# **Research Paper**

# DEVELOPMENT AND *INVIVO* EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF DICLOFENAC POTASSIUM CONTROLLED DRUG DELIVERY SYSTEM.

Maiti Anindya Kishore<sup>1</sup>\*, Dhara Amal Kumar<sup>1</sup>, Nanda Arunabha<sup>2</sup>

<sup>1</sup>Contai Polytechnic, Contai-721401, West Bengal, INDIA

<sup>2</sup>Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032, INDIA.

# Abstract

Inflammation is a patho-physiological response of leaving tissue injury caused by physical trauma, toxic chemical or microbial agents. Non-steroidal anti-inflammatory drugs are used for pain management, and in long term therapy, they carry potential toxic effect significantly associated with gastrointestinal bleeding and ulceration. Hence an attempt was made to formulate a sustained release dosage form with the help of Diclofenac potassium (DP) solid dispersion which provides the initial loading dose and the microsphere of Diclofenac potassium that yields the maintenance dose and minimize the frequent dosing as well as minimize the adverse effects. The objectives of this study to optimise the anti-inflammatory activity of new formulations in Sprague-Dawley rats.

Diclofenac potassium solid dispersion was prepared co-solvent method using PVP and microspheres were prepared by ionotropic gelation methods using sodium alginate and chitosan. Sustained release dosage form was prepared by compiling solid dispersion and microsphere in different ratios. The anti-inflammatory activities were evaluated using formaldehyde induced rat paw edema.

The percentage inhibition activities over 10 days of treatment of different DP formulas were found to be 82.59% and 71.41% respectively. The effect is comparable with the pure diclofenac potassium which produces the percentage of inhibition activity of 70.15%. The diclofenac potassium loaded new formulation exhibited the significant anti-inflammatory activities in compare to pure drug in formaldehyde-induced rat paw (p<0.005) edema.

Key words: Microsphere, Solid dispersion, Diclofenac potassium, Formaldehyde induced

#### **INTRODUCTION:**

Pain is an unpleasant sensation and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. As per International Association for the study of Pain, (IASP) pain is classified into acute pain and chronic pain. The difference between acute and chronic pain has relied on a single continuum of time with some interval since the onset of pain used to

designate the onset of acute pain two most commonly used chronologic markers used to denote chronic pain have been 3months and 6months since the initiation of pain; however, these distinctions are arbitrary. Inflammation is a patho-physiological response to living cell injury caused by physical trauma, infections, toxic and noxious chemicals, allergic reactions and other irritant stimuli. For the management of pain and inflammations, there are several steroidal and non-steroidal drugs prolonged but their available. administration causes various adverse effects like cardiac problems, ulcerations, risk of gastrointestinal bleeding [1],[2],[3].

NSAIDs have different spectrum of activity than the analgesics. Other than analgesic effect they have antiinflammatory and antipyretic activities [4]. In general these agents are most effective in pain associated with inflammation such as arthritis [5]. Diclofenac potassium is a popular non-steroidal most anti inflammatory drug that shows preferential inhibition of the cyclooxygenase-2[COX-2]enzyme [6] and which possesses structural characteristics of aryl-alkanoic acid agents and displays antiinflammatory, analgesic and anti-pyretic properties [7]. In the carrageenan induced rat paw edema assay, it is twice as potent as indomethacin and 450 times as potent as aspirin [8]. Moreover, diclofenac has been shown to undergo considerable firstpass metabolism, limiting its oral bioavailability (50-60%) [9], [10]. But its prolong associated with use gastrointestinal disturbances, hyper acidity, peptic and ulceration gastrointestinal bleeding. Hence an attempt was made to formulate a sustained release dosage form with the help of 19

Diclofenac potassium solid dispersion which provides the initial loading dose and the microsphere of Diclofenac potassium that yields the maintenance dose and minimize the frequent dosing as well as the adverse effects.

From the literature survey it was found that, synthetic polymers materials don't exhibit the biodegradability and bioincompatibility. On the other hand, to dissolve the synthetic polymers, organic solvents are required. Problems of possible toxicity, explosion hazards and especially environmental pollution associated with the use of organic solvents have been raised recently [11]. Natural polymers are devoid of toxicity and biodegradability problems. Hence alginate and chitosan are used as coating materials as they are biodegradable, dissolved without organic solvent and eco-friendly [12].

# MATERIALS AND METHODS

# Chemicals

Diclofenac potassium IP was received as a gift sample from U S Vitamins Ltd, Gujarat, Sodium alginate, calcium chloride (Analytical grade), Polyvinyl pyrrolidone and mannitol were purchased from Loba Chemicals, Qualigens India Ltd, S D Fine chemicals and Qualigens Ltd respectively, chitosan was received as gift sample from India Sea Foods, Cochin, India. All other ingredients were used either reagent or analytical grade.

# Method of preparation of Diclofenac potassium solid dispersion

Solid dispersion of Diclofenac potassium was prepared by common solvent method where drug and polymers were dissolved

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in a common solvent [13]. Here PVP was taken as carrier polymer and methanol was taken as solvent. In a beaker accurately weighted 200mg diclofenac potassium was completely dissolved in to methanol containing 600mg PVP. The solvent was completely evaporated by heating at low temperature on water bath with constant stirring until the mass comes to the constant weight. After evaporation it was stored in a desiccator over fused calcium carbonate for two days for hardening. Lastly it was pulverized through 60-mesh and taken for analysis. It should be kept in cool dry place.

# Method of preparation of Diclofenac potassium microsphere

The microsphere of diclofenac potassium was prepared by ionotropic gelation method followed by Fattah et.al. [14] and Bert et. al. [15]. In a beaker, 200mg of sodium alginate was dispersed in distilled water and homogenized for 1hour. Drug alginate solution was prepared bv dispersing 200mg of drug slowly into the sodium alginate solution with constant stirring at 200rpm for 3 hours. A gelation medium was prepared by dissolving 2% of CaCl<sub>2</sub> in distilled water separately with 200mg of chitosan which is previously dissolved with 3% glacial acetic acid. The pH of the medium was adjusted to 4.5±0.1. Bubble free dispersion medium was extruded drop wise through 22G glass syringe in to the gently agitated gelation medium of chitosan and CaCl<sub>2</sub> solution. The agitation was done by magnetic stirrer at 200 rpm at room temperature. After curing of beads for 2h, they were taken out from the solution and washed with distilled water. The beads were dried at  $30^{\circ}$ C under reduced pressure till they

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attained constant weight.

# Preparation of Diclofenac potassium controlled drug delivery system

The mixture of solid dispersions and microspheres were used for the preparation of controlled drug delivery system of diclofenac potassium. As the objective of the present work is to sustain the action of DP for 24h, we have prepared Formula-A using 7.6 mg of DP containing solid dispersions to provide loading dose which reaches at nearly steady state level with in 3h and 2.4mg DP containing microspheres to prolong the duration of action for another 21h.

Similarly, Formula-B was prepared by compiling 8mg DP containing solid dispersion, to provide loading dose within 4h with 2mg DP containing microspheres are used to prolong the duration of action for another 20h.

# **Test Animals**

The experimental animals were 24 male Sprague-Dawley rats (250-300 g in body weight). They were housed in plastic boxes in 4 groups (6nos. in each group) and fed food and water *ad libitum* in a room under natural light. All tests were conducted between 09:00 am and 15:00 p.m. To habituate them to the formalin test environment, rats were placed in the test chambers in 3 groups for 15 min a day for 4 days and alone on the  $5^{th}$  day. Each animal was used only once and sacrificed at the end of the experiment. The testing room was maintained at 22-24<sup>o</sup>C. The guidelines on ethical standards for investigations of experimental pain in animals were followed The following experiments were [16]. performed under protocols approved by the University Animal Ethics Committee, Jadavpur University, Kolkata, India.

### **Acute Toxicity Studies**

Acute oral toxicity studies were performed according to Organisation for Economic **Co-operation** and Development (OECD/OCDE) guidelines. Three rats of either sex were selected by random sampling method for the study. The new formulated drug was administered with a dosing of 10mg kg<sup>-1</sup>. The rats were fasted over-night for food with free access of water prior to test. After dosing the individual rat was observed periodically for 24h and thereafter for 14 days. If the mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in one animal, then the same dose was repeated again for confirm the toxic effect. If no more mortality was observed, then higher dose (100mg kg<sup>-1</sup>) was applied for toxicity studies. At the end of toxicity study, the survived animals were sacrificed.

#### Anti-inflammatory activity

#### Formaldehyde induced inflammation

Experimental inflammation was induced in male rats according to the method described by Hossienzadeh H and Younesi H [17]. On the day of testing, animals were randomly assigned to 4 groups of 6 animals each group. The first group rats were given 10mL kg<sup>-1</sup> of normal saline orally and served as control, group-2 new formulated DP received beads(Formula-A) equivalent to 10 mg kg<sup>-</sup> diclofenac potassium IP, group-3 formulated received new DP beads(Formula-B) equivalent to 10 mg kg<sup>-</sup> diclofenac potassium IP whereas, the was received 10mg kg<sup>-1</sup>of group-4 the pure diclofenac potassium IP, drug(Table-1). Inflammation was produced by sub-aponeurotic injection of 21

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0.1ml of 2.0% formaldehyde in normal saline (The formalin was made of commercially available 37% formaldehyde solution further diluted in isotonic saline) in right hind paw of the rats with the help of 26G needle, on the first day and third day. Each animal was kept singly in the experimental room. The formalin test was carried out in a 30×30×60 cm-sized clear transparent plastic chamber. A mirror placed behind the box allowed for an unobstructed view of the rat's body and the rat's behaviour was recorded on a videotape. The paw volume was measured at daily using vernier callipers [18]. Percentage inhibition of the mean increase in the paw oedema of each group was calculated on the tenth day and compared with the control.

#### Measurement of oedema of the injected paw

The baseline diameters of the hind paws were measured before the formalin injection using a calliper; at the metatarsal level. Those of the hind paws that developed oedema were determined at 4 hr after the injection by measuring the dorsal plantar foot thickness at the metatarsal. Both of the hind paws were measured simultaneously. The 4 hr interval from the formalin injection to the measurement of the paw oedema was set from the literatures for the maximum time to develop an oedema [19], [20].

Percentage of inhibition of oedema = (  $V_{C}-V_{t}$ ) X100

# $V_{C}$

Where,  $V_c$  and  $V_t$  are the volume of oedema in control and drug treated rats.

#### **Statistical analysis**

Biochemical and pharmacokinetic parameters determined in normal and febrile rats were subjected to statistical analysis of paired t-test to observe the difference. Level of significance was set at P < 0.05. Mean values (n=6) and their standard error of mean (±SEM) were computed. Statistical analysis was performed using one way analysis of variance [ANOVA].

#### **RESULTS AND DISCUSSIONS**

#### **Acute Toxicity Studies**

All the doses (10mg kg<sup>-1</sup> and 100mg kg<sup>-1</sup>) of DP microsphere formulations were employed for acute toxicity studies were found to be non-toxic. After analysing the videotape, some licking of paws was seen. It is due to inflammation formation by formaldehyde injection. The sub maximal dose (10mg kg<sup>-1</sup>) which was found to be safe for rats was employed for further pharmacological investigations.

# Formaldehyde induced Inflammation in Rats

Continuous oral treatment for 10 days with newly formulated diclofenac potassium controlled drug delivery system and pure DP (10mg kg<sup>-1</sup>) remarkably reduced the paw edema induced by formaldehyde in rats. The significant difference (p < 0.05) in paw thickness from first day and throughout the period of experiment as compared with normal saline treated group. The results obtained as mean increase in paw volume and inhibition percentage are shown in Table-2. The percentage inhibition activity over 10 days of treatment of different DP formula was found to be 82.59% and 71.41% respectively. The effect is comparable with the pure diclofenac potassium which produces the percentage of inhibition activity of 70.15 %.

It is well known that, formaldehyde induced paw edema in rats is one of the most suitable test procedure to study the anti-inflammatory activity of any formulation or drug as it closely resembles to human arthritis. It is also known to be the acute inflammatory model sensitive to cyclo-oxygenase (COX) inhibitor and has been used to evaluate the effect of nonsteroidal anti-inflammatory agents (NSAIDs), which primarily inhibits the cyclo-oxygenase involved in prostaglandin synthesis [21]. In this study, formalin administration on 1<sup>st</sup> and 3<sup>rd</sup> day produces overgrowth of fibroblast and causes arthritis which is inhibited by new formulated DP.

In this study, the two new formulations of Diclofenac Potassium (Formula-1 and Formula-2) showed the significant protection against formaldehyde induced pain and it is also found that, the new formulations are more active in antiinflammatory activities as well as antirheumatic activities in compare with pure drug.

# **Table-1 Grouping for animal studies**

<b>Controlled Group</b>		
Group-1	Formaldehyde[ Injected ] + Normal Saline [ Orally administered ]	
Test Groups		
Group-2	2 Formaldehyde[Injected] + Formula-A [Orally administered]	
Group-3	Formaldehyde[ Injected ] + Formula-B [ Orally administered ]	
Standard Group		
Group-4	Formaldehyde[ Injected ] + Diclofenac potassium [ Orally administered ]	

# Table- 2 : Percentage of inhibition of Paw volume produced by different formulation of Diclofenac potassium.

Group	Paw Volume	Mean ± SEM	% of Inhibition
	0 <sup>th</sup> Day (mm)	10 <sup>th</sup> Day (mm)	(10 <sup>th</sup> Day)
Group-1	$0.9433 \pm 0.0284$	$2.002 \pm 0.0104*$	
Group-2	0.9317 ± 0.713*	$1.1116 \pm 0.0221*$	82.59 %
Group-3	$0.8923 \pm 0.265*$	1.195 ± 0.0423*	71.41 %
Group-4	$0.9400 \pm 0.392*$	$1.256 \pm 0.0341*$	70.15 %

Values are mean  $\pm$  SEM (n=6). \*p < 0.05

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