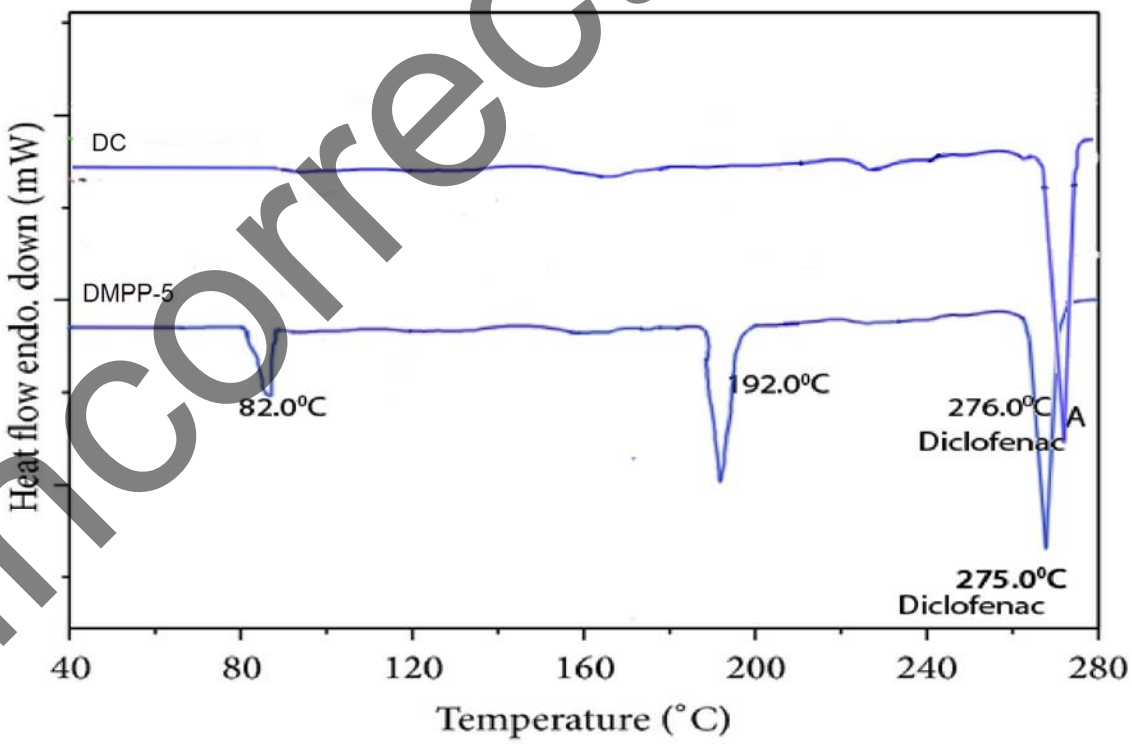

**Figure 3**

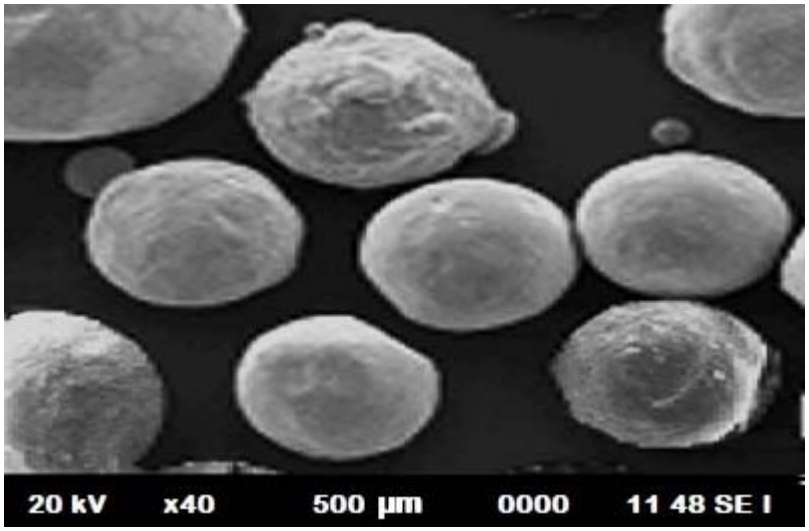


**Figure 4**

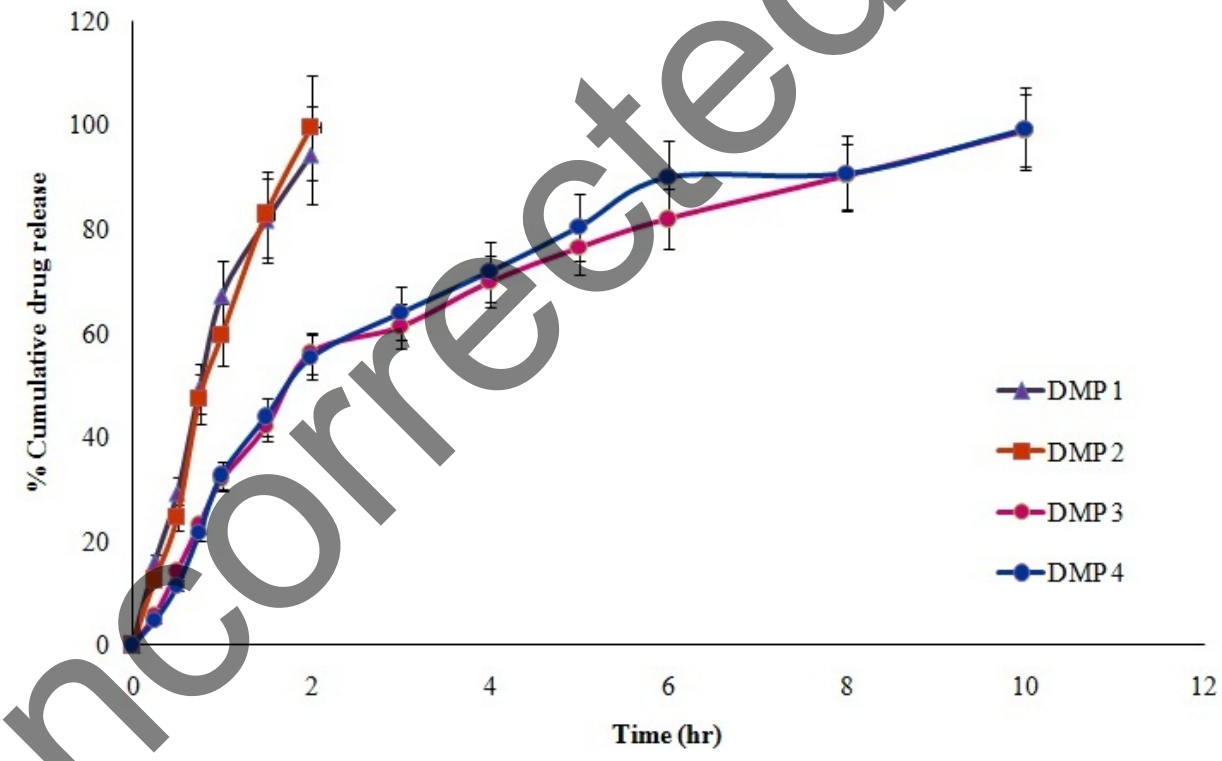

**Figure 5**

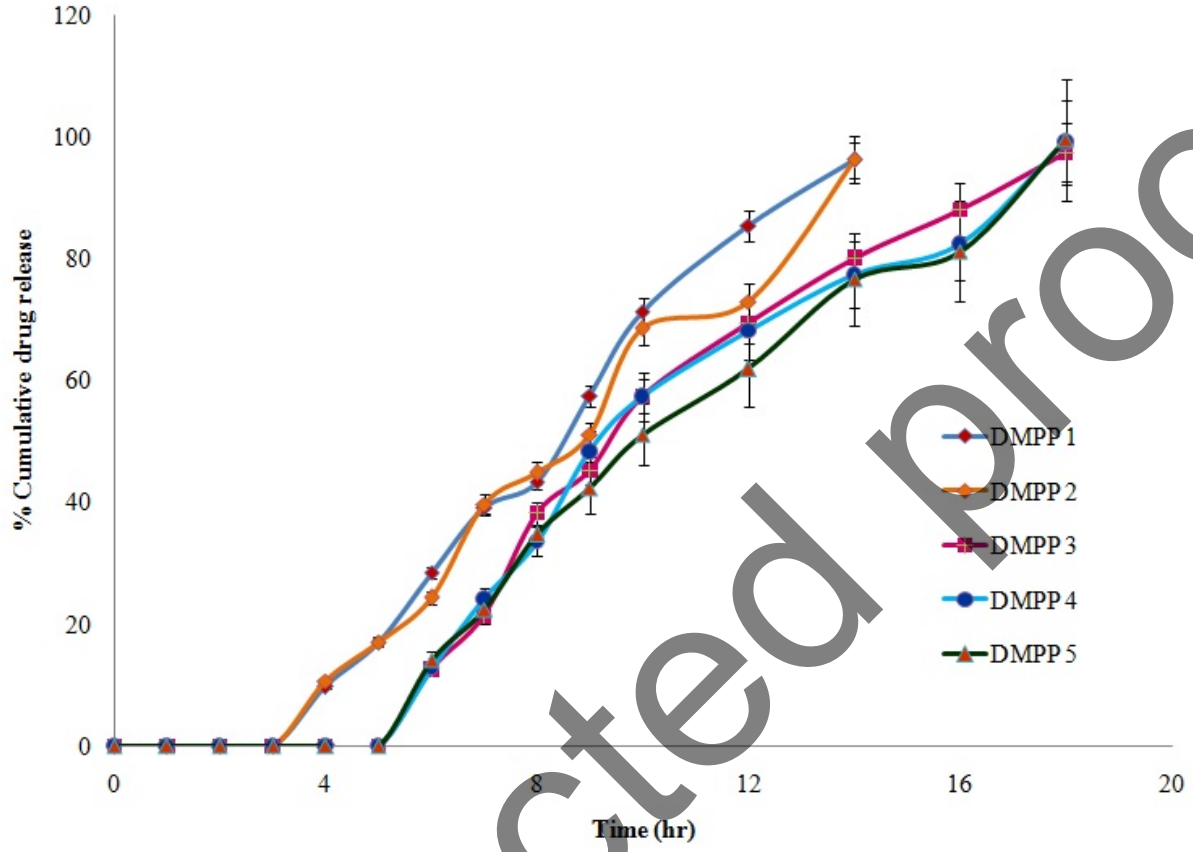


**Figure 6**

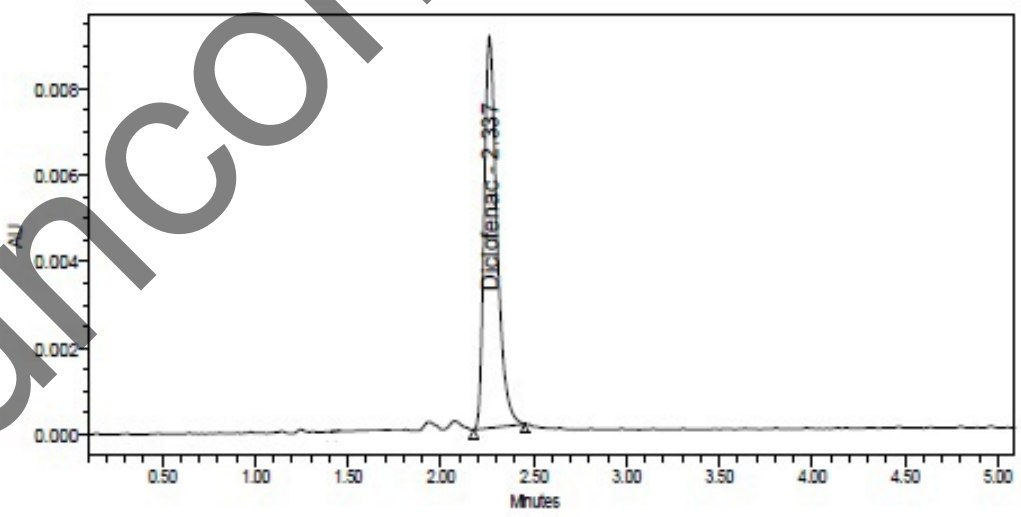

**Figure 7**