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Polymeric Prodrugs of Diclofenac and *in vivo* Evaluation

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Abstract: The present study deals with the synthesis of prodrugs of diclofenac through an ester and amide linkage to PEG 1500 (polyethylene glycol) and PEG 6000. Diclofenac is most commonly and widely used OTC drug for analgesic and anti inflammatory activity. The prodrugs synthesized were characterized by FT-I.R and N.M.R. *In vitro* dissolution studies were carried out for drug release at pH 1.2 and 7.2. The drug release profile indicated release of more drug at pH 7.2. Anti inflammatory activity was carried out on Male Sprague-Dawley rats and found to be equipotent as standard drug. Ulcer protecting activity was performed using pylorus-ligation method and found to be more protective than standard drug.
Key Words: Diclofenac, polyethylene glycol, polymeric prodrugs, anti inflammatory activity, ulcer protective.

Introduction:

Diclofenac[1-4] is a commonly used drug for anti inflammatory activity[5]. It is phenyl acetic acid derivative and chemically [2-(2,6-dichloro phenyl)amino phenyl]ethanoic acid. The drug was commonly used in India as OTC[6] NSAID pain killer. The recommended dose of diclofenac is 100-150 mg/day in divided doses[1-2]. The mechanism of NSAIDs is by their inhibition of COX (cyclooxygenase) enzymes required for the production of prostaglandins[5,7,8]. The major adverse effect associated with the use of diclofenac is ulcer formation. Hence, in prescription containing diclofenac an anti histaminic drug is recommended[1-2, 6]. Diclofenac sustained release tablets were developed using HPMC (hydroxy propyl methyl cellulose)[9]. By direct compaction and compression using xanthan gum as sustained release agent[10], diclofenac tablets were developed by another group. Based on the available literatures, it was thought to develop a novel drug delivery system by polymeric prodrug approach.

Prodrugs

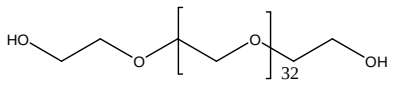
Prodrug[11] as such is inactive, after entering into body the drug is released in active form[12-15]. Prodrugs are classified as carrier linked, Tripartite, mutual, polymeric, bioprecursor[16]. The word polymeric prodrug was

proposed and introduced by Prof. H. Ringsdorf. The model consisted of polymeric backbone drug, linker, spacer and solubilizing agent[17]. The polymers which are used as polymeric backbone are PEG (polyethylene glycol), PVP (poly vinyl pyrrolidone)[18]. In the study, PEG 1500 and PEG 6000[19-20] were selected as polymeric backbone. The drugs are bonded covalently to the polymer via ester, amidic linkage[21]. The attachment of drug to polyethylene glycol is known as PEGylation for drug delivery[22].

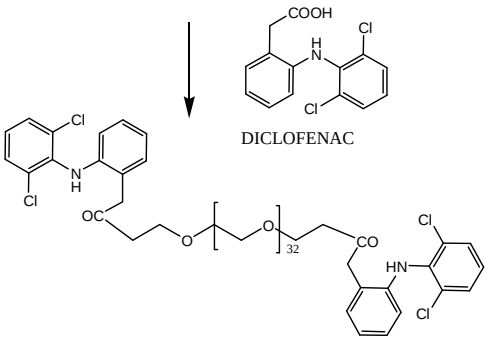
Materials and Methods:

Diclofenac drug was obtained from Hetero Drugs, Hyderabad. PEG 1500, PEG 6000, DMF (Di methyl formamide), DCC (Dicyclo hexyl carbodiimide), DMAP (Dimethyl amino pyridine), Glycine and other solvents of reagent grade were purchased from SD Fine chemicals.

Animal studies were done in the department of pharmacology, Geethanjali College of Pharmacy, Keesara, Ghatkesar and approved by Animal Ethics Committee, Regd no: 1648/PO/a/12/CPCSEA-GCOP-IAEC-03/2013 for anti inflammatory and ulcer protecting activity.



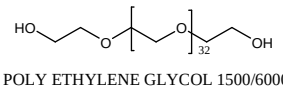
POLY ETHYLENE GLYCOL 1500/6000



POLY ETHYLENE GLYCOL 1500/6000- DICLOFENAC

Synthesis of PEG 1500/6000-Diclofenac

PEG 1500/6000 1.5gms and 1.6 ml of pyridine were taken in a round bottom flask, to it a solution of DCC 1gm and 0.6 gms of DMAP in 10 ml DMF was added. The flask was kept in an ice bath and temperature was maintained 0^{o} C, to this diclofenac was added for 10 mints. The flask was then placed on a magnetic stirrer, attached with a condenser and was allowed for coupling reaction for 7 days at room temperature. The residue obtained was dissolved in DCM (dichloro methane) and reprecipitated by excess of cold diethyl ether[23]. The product obtained was confirmed by TLC (Thin Layer Chromatography) using mobile phase DCM: methanol 3:2. Finally, confirmed by FT-I.R and N.M.R.



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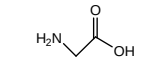
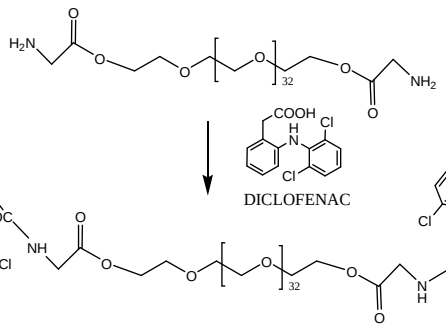


GLYCINE



POLY ETHYLENE GLYCOL 1500/6000-GLYCINE-DICLOFENAC

Synthesis of PEG1500/6000-Glycine

PEG 1500/6000 1.5 gms and 1.6 ml of pyridine were taken in a two necked round bottom flask and 20 ml of DMF was added and placed on a magnetic stirrer. To this 0.18 gms of glycine was added in small portions for 3 hrs maintaining at room temperature. Then the contents were refluxed by attaching a condenser, maintaining a temperature of 130^{o} C for 21 hrs. The residue obtained was dissolved in DCM and reprecipitated by excess of cold diethyl ether. The formation of the product was confirmed by TLC and mobile phase DCM : methanol 3:2 and structures were confirmed by FT-I.R and N.M.R.

Synthesis Of PEG1500/6000-Glycine-Diclofenac

A solution of DCC 1 gm in 10 ml DMF and 0.6 gms of DMAP in 10 ml of DMF were taken in a beaker. The mixture was added drop by drop to another beaker containing a solution of 0.4 gms of PEG 1500/6000-glycine in 20 ml of DMF. Diclofenac 1 gm was added in portions to the above mixture at 0^{o}C for 10 mints. The contents were transferred to a round bottom flask fitted with a condenser and placed on magnetic stirrer. The coupling reaction was carried out for 7 days at room temperature. The residue obtained was dissolved in DCM and reprecipitated by excess of cold diethyl ether. The product obtained was confirmed by TLC and mobile phase DCM : Methanol 3:2 and structures were confirmed by FT-I.R and N.M.R.

***In vitro* drug Release Studies**

The synthesized prodrugs were subjected for dissolution studies at pH 1.2 and 7.2, temperature was maintained at 37^{o} C. The λ_{max} was determined for diclofenac and found to be 240 nm, aliquots of 5 ml were collected at intervals of 0, 5, 10, 15, 30, 45, 60 mints, sink conditions were maintained. A standard graph was plotted and % drug release was found. A graph of time v/s cumulative drug release was plotted at pH 1.2 and 7.2.

Anti Inflammatory Activity

Male Sprague-Dawley rats weighing 100-150 gms were divided into 6 groups. The method for anti inflammatory activity followed was Carrageenan induced Rat Paw Edema. Ist group control, IInd group standard drug, IIIrd, IVth, Vth and VIth groups received diclofenac prodrugs, injected in subplantar region in the left and right hind paws and the swelled volume was measured by Dolphin, India Plethysmometer. The measurements were taken at intervals of 1, 3, 6 hrs[24]. The % inhibition was calculated by formula

% Inhibition = $(v_t\text{-}v_0)_{control}\text{-}(v_t\text{-}v_0)_{test}/(v_t\text{-}v_0)_{control}$ X 100

Ulcer Protecting Activity

For ulcer protecting activity Pylorus-ligation method was followed. The animals weighing 100-150 gms were fastened overnight, anaesthetized, incised 1 cm long in abdomen below the sternum. The stomach was exposed and a thread was passed round pyloric sphincter, a knot was tied. Abdomen was closed with sutures and animals were kept in a separate cage, allowed to recover. Ist group control, IInd group received standard drug diclofenac 10 mg/kg, IIIrd, IVth, Vth, VIth groups were injected prodrugs of diclofenac and after performing pylorus ligation kept in separate cages, after 4 hrs the animals were sacrificed and abdomen was cut open, stomach was removed and washed under running tap water, then placed on glass slide and observed on microscope at 10 X magnification for ulcers[25].

Ulcer Index was calculated by

% Inhibition of Ulceration= $\text{Ulcer Index}_{control}\text{-Ulcer Index}_{test}/\text{Ulcer Index}_{control}$ X 100.

Results and Discussion:

Table 1: Physical Properties of Diclofenac prodrugs

S.No	Compound	Solubility	Colour	Melting Point	I.R Spectra	N.M.R Spectra
1	Diclofenac	Methanol	White	262-270^{o} C	OH of COOH-3259, 2^{0} NH-3387, C=O-1602.	2.5δ-3.5 δ (s, 2H) 3.6-7.2δ-(m, 7H) 7.5δ-(s, 1H) 10.1 δ (s, 1H)
2	PEG 1500-Diclofenac	Dichloro methane	White	228-237^{o} C	C=O-1626, C-O-C-1159, CH-str-2850, C-O str- 1186, 2^{0}NH-3327.	1.7-1.9δ-(m, 44H), 2.2-2.5δ (d, 4H), 3.3-7.4δ (m, 14H), 7.5-7.9 δ (s, 2H).
3	PEG1500-Glycine-Diclofenac	Dichloro methane	Red	229-239^{o} C	2^{0}NH-3124, 3327, C-O-C-1157, C=O 1626, CH-str-2850, C-N str-1311.	1.1-1.9δ (m, 34H) 2.5-3.3δ (s, 4H) 3.5-7.9δ (m, 14H) , 8.2-8.4 δ (d, 4H)
4	PEG6000-Diclofenac	Dichloro methane	White	210-222^{o} C	C=O-1626, C-O-C- 1159, CH-str-2850, C-O str-1186, 2^{0} NH-3327.	1.7-1.9δ-(m, 44H), 2.2-2.5δ (d, 4H), 3.3-7.4δ (m, 14H), 7.5-7.9 δ (s, 2H).
5	PEG6000-Glycine-Diclofenac	Dichloro methane	Brick Red	225-236^{o} C	2^{0}NH-3124, 3327 C-O-C-1159, CH str-2850, C-O str-1186, C=O-1626, C-N str 1311.	1.1-1.9δ (m, 34H) 2.5-3.3δ (s, 4H) 3.5-7.9δ (m, 14H) , 8.2-8.4 δ (d, 4H)

The spectral data (2 and 4 of table 1) confirms the formation of ester and spectral data (3 and 5 of table 1) confirms the formation of amide with respect to COOH group of diclofeanc.

Figure 1: I.R Spectra of Diclofenac

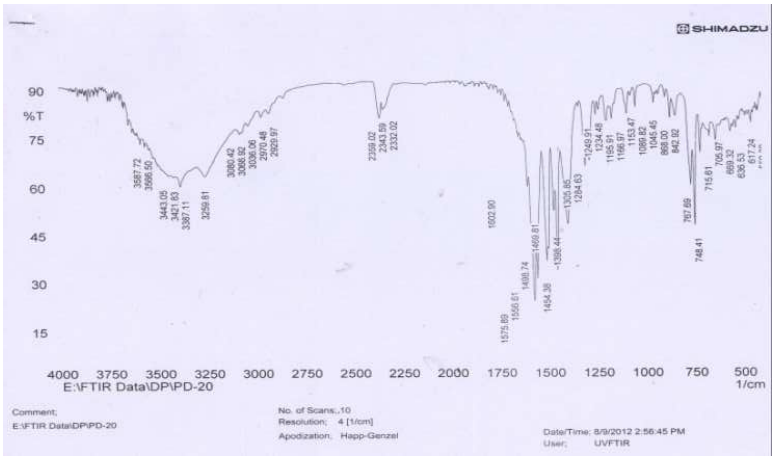


Figure 2: I.R Spectra of PEG 1500/6000-Diclofenac

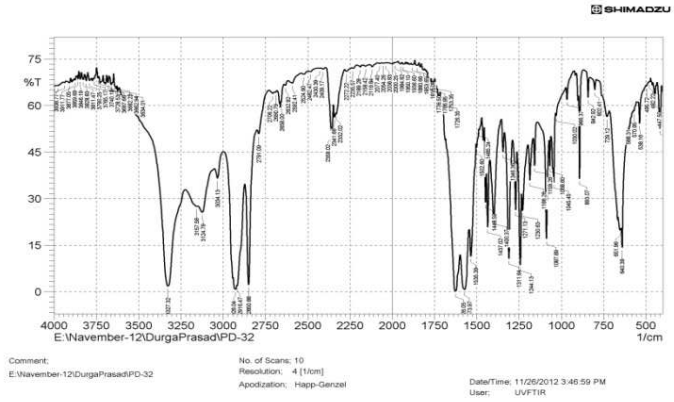


Figure 3: I.R Spectra of PEG 1500/6000-Glycine-Diclofenac

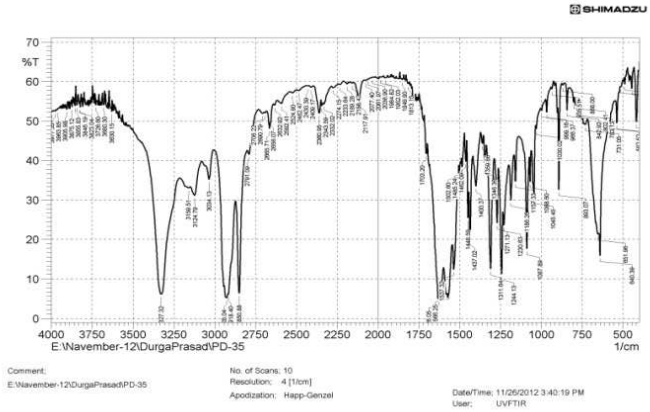


Figure 4: N.M.R Spectra of Diclofenac

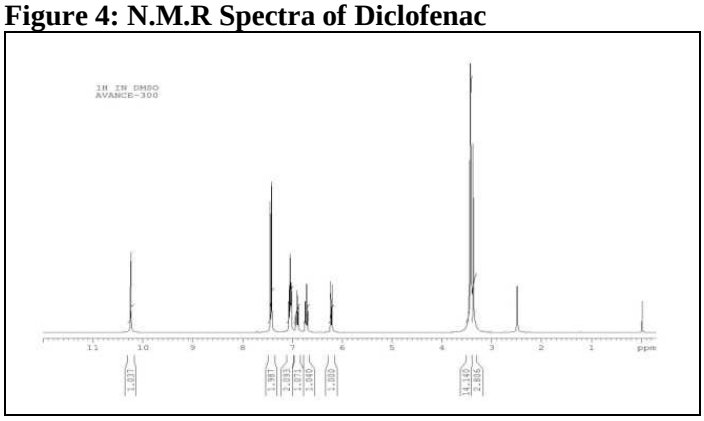


Figure 5: N.M.R Spectra of PEG 1500/6000-Diclofenac

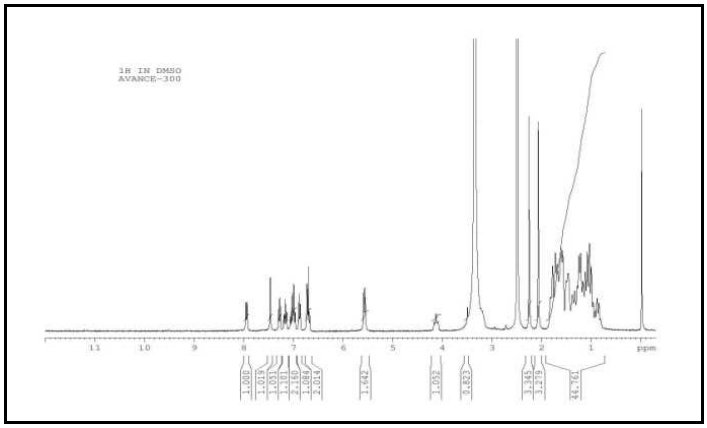


Figure 6: N.M.R Spectra of PEG 1500/6000-Glycine-Diclofenac

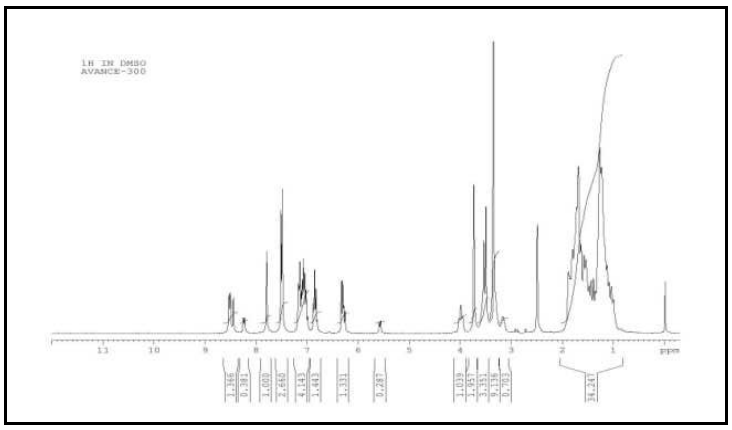


Figure 7: *In vitro* drug release profile of PEG 1500 prodrugs at pH 1.2 and 7.2.

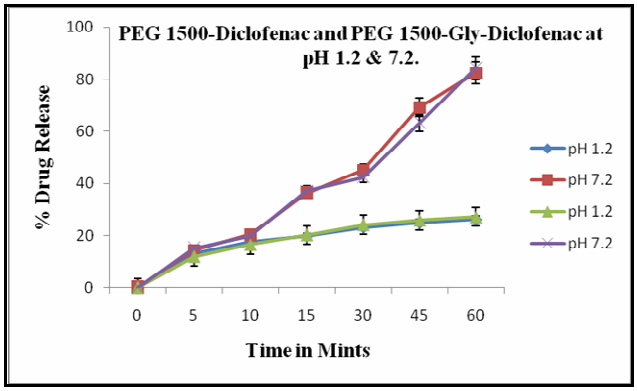
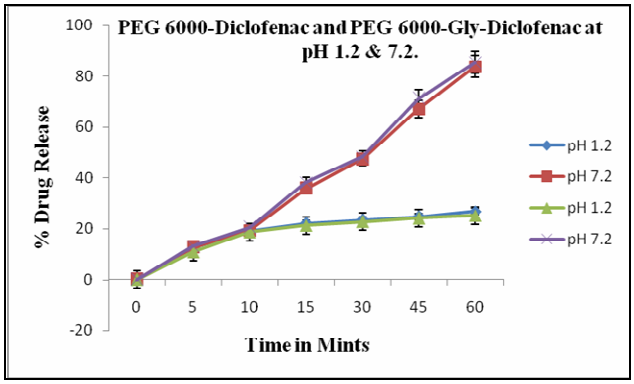


Figure 8: *In vitro* drug release profile of PEG 6000 prodrugs at pH 1.2 and 7.2.



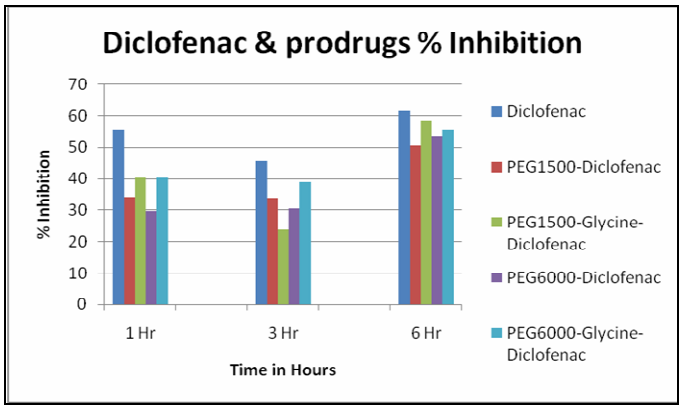
From the data obtained regarding drug release studies of prodrugs of diclofenac, it clearly indicated that release was more at a pH of 7.2 than at pH 1.2.

Table-2: Anti inflammatory Activity

Groups	Change in Paw volume (ml) mean±SEM & % Inhibition		
	1 hr	3 hr	6 hr
Control	0.47±0.01	0.59±0.01	0.65±0.02
Diclofenac	0.21±0.02 (55.32)	0.32±0.02 (45.77)	0.25±0.02 (61.54)
PEG 1500-Diclofenac	0.31±0.03 (34.04)	0.39±0.02 (33.90)	0.32±0.02 (50.77)
PEG 1500-Gly-Diclofenac	0.28±0.02 (40.43)	0.45±0.01 (23.73)	0.27±0.02 (58.46)
PEG 6000-Diclofenac	0.33±0.02 (29.78)	0.41±0.02 (30.51)	0.30±0.02 (53.38)
PEG 6000-Gly-Diclofenac	0.28±0.02 (40.43)	0.36±0.01 (38.90)	0.29±0.01 (55.38)

Values are mean±SEM, n=6, one way ANOVA $p<0.05$ vs control.

Figure 9: Anti inflammatory activity of Diclofenac prodrugs

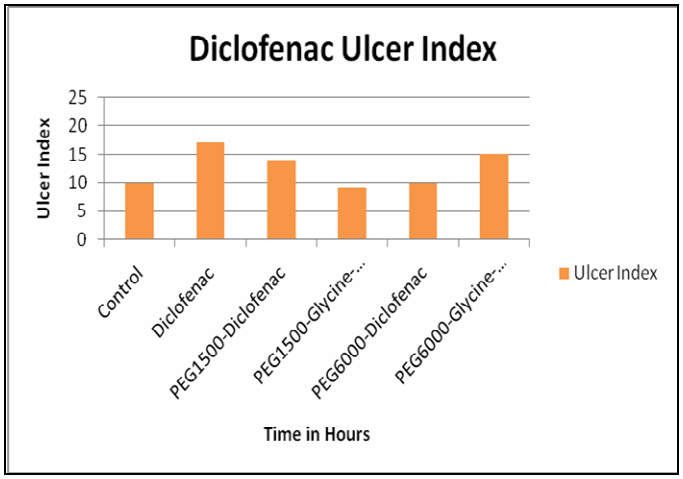


From the data obtained for anti inflammatory activity all of the polymeric prodrugs are equally potent as that of standard drug. From the data of ulcer index also clearly indicated that all of the prepared polymeric drug had a good ulcer protecting activity than the standard drug diclofenac.

Table-3: Gross Ulcer Index of Aceclofenac prodrugs

S.No	Treatment	Ulcer Index
1	Control	10
2	Diclofenac	17
3	PEG 1500-Diclofenac	14
4	PEG 1500-Gly-Diclofenac	09
5	PEG 6000-Diclofenac	10
6	PEG 6000-Gly-Diclofenac	15

Figure 10: Ulcer Index of Diclofenac prodrugs



Conclusion:

Hence from this study, we can conclude that the polymeric prodrugs of diclofenac can be useful as anti inflammatory as well as ulcer protective there by overcoming the adverse effect of the drug as such.

Acknowledgements:

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