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Synthesis of nimesulide conjugates, *in vitro* and *in vivo* evaluation

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ABSTRACT

In this study nimesulide drug conjugates were synthesized using polyethylene glycol 1500 and 6000, covalently bonded through ester and amidic linkages. Major complications of nimesulide included upper G.I tract irritation and it is used with an Antihistaminic drug. Hence to overcome the complications PEG 1500-Nimesulide, PEG 1500-Glycine-Nimesulide, PEG 6000-Nimesulide, PEG 6000-Glycine-Nimesulide were synthesized. The prodrugs synthesized were characterized by I.R, N.M.R, in vitro dissolution, in vivo anti inflammatory activity and ulcer protecting activity. I.R and N.M.R confirmed the structure of prodrugs. In vitro dissolution study revealed that the release of drug from drug conjugates was high at pH 7.2 as compared to pH 1.2. In vivo anti inflammatory activity was retained after conjugation of drug with polymers. Ulcer protecting activity was compared with standard nimesulide. Drug conjugates had more ulcer protecting activity.

Key words: polyethylene glycol, nimesulide drug conjugates, anti inflammatory activity, ulcer Index.

INTRODUCTION

Nimesulide is chemically N-(4-Nitro-2-phenoxyphenyl) methane sulfonamide [1], NSAID which is selective COX-2 inhibitor and used widely for anti inflammatory activity with a maximum dose of 200 mg per day [2]. The common adverse effect associated with usage of NSAID's is they irritate gastric mucosa and increase formation of ulcers by inhibiting the action of prostaglandins, which protect gastric mucosa [3]. Long term usage of NSAID's, cause gastric erosion. The mechanism of action of NSAID's is inhibition of prostaglandin synthesis via blocking of COX-1 and COX-2 [3]. NSAID's inhibit conversion of Arachidonic acid to PGE_2 (Prostaglandin E_2) [4]. Hence, the use of NSAID's are associated with Anti histaminic drugs [1-3]. To overcome the concurrent usage of Antihistaminic drugs with NSAID's a prodrug approach was initiated.

Prodrugs
Polymeric prodrugs was proposed and identified by Prof. H. Ringsdorf in the year 1975. Biodegradable polymers were used as carrier and the drug is directly or indirectly attached through a spacer or without spacer onto the

polymeric backbone. The polymers used were HPMA (Hydroxy propyl methacrylamide), Dextrans, Proteins and Polyethylene glycol [5].

Ideal properties of prodrugs include enzymatically cleavable, no pharmacological activity, non toxic [6-8]. Prodrugs are classified as carrier linked prodrugs, Tripartite prodrugs, mutual prodrugs, polymeric prodrugs and Bioprecursor [6]. A special type of drug delivery system is covalent polymer-drug conjugate where in the drug is covalently linked to a polymeric backbone [9]. The drugs which are covalently attached to polyethylene glycol, is known as PEGylation. Polyethylene glycols of different molecular weights were used. Prodrugs of indomethacin were synthesized by using HEMA (Hydroxy ethyl methacrylate) as polymeric backbone [10]. Acyclovir and Valacyclovir prodrugs were synthesized by using polyethylene glycol and found to be stable at pH 7.4 and 5.5 [11]. PEG-metronidazole conjugates were synthesized by ester linkage [12]. Linker molecules used were Aminoacids, Chymotrypsin, Glycosidase and Phosphodiesterase [13]. Ester, Amide, Anhydride prodrugs were found to have improved G.I (Gastro intestinal) tolerance of NSAID's [14]. Nimesulide dissolution was enhanced by using modified PEG4000 and PVP K-30 [15]. In this study polyethylene glycol 1500 and polyethylene glycol 6000 prodrugs were developed.

Based on the literature the study was aimed at synthesizing prodrugs of nimesulide using polyethylene glycol 1500 and 6000 as polymeric backbone, as well as with spacer, an aminoacid Glycine. The synthesized drug conjugates were characterized by FT-IR and ^{1}HNMR. *In vitro* dissolution studies were conducted for the prodrugs synthesized. *In vivo* anti inflammatory activity and ulcer protecting activity was also performed for the drug conjugates.

MATERIALS AND METHODS

Nimesulide drug sample was obtained from Dr Reddy's Labs, Hyderabad, Polyethylene glycol 1500 and 6000, were purchased from Merck, Mumbai. DMF (Dimethyl formamide), was purchased from Finar, Ahmedabad, DCC (Dicyclohexyl carbodiimide) was purchased from Arva synthesis, Hyderabad, 4,4/ DMAP (Dimethyl amino pyridine) was purchased from SD Fine chemicals, Mumbai. All other reagents and solvents of reagent grade were purchased from SD Fine chemicals, Mumbai.

Melting points were recorded using Melting point apparatus Biotech India, Mumbai.
I.R spectras were recorded on Schimadzu 8400 S using KBr pellet in range of 4000 cm^{-1} to 400 cm^{-1}. ^{1}HNMR were recorded on Bruker 300 Spectrometer in DMSO (Dimethyl sulphoxide). Dissolution studies were performed on Electrolab TDT-08L 8 basket type. Wavelength (λ_{max}) and absorbance values were determined by using Thermoscientific UV-10 spectrophotometer.

In vivo Evaluation
All the animals were obtained from Department of Pharmacology, Geethanjali College of Pharmacy, Keesara, and approved by Animal Ethics Committee, Regd no: 1648/PO/a/12/CPCSEA-GCOP-IAEC-03/2013 for anti inflammatory activity and ulcer protecting activity. *In vivo* studies were carried out on Male Sprague-Dawley rats.
Synthesis of PEG 1500/6000-Nimesulide

PEG 1500/6000 1.5gms and 1.6 ml of pyridine were taken in a round bottom flask, to it a solution of DCC (Dicyclohexyl Carbodiimide) 1gm and 0.6 gms of DMAP (Dimethyl amino pyridine) in 10 ml DMF (Dimethyl formamide) was added. The flask was kept in an ice bath and temperature was maintained 0^{o} C, to this 0.5 gms of nimesulide 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 dichloro methane and reprecipitated by using excess of cold diethyl ether. The product obtained was confirmed by TLC (Thin Layer Chromatography) using dichloro methane : methanol in ratio 3:2 as mobile phase.

Further the prodrugs were characterized by I.R and N.M.R.

Synthesis of PEG 1500/6000-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 magnetic stirrer. To this 0.18 gms of glycine was added in small portions for 3 hrs maintaining 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 dichloro methane and reprecipitated by using excess of cold diethyl ether. The product was confirmed by TLC using mobile phase dichloro methane : methanol 3:2.

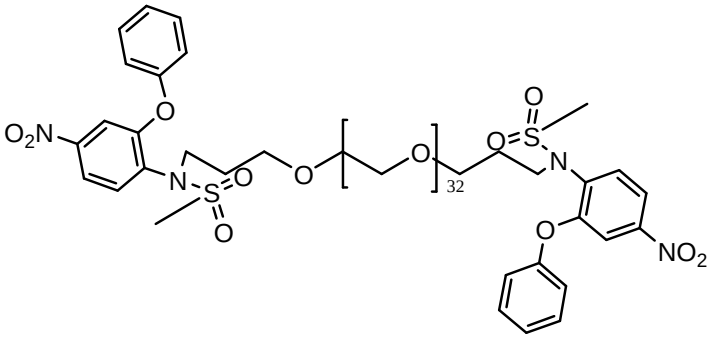
The polymeric backbone with spacer was characterized by I.R and N.M.R.

Synthesis of PEG 1500/6000-Glycine-Nimesulide

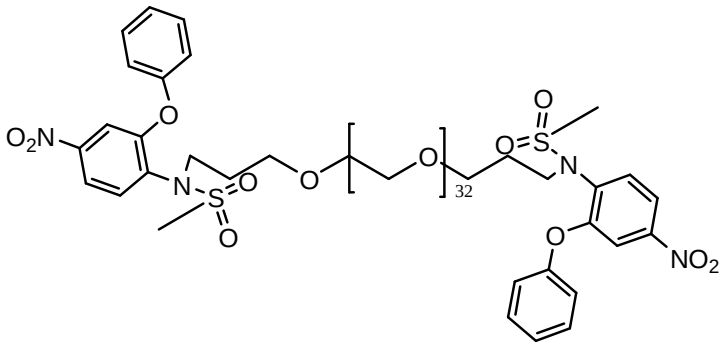
A solution of DCC 1 gm in 10 ml of DMF and 0.6 gms of DMAP in 10 ml DMF were taken in a beaker. The mixture was added drop by drop to another beaker containing a solution of 0.4 gms of PEG 1500/6000-Glycine in 20 ml of DMF. Nimesulide 1 gm was added in portions to the above mixture at 0^{o} C for 10 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 dichloro methane and reprecipitated by using excess of cold diethyl ether. The product obtained was confirmed by TLC Using dichloro methane : methanol 3:2 as mobile phase [16].

The prodrugs were further characterized by I.R and N.M.R.

Fig No.1 Structures of synthesized prodrugs of Nimesulide



POLY ETHYLENE GLYCOL 1500- NIMESULIDE



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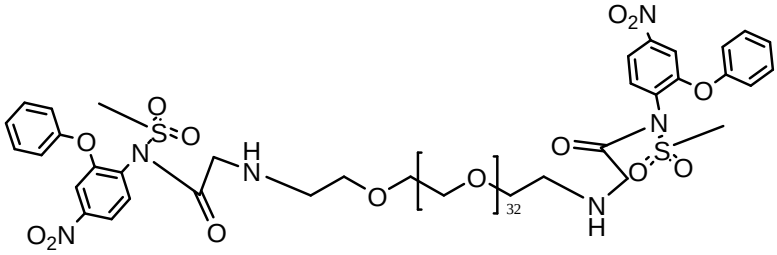
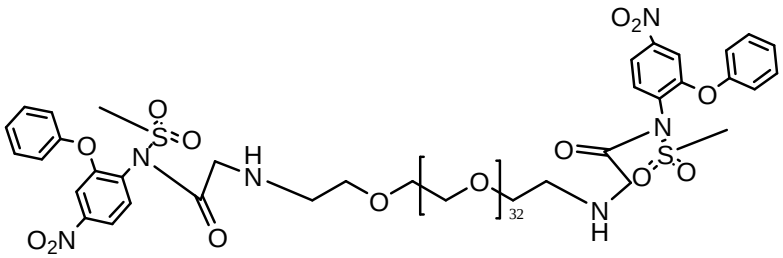


POLY ETHYLENE GLYCOL 1500-GLYCINE-NIMESULIDE



POLY ETHYLENE GLYCOL 6000-GLYCINE-NIMESULIDE

In vitro Drug Release studies

The synthesized prodrugs weight equivalent to 10 mg were taken and placed in baskets of Electrolab and 900 ml of 1.2 pH buffer was taken and dissolution was carried out at 37^{o} C. 5 ml of aliquots were collected at intervals of 0, 5, 10, 15, 30, 45, 60 mints and sink conditions were maintained by 1.2 pH buffer. λ_{max} was determined for nimesulide and found to be 220 nm. A standard graph was plotted and % drug release for the prodrugs of nimesulide was found. A graph was plotted time v/s cumulative % drug release.

The same procedure was followed for release studies at pH 7.2.

Anti inflammatory Activity

The method followed was Carrageenan induced Rat Paw Edema. The animals weighing 100-150 gms were divided into 6 groups. Ist group received no drug, IInd group received 10 mg/kg stock solution of Nimesulide standard drug, IIIrd, IVth, Vth and VIth groups received polymeric prodrugs injected in subplantar region in the left and right hind paws. The swelled volume of paws was measured by Dolphin, India Plethysmometer before injection and noted as V_o and after injections as V_t at 1, 3, 6 hrs. The % swelling inhibition was calculated by (n=6)

% Inhibition = $(^{V}{}_{t}\text{-}^{V}o)_{control}\text{-}(^{V}{}_{t}\text{-}^{V}{}_{o})_{test} / (^{V}{}_{t}\text{-}^{V}{}_{o})$ X 100 [17], [19], [20].

Statistical Analysis

The results were expressed as mean± S.E.M using One way ANOVA, p value < 0.05 was considered significant.

Ulcer Protecting Activity

The method used for ulcer protecting activity was Pylorus-ligation method. The animals weighing 100-150 gms were fastened overnight, anaesthetized, incised 1 cm long in abdomen below the sternum. Stomach was exposed and a thread was passed round pyloric sphincter, a knot was tied. Abdomen wall was closed with sutures. The animals were kept in a separate cage and allowed to recover. Next group was injected standard drug nimesulide 10 mg/kg after performing pyloric ligation and kept in separate cage. Next groups were injected polymeric prodrugs 10 mg/kg dose and kept in separate cages. After 4 hrs, all the animals were sacrificed and abdomen was cut open and entire stomach was removed and washed under running tap water, then placed on glass slide and observed on microscope at 10 X magnification for ulcers (n=6).

Area of gross damage (ulceration) was measured by Computerized Video analysis system Metamorph 7.0 Molecular devices Downington, PA USA.

Mean ulcer score was expressed as Ulcer Index.

% Inhibition of Ulceration= $\text{Ulcer Index}_{control}\text{-Ulcer Index}_{test} / \text{Ulcer Index}_{control}$ X 100 [18-20].

RESULTS AND DISCUSSION

I.R and N.M.R spectras confirmed the structures of synthesized prodrugs and were given in table no.1 and representative structures in fig no.1. FT-IR spectra of PEG-prodrugs are shown in fig nos 2, 3, & 4. I.R spectra of the synthesized compounds indicated presence of carbonyl group, ether linkage and aliphatic amine group. N.M.R spectra of the compounds are shown in fig nos 5, 6, & 7. N.M.R spectra have shown presence of methylene protons

and aliphatic amine protons, but absence of aromatic amine protons. *In vitro* dissolution drug release for prodrugs was given in fig nos. 8, & 9. The study clearly indicated the drug release profile of prodrugs at pH 7.2 was more rather than at pH 1.2. *In vivo* anti inflammatory activity for the synthesized prodrugs was given in fig no. 10, which indicated that the prodrugs had similar activity as standard nimesulide. Ulcer index for the prodrugs synthesized was shown in fig no. 11, and were found to have better ulcer protecting activity than nimesulide standard drug.

Table No:1 Physical Properties of synthesized prodrugs

S.No	Compound	Solubility	Colour	Melting Point	I.R Spectra	N.M.R Spectra
1	Nimesulide	Methanol	Yellow	120-126^{o} C	SO_2NH-3481, 3462 2^{0} NH-3524.	3.2δ-(t, 6H) 7-10.5δ-(m, 8H) 10.1δ-(s, 1H).
2	PEG 1500-Nimesulide	Dichloro methane	Yellow	128-136^{o} C	SO_2N-3390, 3327 C-O-C-1116, CH-str-2850, C-O str- 1147.	1-1.5δ-(t, 6H), 1.6-3.5δ (m, 38H), 5.5-10.1δ (m, 18H).
3	PEG1500-Glycine-Nimesulide	Dichlor methane	Yellow	137-146^{o} C	SO_2N-3390, C-O-C-1116, CH-str-2850, C-O str-1147.	1-1.3δ (t, 6H) 1.4-3.5δ (m, 25H) 5.5-7.8δ (m, 16H) 8.0-8.2δ(s, 2H).
4	PEG6000-Nimesulide	Dichloro methane	Yellow	158-167^{o} C	SO_2N-3390, C-O-C- 1116, CH-str-2850, C-O str-1147, C=O-1686, Aliphatic NH-3524.	1-1.3δ (t, 6H) 1.3-3.6δ (m, 46H) 5.5-7.9δ (m, 16H).
5	PEG6000-Glycine-Nimesulide	Dichloro methane	Yellow	132-140^{o} C	SO_2N-3389, C-O-C-1116, CH str-2850, C-O str-1145, C=O-1627, Aliphatic NH-3524.	1-1.3δ (t, 6H) 1.4-4.5δ(m, 17H) 5.5-7.9δ (m, 16H) 8.0-8.2δ (s, 2H).

I.R and N.M.R spectras of synthesized compounds were given below.

Fig No:2 I.R Spectra of Nimesulide

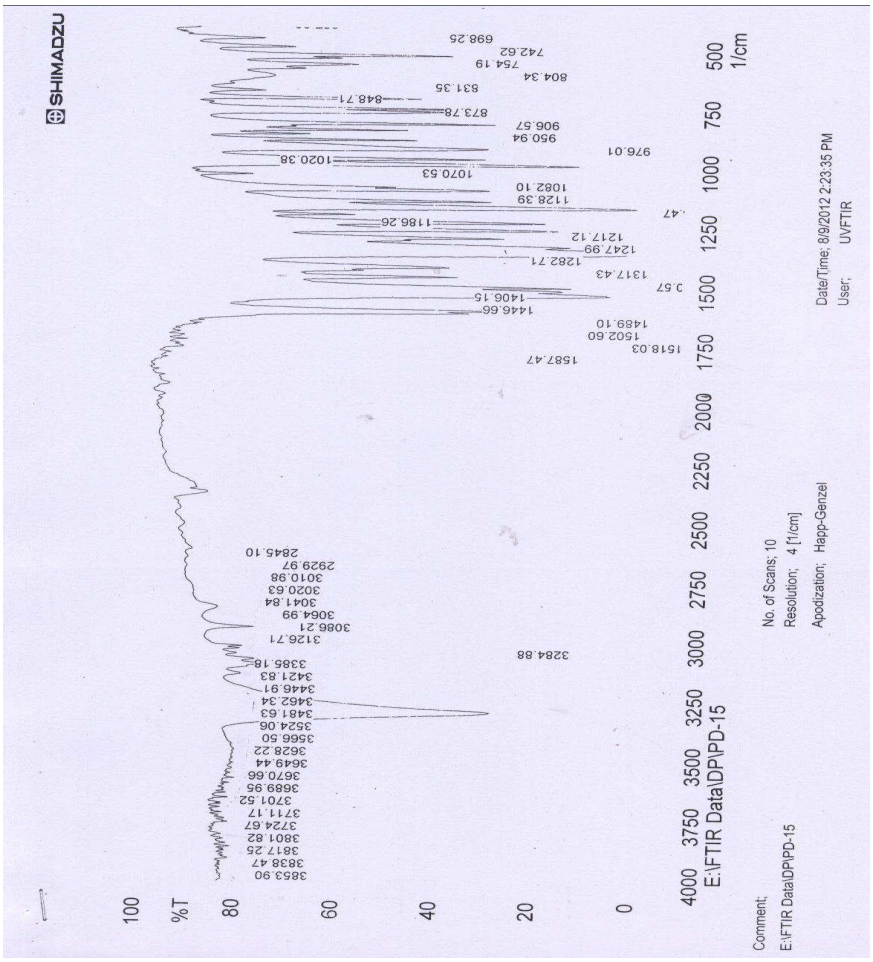


Fig No:3 I.R Spectra of PEG-Nimesulide

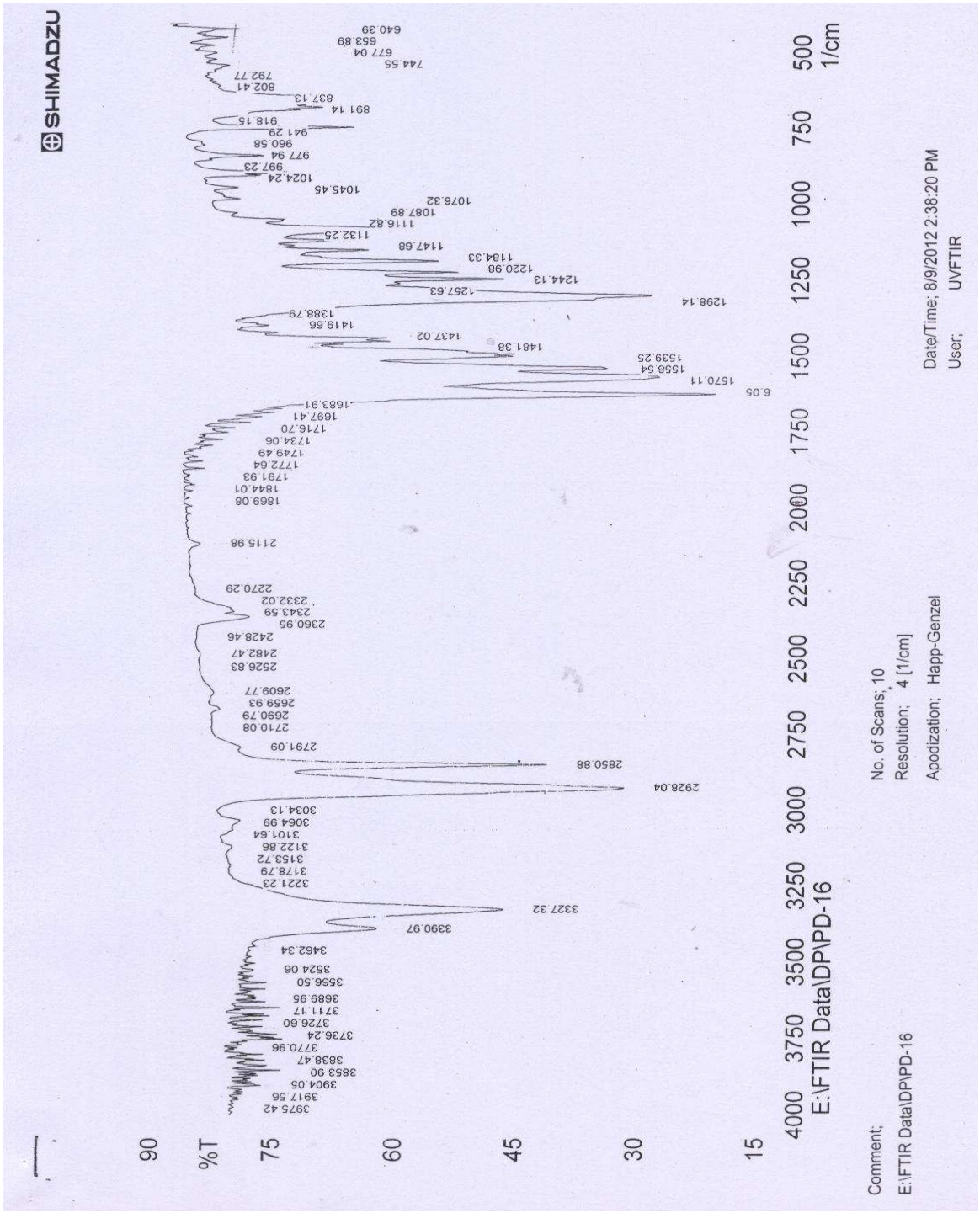


Fig No:4 I.R Spectra of PEG-Gly-Nimesulide

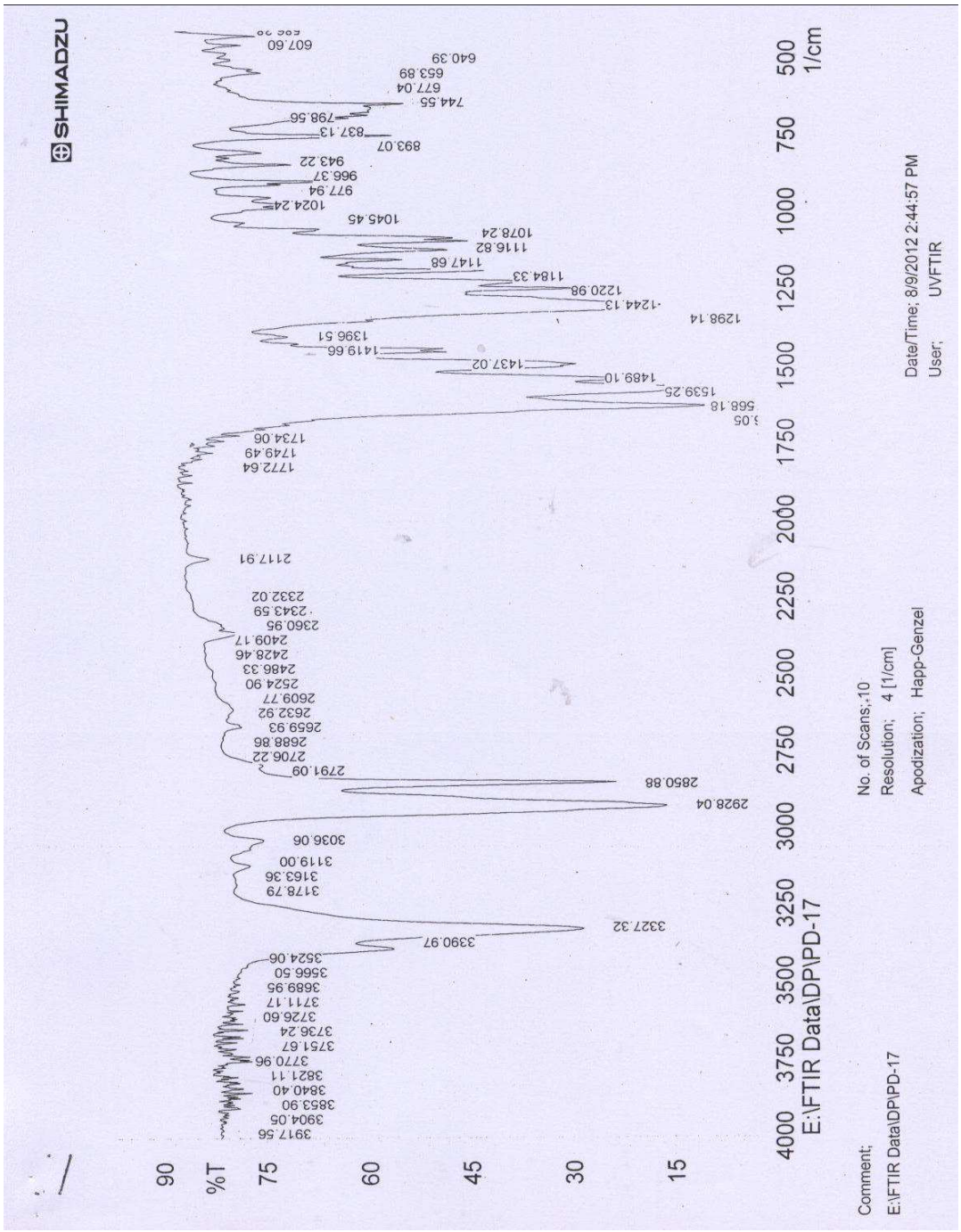


Fig No:5 N.M.R Spectra of Nimesulide

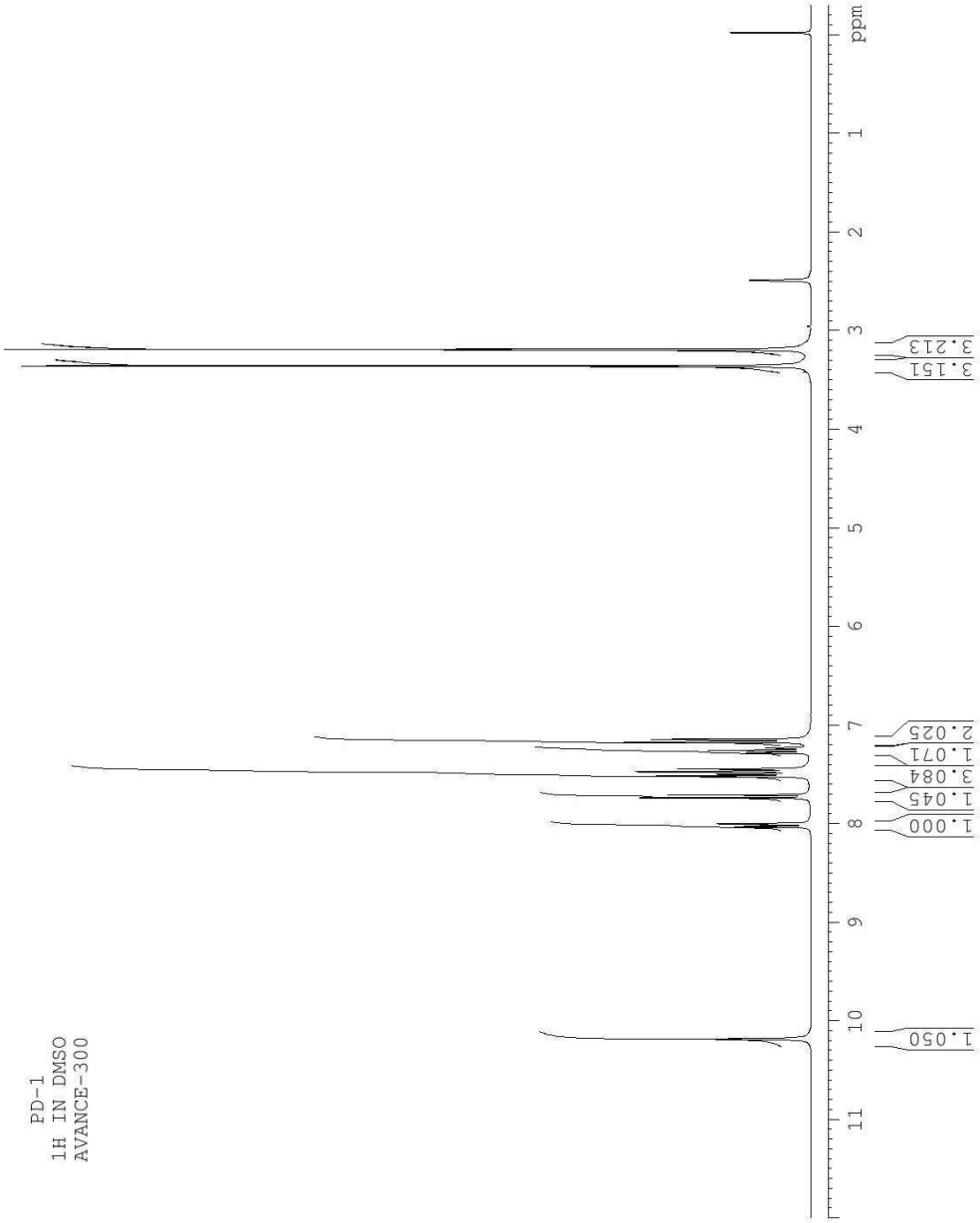


Fig No:6 N.M.R Spectra of PEG -Nimesulide

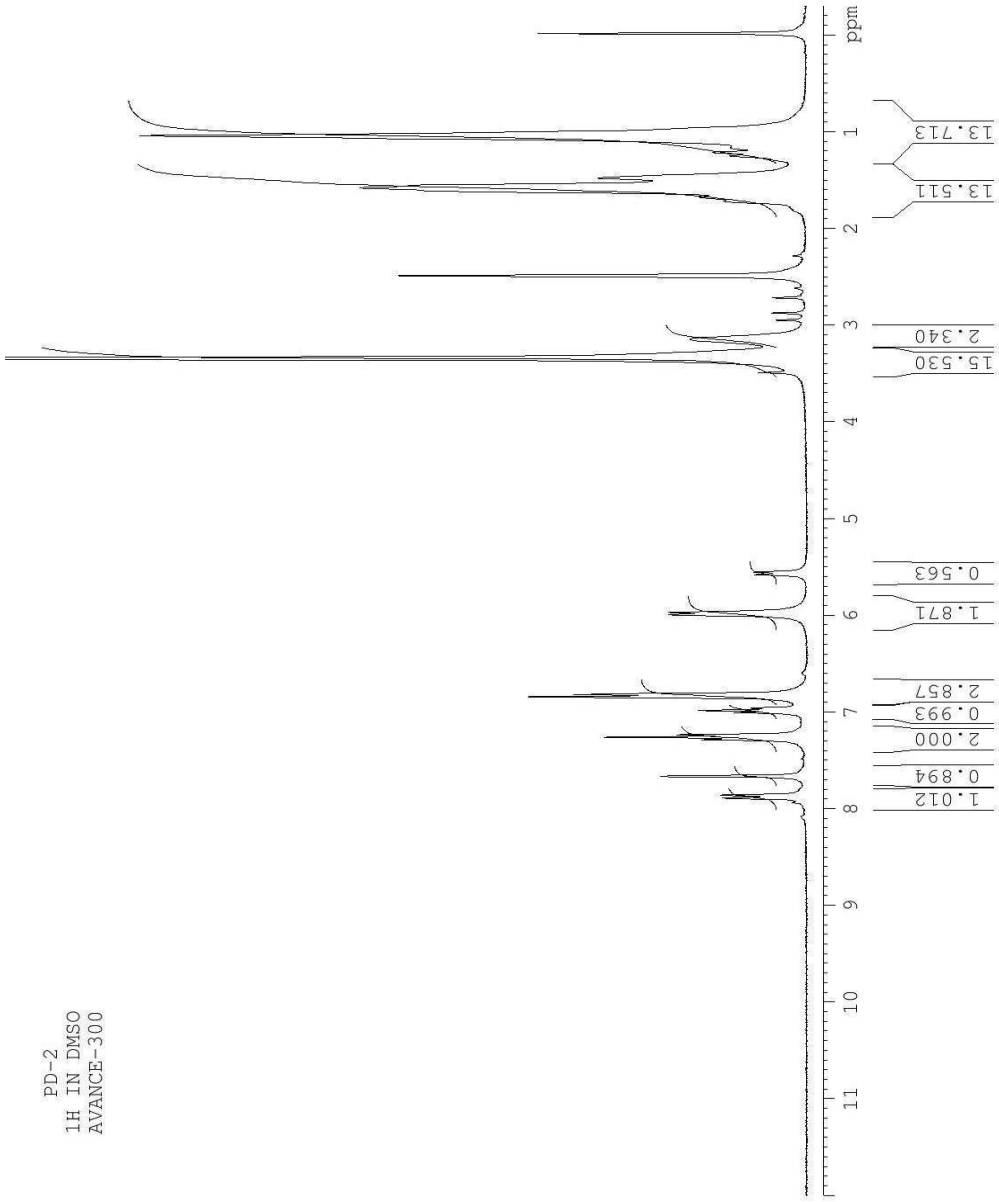


Fig No:7 PEG -Gly-Nimesulide

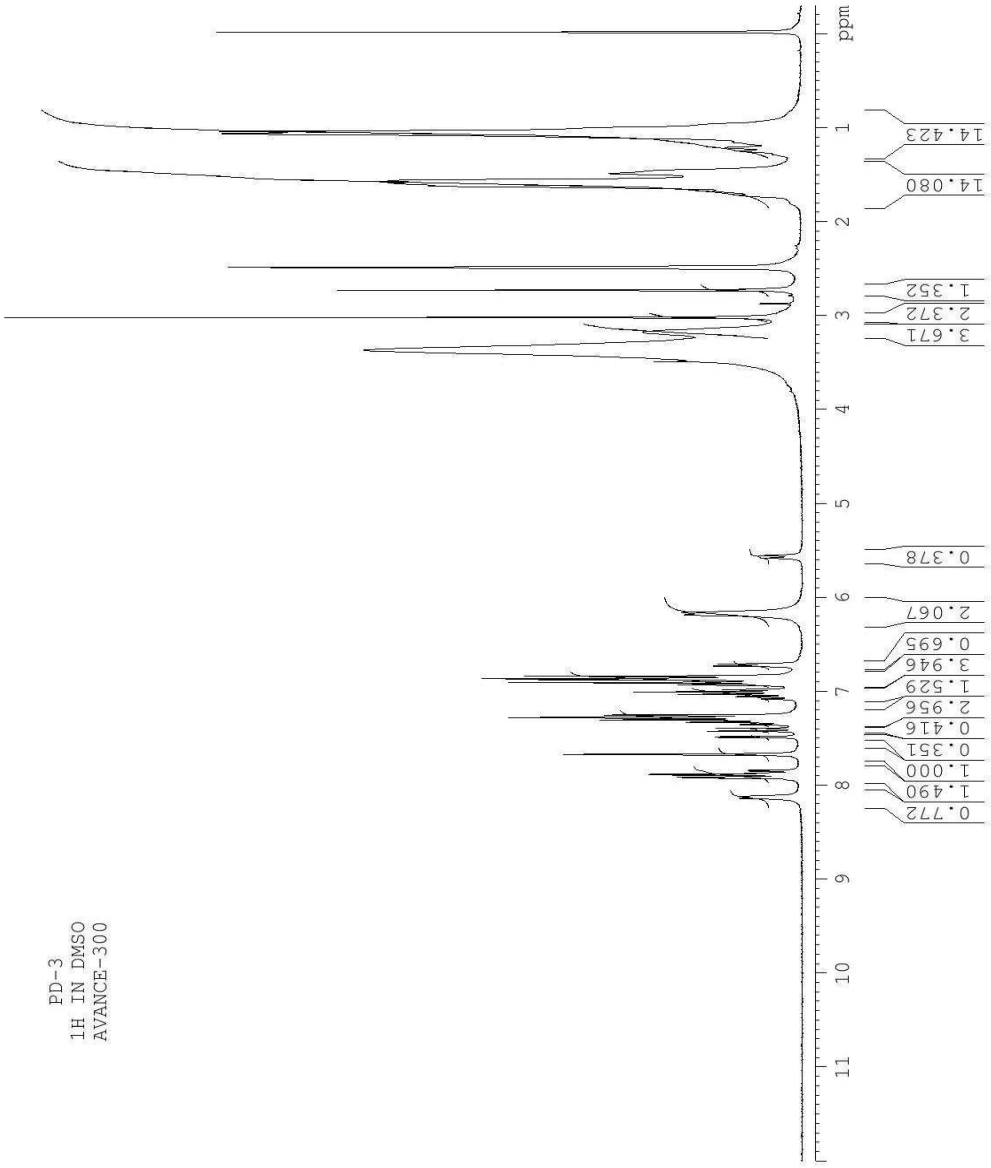


Fig No: 8 *In vitro* drug release of PEG1500-Nimesulide and PEG1500-Gly-Nimesulide at pH 1.2 & 7.2

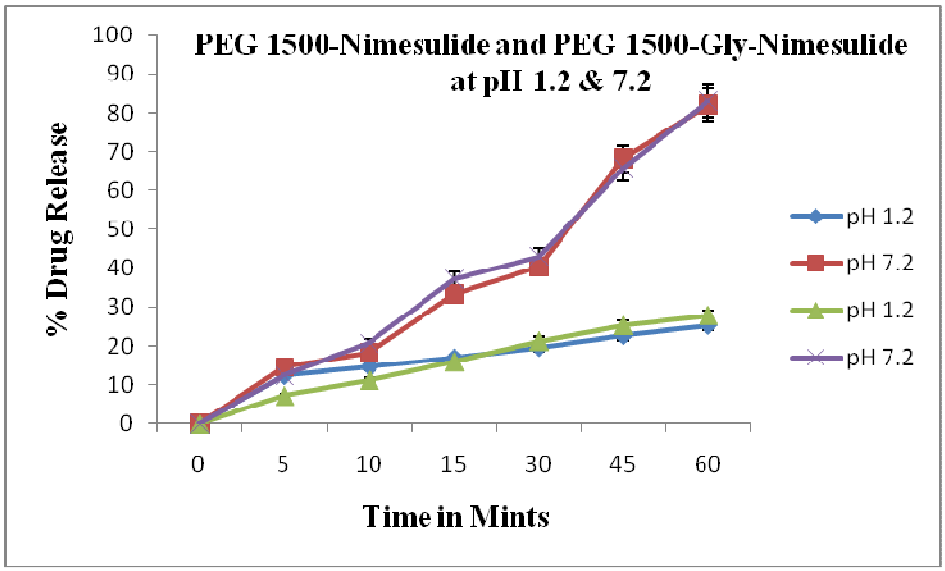


Fig No: 9 *In vitro* drug release of PEG6000-Nimesulide and PEG6000-Gly-Nimesulide at pH 1.2 & 7.2

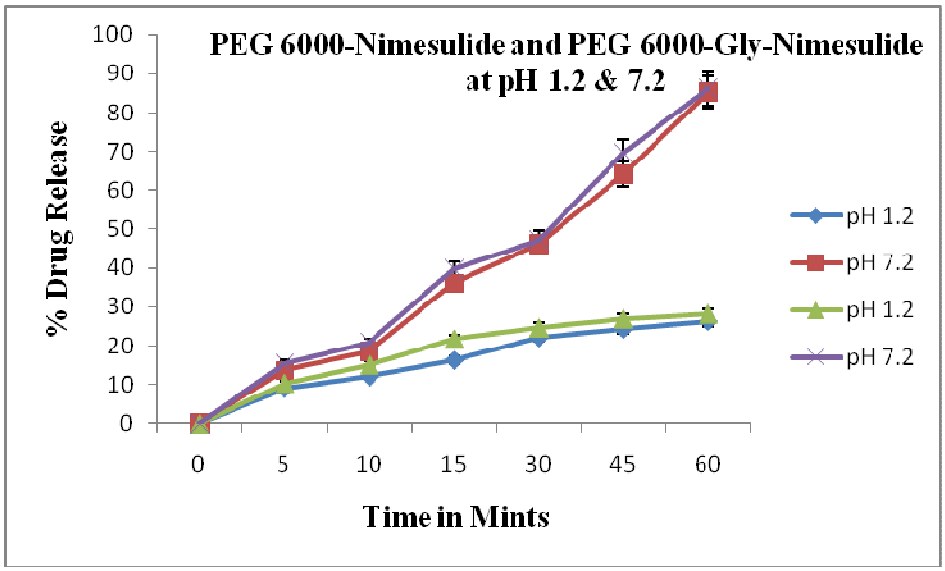


Fig No: 10 *In vivo* Anti inflammatory Activity

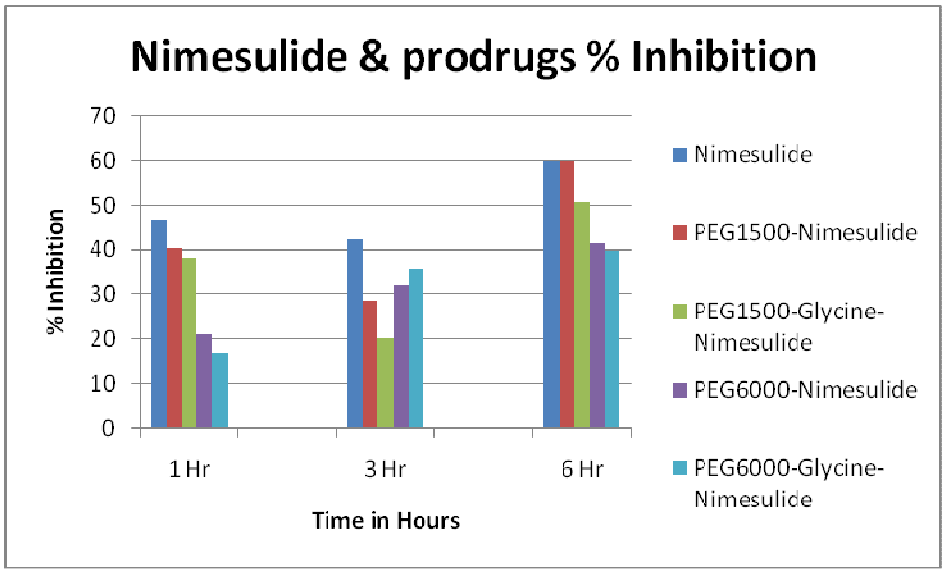
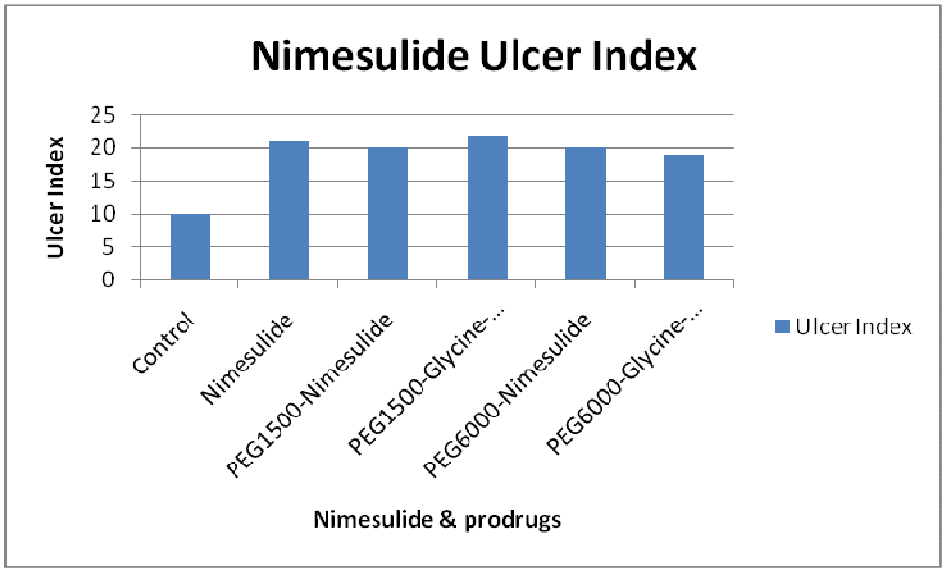


Fig No: 11 *In vivo* Ulcer protecting Activity



CONCLUSION

Out of the synthesized prodrugs of nimesulide all of them had retained their anti inflammatory activity as well some of them shown good ulcer protecting activity when compared to standard drug nimesulide. Hence, the prodrugs of nimesulide synthesized can avoid the adverse effects associated with the use of NSAID's by this approach along with ulcer protecting activity.

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REFERENCES

[1] Swarnalata Saraf, NSAIDS-An Overview, Pharma Book Syndicate, Hyderabad, **2008,** 1, 153-154.
[2] Martindale, The Complete Drug Reference, PhP Pharmaceutical Press, Sean C. Sweetman, London, **2005,** 34, 67-68.
[3] D.V. Derle, K. N. Gujar, B. S. H. Sagar, *Indian J.Pharm.Sci*. **2006,** 409-414.
[4] Wilson and Gisvold, Text book of Organic Medicinal and Pharmaceutical Chemistry, Lippincotts, Williams and Wilkins, Philadelphia, **2004,** 11, 757-761.
[5] Carlos Elvira, Alberto Gallardo, Julio San Roman, Alejandro Cifuentes, *Molecules*. **2005,** 10, 114-125.
[6] N.K Jain, Introduction to Novel Drug Delivery Systems, Vallabh Prakashan, **2010,** 1, 282-291.
[7] Povl Krogsgaard-Larsen, Kristian Stromgaard, Ulf Madsen, Text Book of Drug Design and Discovery, CRC Press, New Delhi, **2011,** 4, 137-146.
[8] Graham. L. Patrick, An Introduction to Medicinal Chemistry, Oxford University Press, New Delhi, **2009,** 4, 252-258.
[9] K. Hoste, K. De Winne, E. Schacht, *Int J Pharm*. **2004,** 277, 119-131.
[10] M.J.N Chandrasekar, M.J Nanjan, B.Suresh, *Indian J Pharm.Sci*. **2004,** 66, 1, 69-71.
[11] M. Zacchigna, G. Di Luca, V. Maurich, E. Boccu, *Farmaco*. **2002,** 57, 207-214.
[12] Cinzia Bersani, Manuela Berna, Gianfranco Pasut, Francesco Maria Veronese, *Farmaco*. **2005,** 60, 783-788.
[13] Reinhard Reents, A. Duraiswamy, Jeyaraj, Herbert Waldmann, *DDT*. **2002,** 7, 1, 71-76.
[14] Dinesh J. Makhija, Rakesh R. Somani, *Der Pharmacia Lettre*, **2010,** 2, 2, 300-309.
[15] K.P.R. Chowdary, Veeraiah Enturi, *IJPRD*. **2011,** 3, 1, 224-230.
[16] Anjali Nayak, Anurekha Jain, *Sci Pharm.* **2011,** 79, 359-373.
[17] S.K. Kulkarni, Hand Book of Experimental Pharmcology, Vallabh Prakashan, New Delhi, **1999,** 3, 128-130.
[18] S.K. Kulkarni, Hand Book of Experimental Pharmacology, Vallabh Prakashan, New Delhi, **1999,** 3, 148-150.
[19] Francesco Giuliano, Timothy D. Warner, *British Journal of Pharmacology*. **1999,** 126, 1824-1830.
[20] M.J.N Chandrasekar, B. Duraiswamy, Polwalkar Nitin, M.J Nanjan, *Indian Drugs*. **2001,** 38, 7, 351-354.