International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (3): 539-547
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Pharmaceutical Sciences | Open Access | MCI Approved
UGC Approved Journal

Conjugation of Naproxen to Polyethylene Glycol (PEG) and to Evaluate Drug Release Study

S. Sourin[1] and DG. Debaprotim[2]

[1]Department of Pharmaceutical Chemistry, Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Assam, India.

[2]Assistant Professor, Department of Pharmaceutical Chemistry, Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Assam, India.

Received: 12 Mar 2019 / Accepted: 21 Apr 2019 / Published online: 1 Jul 2019
***Corresponding Author Email:** mobthepbl@gmail.com

Abstract

The field of polymer therapeutics has evolved over the past decade and has resulted in the development of polymer-drug conjugates with a wide variety of architectures and chemical properties**. Aim:** Conjugation of naproxen to Poly Ethylene Glycol and to evaluate drug release study. **Method:** Poly (ethylene glycol) $_{4000}$(PEG) was obtained from M/s. Merck. Mumbai. Thionyl chloride, and glycine were obtained from M/s. SD Fine Chemicals and used as such. Glycine and leucine was being obtained from M/s. Loba Chemicals, Mumbai, and used as such. Glycine-leucine, leucine-glycine, were obtained from M/s. Sigma-Aldrich Germany. Dicyclohexyl carbidomide (DCC) and 4-dimethyl amino pyridine (DMAP) were obtained from M/s. Merck, Germany. Triethylamine, Dimethylformamide (DMF) were obtained from M/s. Ranbaxy fine Chemicals. Naproxen was obtained from M/s. Matrix Laboratories Limited, Secunderabad as gift sample, and was used after confirming its purity by its melting point and IR spectrum. All other solvents used were purified by standard techniques. **Result:** The conjugation of Naproxen to PEG was performed and evaluated for drug release study along with the characterization and the values obtained is discussed and explained below. **Conclusion:** The use of simple synthetic procedures and the quite general applicability of the drug anchoring strategy render the entire approach suitable for the synthesis of similar conjugates, varying the structure of the NSAID and the polymer length. It is noticeable that, in all cases, the drug-polymer conjugates were obtained with a high degree of purity, avoiding expensive and time consuming purification procedures.

Keywords

Dicyclohexyl carbidomide (DCC), Dimethylformamide (DMF), Drug-Polymer Conjugates, Naproxen, NSAID.

*****

INTRODUCTION

The word "polymer" is derived from two Greek words, 'poly" that means many (numerous) and "Mer" which means units. In basic terms, a polymer is a long-chain molecule that is composed of a large number of repeating units of identical structure.

The Concepts utilizing polymers in the design of therapeutic agents have been widely investigated for a number of decades. Initial work of the 1960s focused on utilizing polymers as blood plasma expanders, wound dressings, and injectable or implantable depots1,2. In 1975, a rational model for pharmacologically active polymers was first proposed by Helmut Ringsdorf 3. His concept of covalently bound polymer-drug conjugates still forms the basis for much of the work in this area performed today[1].

The Ringsdorf model primarily consists of a biocompatible polymer backbone bound to three components: 1) a solubilizer, which serves the purpose of imparting hydrophilicity and ensuring water solubility, 2) a drug, usually bound to the polymeric backbone via a linker, and 3) a targeting moiety whose function is to provide transport to a desired physiological destination or bind to a particular biological target.

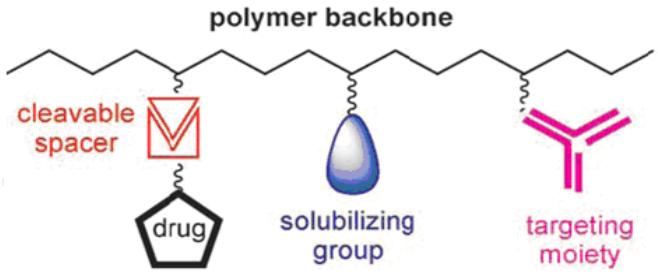


Fig. 1- Ringsdorf's model for drug-delivery systems based on synthetic polymer

Conjugates of drugs and polymers as well as other polymeric carrier systems have collectively been termed polymer therapeutics, which primarily encompass polymer–protein conjugates, drug–polymer conjugates and more recently supramolecular drug-delivery systems as well as nanosized systems[9,10]. Anchoring of enzymes or biological response modifiers to polyethylene glycol components (PEGylation) has led to numerous polymer–protein conjugates with improved stability and pharmacokinetic properties. Several polymer–protein conjugates have received market approval. The coupling of low molecular weight anticancer drugs to polymers through a cleavable linker has been an effective method for improving the therapeutic index of clinically established agents and today the first candidates of anticancer drug–polymer conjugates are being evaluated in clinical trials.

Naproxen

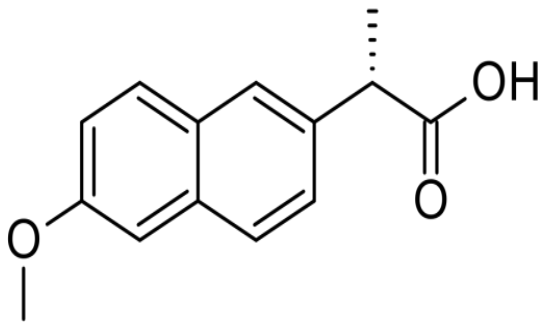


Fig.2: Structure of naproxen moiety

NAPROXEN, the NSAIDS drug which is used to relieve pain from various conditions such as headaches, muscle aches, tendonitis, dental pain, and menstrual cramps. It is a propionic acid derivative with analgesic and anti-inflammatory activity which has been widely used in the treatment of rheumatic

diseases. Naproxen has been well studied in rheumatoid arthritis and is as effective as aspirin but better tolerated, thus enabling more patients to continue with treatment.

The **Naproxen** drug is used for the characterization and synthesis the three naproxen derivative which are conjugated with the polymer and which show the great property on the drug release system. A versatile and general approach is employed to link the drug to polymers, affording

the derivatives with a very high degree of purity. The release of the drug from the conjugates proved to be exceptionally slow, even in acidic aqueous media, and the kinetic of the process seems to be triggered by their solubility in water. On the other hand, the interesting outcome of the first *ex-vivo* drug release experiments on human blood samples makes this preliminary study valuable for future investigations on the use of these polymeric prodrugs in *in-vivo* treatment of inflammatory states.

In case of polymer, Polyethylene glycol (PEG) derivatives are undoubtedly one of the best candidates to be used for this purpose. PEG, indeed, is easily available in a wide range of molecular weights and it is the polymer with stealth behavior [9] most commonly used for biomedical applications. [10] As a matter of fact, PEGylated drugs have been extensively studied and successfully employed to improve the TI of active ingredients.

Advantage:

- Naproxen has generally been better tolerated than aspirin or indomethacin at the dosages used.
- It has been widely used in the treatment of rheumatic diseases.
- Naproxen is also effective in degenerative joint diseases of the hip and knee.
- Naproxen appears to be effective in reducing pain and swelling in acute gout and is an effective analgesic in patients with pain following surgery or trauma and in pain of dysmenorrhoea.

Side effect:

The drug, naproxen which is basically used to relieve the pain from various condition, but sometime it may cause some unwanted effects. such are described below-

- shortness of breath (even with mild exertion).
- swelling or rapid weight gain.
- the first sign of any skin rash, no matter how mild.
- signs of stomach bleeding - bloody or tarry stools, coughing up blood or vomit that looks like coffee grounds.
- liver problems - nausea, upper stomach pain, itching, tired feeling, flu-like symptoms, loss of appetite, dark urine, clay-colored stools, jaundice (yellowing of the skin or eyes).
- kidney problems - little or no urinating, painful or difficult urination, swelling in your feet or ankles, feeling tired or short of breath.
- low red blood cells (anemia) - pale skin, feeling light-headed or short of breath, rapid heart rate, trouble concentrating; or
- severe skin reaction - fever, sore throat, swelling in your face or tongue, burning in your eyes, skin pain followed by a red or purple skin rash that spreads (especially in the face or upper body) and causes blistering and peeling.

Common naproxen side effects may include:

- indigestion, heartburn, stomach pain, nausea;
- headache, dizziness, drowsiness, bruising, itching, rash.

MATERIALS AND METHOD

Preparation of the polymeric pro-drugs

i) Preparation of chloro derivative of peg (peg-cl_2)

Poly (ethylene glycol)$_{1500}$ (10mmol) and pyridine (20mmol) were taken in a 250 ml two-necked round bottom flask fitted with a stirrer and condenser. Thionylchloride (60mmol) was added drop by drop for 30 minutes under reflux maintaining the temperature at 40^{0}C. When the addition was complete, the temperature of the flask was raised to 70^{0}C and the reaction was carried out for 5h. The temperature was then brought to room temperature and the contents were filtered to remove pyridine hydrochloride. The residue was dissolved in methylene chloride, dried over anhydrous potassium carbonate and filtered. The filtrate was treated with alumina (50g, activated at 120^{0}C for 1h), precipitated by cold diethyl ether and crystallized from ethanol.

ii) Covalent binding of glycine to peg – cl_2

To a solution of PEG-Cl_2 (6mmol) and pyridine (6mmol) in DMF (20ml), glycine (30mmol) was added in small portions for 3h under stirring at room temperature. The mixture was then refluxed at 130^{0}C for 8h with continuous stirring. The solvent was removed under reduced pressure and the residue was dissolved in dichloromethane. The solution was cooled, filtered and diethyl ether was added. The PEG-(Glycine-COOH)$_2$ precipitated was recrystallized from ethanol and purified by column chromatography using methanol and water in the ratio of 3:1.

iii) Synthesis of peg-[(glycine)-naproxen]$_2$

A solution of DCC (15mmol), DMAP (6mmol) in 10ml of DMF was taken in a 250ml beaker. The solution was then added drop by drop to another 250ml beaker containing a solution of PEG-(glycine-COOH)$_2$ (6mmol) dissolved in DMF (20ml). Naproxen (15mmol) was added in small portions to this mixture, maintained at 0^0C for 10 minutes. The coupling reaction was carried out for 4 days (96h) at room temperature with continuous stirring. At the end of the fourth day, the solvent was removed under reduced pressure and the residue was dissolved in dichloromethane and washed with brine.

The organic layer was separated and dried over anhydrous potassium carbonate. The organic layer was concentrated and diethyl ether was added. The precipitated product, namely PEG-[(glycine)-Naproxen] $_2$, was recrystallized twice from dichloromethane and was further purified by column chromatography using methanol and water in the ratio of 3:1.

Evaluation study

Estimation of drug content

A stock solution of naproxen was prepared by dissolving 100 mg of the drug in 100 ml of 0.1N NaOH. From the stock solution 10, 20, 30, 40 and 50 µg/ml dilutions were prepared using phosphate buffer of pH 7.4. The λ_{max} of the drug was determined by scanning the diluted solution between 200-400nm against a blank reagent in a double beam spectrophotometer (model UV 160 A). The λ_{max} was found to be 273nm. A standard curve between concentration and absorbance was then plotted and its intercept (B) and slope (k) were calculated.

The amount of naproxen in the synthesized polymeric pro-drugs were determined by UV spectrophotometry. The pro-drug (100 mg) was taken in 100 ml 0.1N NaOH. The resulting solution was sonicated for 10 minutes and filtered. The filtrate (5 ml) was diluted to 50ml using 0.1N NaOH and kept for 24h at room temperature for the complete release of the drug from the polymer backbone. The absorbance was measured at 273 nm using 0.1N NaOH solution as a blank.

***In vitro* drug release studies**

The synthesized PEG-drug conjugates on oral administration should undergo drug release in the biological media followed by absorption of the drug into the systemic circulation before eliciting their action. The rate and the extent of the drug release will decide the intensity and duration of the drug action in the system. *In vitro* drug release testing should provide the means to evaluate bioavailability and the information necessary for the development of more efficacious and therapeutically optimal dosage forms.

In short the *in vitro* release studies serve the following;

- It adequately mimics the *in vivo* situation as a result of which one may obtain a better understanding of the *in vivo* environment.
- It can be used to screen potential drug candidates for their drug absorption characteristics. Qualitative screenings of the effect of structure modifications can readily be undertaken once successful *in vitro – in vivo* correlations have been established. Even in the absence of *in vitro – in vivo* correlations, purely on the basis of the *in vitro* data alone, one can predict the form of the drug that would lead to the optimization of a particular desired effect.

In vitro drug release testing was, therefore, carried out to assess the potential of the PEG drug conjugates synthesized.

The *in vitro* drug release from the polymeric pro-drug was evaluated using USP XXIII dissolution apparatus (type II, paddle).

- **Studies in different buffers**

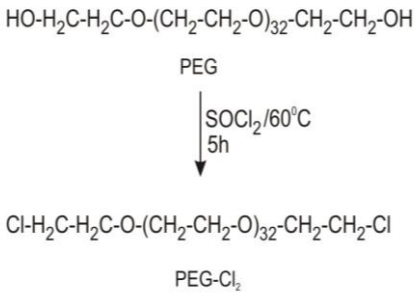
100mg each of the pro-drug containing a known quantity of the drug was taken in 900ml dissolution media of pH 1.2 (HCl buffer), 5.5 (Phthalate buffer), 6.8 (Phosphate buffer) and pH 7.4 (Phosphate buffer). It was stirred at 100 rpm over a period of 24h. Samples (5ml) were withdrawn at time intervals of 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18 and 24 h. After each sample was withdrawn, an equal quantity of fresh dissolution media was replaced. The absorbance of the samples withdrawn after suitable dilution, were measured against the blank at the λ_{max} of the drug, namely, 273 nm. The amount of the drug released at different time intervals and percentage release was calculated using the formula,

$$\text{Percentage release} = \frac{\text{Concentration (mg/ml) x bath volume}}{\text{Drug content}} \times 100$$

RESULT

Scheme 1- Synthesis of the chloro derivative of PEG(PEG-Cl_2)

The synthesis of chloro derivative of PEG is given in Scheme 1.



Scheme 1- Synthesis of PEG- Cl_2

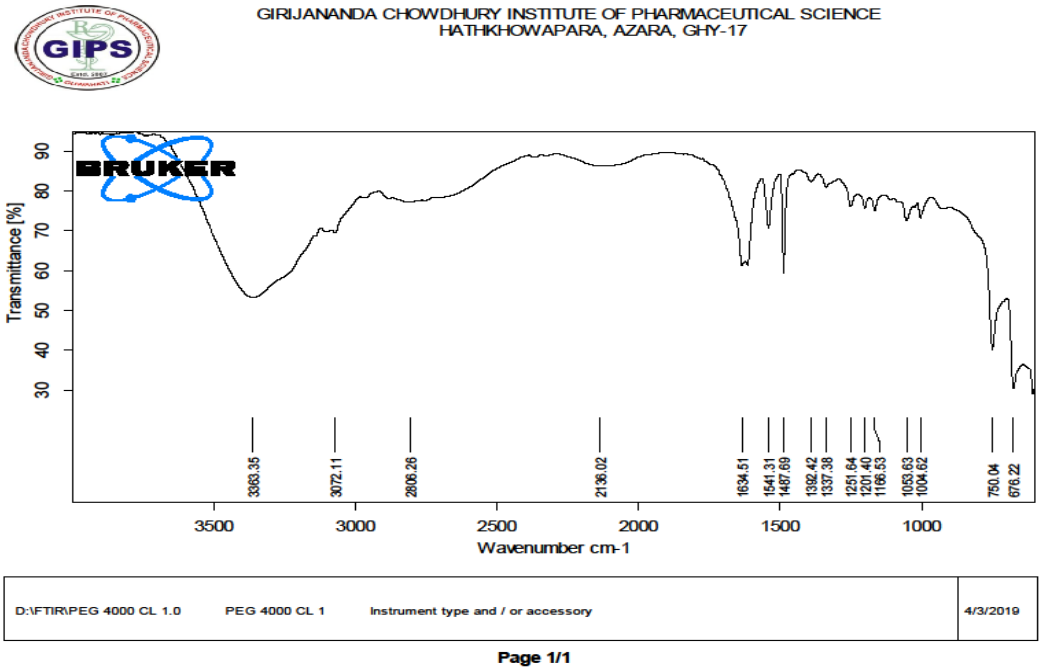


Fig. 3- FTIR of PEG4000-Cl_2

PEG-Cl_2 was obtained in good yield (yield 85%, mp. 41° C). The IR spectrum for PEG and PEG-Cl_2 are given in Figures 5 and 6, respectively. PEG exhibits a characteristic band at 3450 cm^{-1} (OH), 2881 cm^{-1} (-CH_2) and at 1108cm^{-1} (– CH_2 – O – CH_2 stretching). PEG-Cl_2 exhibits bands at 1112cm^{-1} (–CH_2–O–CH_2 stretching), 662cm^{-1} (–C–Cl stretching), 2898cm^{-1} (-CH_2-) and no absorption for –OH at 3300 – 3500 cm^{-1}, indicating the replacement of –OH by –Cl.

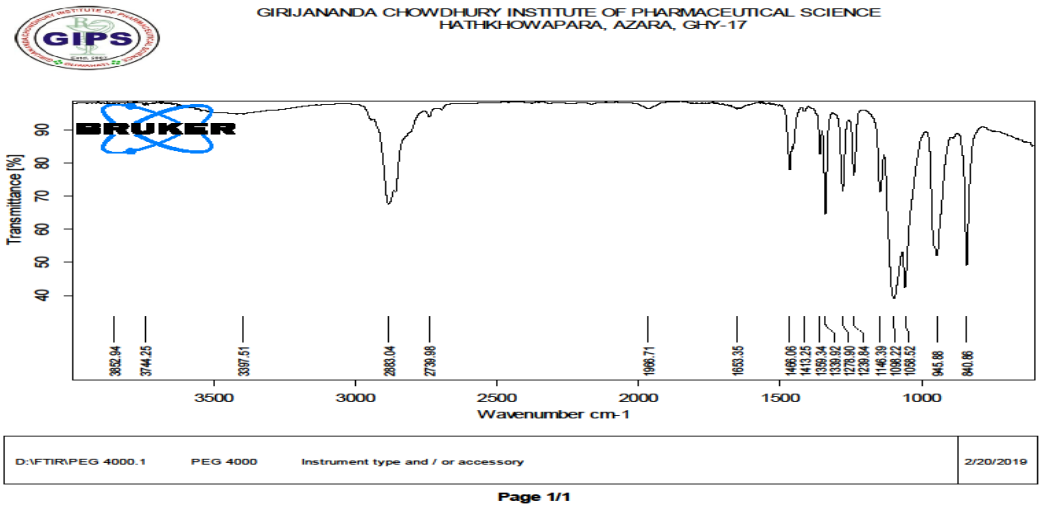


Fig.4- FTIR of PEG4000

SCHEME 2 - ***Covalent binding of Glycine to PEG – Cl₂***

The coupling of PEG-Cl_2 to glycine is given in Scheme 2.

$$Cl\text{-}H_2C\text{-}H_2C\text{-}O\text{-}(CH_2\text{-}CH_2\text{-}O)_{32}\text{-}CH_2\text{-}CH_2\text{-}Cl \quad + \quad H_2N\text{-}CH_2\text{-}COOH$$

PEG-Cl_2 Glycine

DMF / 8h reflux at 130°C

$$HOOC\text{-}H_2C\text{-}NH\text{-}H_2C\text{-}H_2C\text{-}O\text{-}(CH_2\text{-}CH_2\text{-}O)_{32}\text{-}CH_2\text{-}CH_2\text{-}NH\text{-}CH_2\text{-}COOH$$

PEG-(Glycine-COOH)$_2$

Scheme 2 - Covalent binding of Glycine to PEG – Cl_2

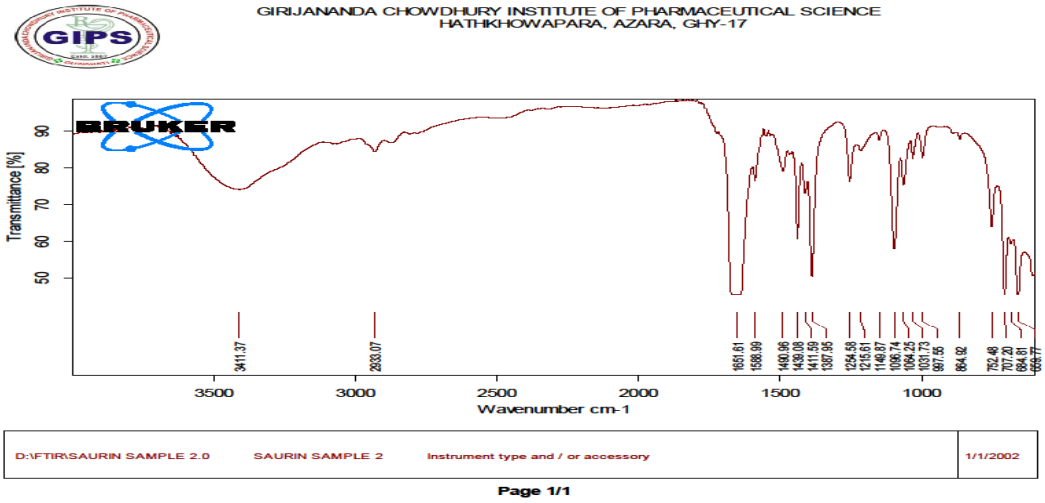
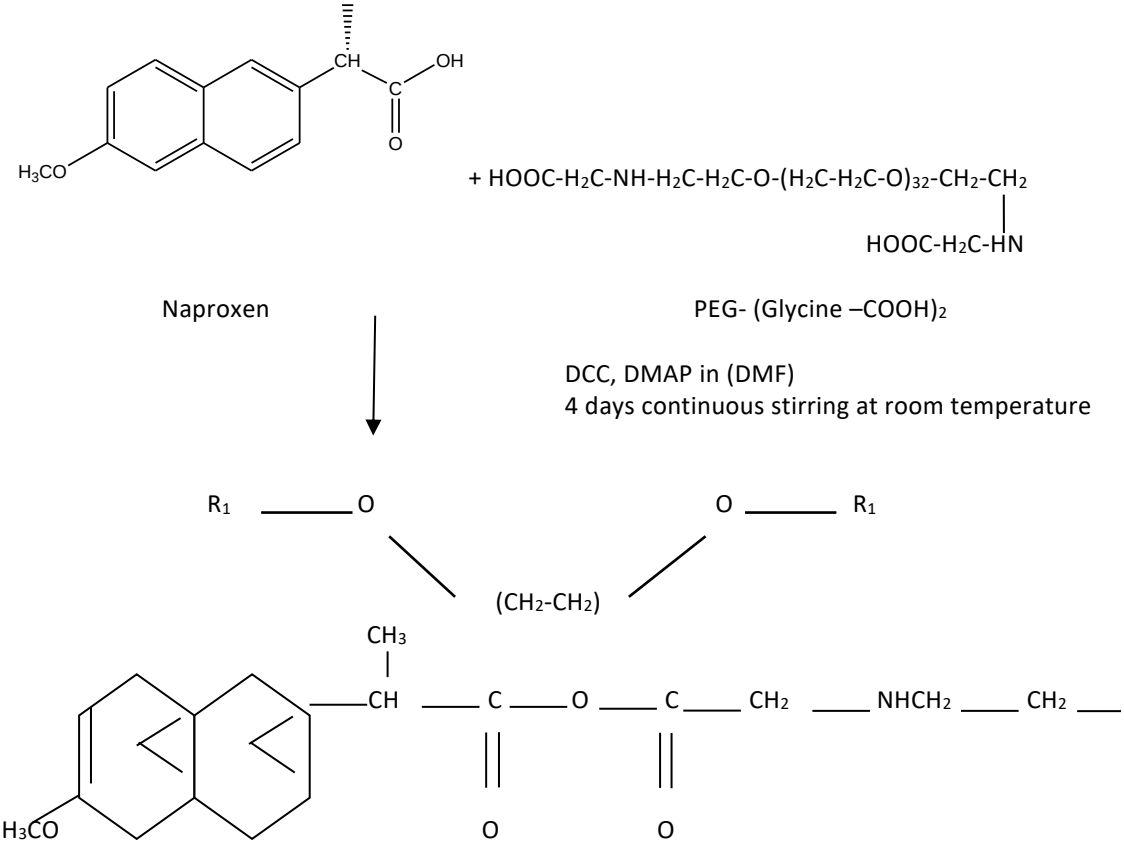


Fig. 5- FTIR of PEG(Glycine-COOH)

PEG – (glycine – COOH) $_2$ was obtained in good yield (yield 82%, mp. 62° C). The **IR (nujol)** spectrum shows a broad band between 2300-3500cm^{-1} (-COOH, NH), 1680cm^{-1} (C=O) and 1100cm^{-1} (-C-O-C).

SCHEME 3 – *Synthesis of PEG-[(Glycine) – Naproxen]$_2$* –The synthesis of PEG-[(Glycine) – Naproxen]$_2$ obtained in good yield (yield 74%, mp. 141° C) is given in Scheme 3



PEG {(Glycine) –Naproxen}$_2$

Scheme 3: Synthesis of PEG –[(Glycine) – Naproxen]$_2$

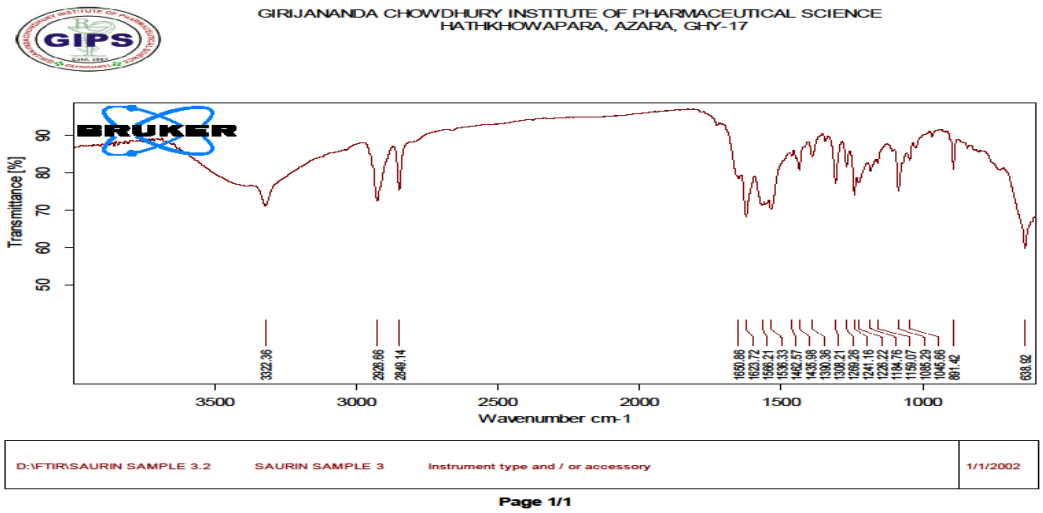


Fig. 6- FTIR of PEG –[(Glycine) – Naproxen]

PEG –[(Glycine) – Naproxen] was obtained in good yield (yield 74%, mp. 141° C). The **IR (nujol)** spectrum exhibits a broad band at 3322cm^{-1} (-CONH, OH), 1720cm^{-1} (-CONH), 1600cm^{-1} (C=N) and 1100cm^{-1} (-C-O-C-).

Estimation of Drug content

The amount of drug present in each of the pro-drugs was estimated spectrophotometerically and is given in Table. It was found that as the spacer length increases, the amount of drug incorporated into the polymer backbone decreases.

Table 1- Estimated drug content of PEG - pro - drugs

Sl No	PEG pro-drugs	Drug content/100mg of pro-drug
1	PEG-Cl_2	69.75mg
2	PEG [(Glycine) – $COOH]_2$	66.80mg
3	PEG [(Glycine) – $Naproxen]_2$	58.25mg

Table 2- *In vitro* drug release from PEG-[(GLYCINE) – NAPROXEN]$_2$ at different pH

Time	pH 1.2	pH 5.5	pH 6.8	pH 7.4
0	0	0	0	0
0.5	2.02	3.89	4.01	5.63
1	5.68	8.62	9.23	10.62
1.5	6.48	16.59	17.18	18.51
2	7.56	19.82	20.34	22.63
3	8.98	28.93	29.14	30.62
4	11.23	32.12	35.34	40.81
6	13.68	39.62	42.18	46.23
8	14.92	42.83	45.34	52.16
10	15.23	48.23	50.12	58.83
12	17.18	50.34	55.18	60.24
18	20.34	53.18	60.24	64.97

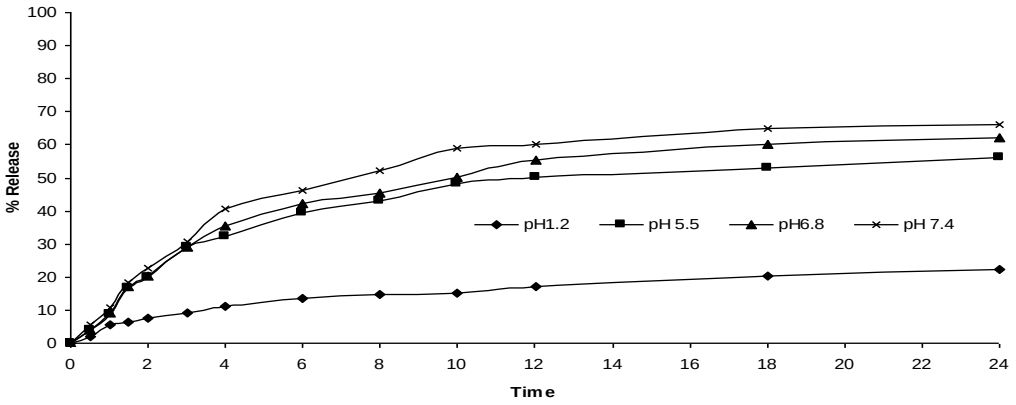


Fig. 6 - *In vitro* drug release from PEG-[(Glycine) – NAPROXEN]$_2$

DISCUSSION

PEG-Cl_2 was obtained in good yield (yield 83%, mp. 41° C). The IR spectrum for PEG and PEG exhibits a characteristic band at 3375 cm^{-1} (OH), 2878 cm^{-1} (-CH_2) and at 1086cm^{-1} (– CH_2 – O – CH_2 stretching). PEG-Cl_2 exhibits bands at 1179cm^{-1} (–CH_2–O–CH_2 stretching), 753cm^{-1} (–C–Cl stretching), 2898cm^{-1} (-CH_2-) and no absorption for -OH at 3300 – 3500 cm^{-1}, indicating the replacement of -OH by -Cl.

PEG – (glycine - COOH) $_2$ was obtained in good yield (yield 80%, mp. 62° C).

IR (nujol) spectrum shows a broad band between 2500-3400cm^{-1} (-COOH, NH), 1651cm^{-1} (C=O) and 1064cm^{-1} (-C-O-C).

CONCLUSION

This thesis deals with the investigations carried out by the writer on the synthesis, characterization and evaluation of some polymeric pro-drugs.

The first chapter of the thesis deals with a brief introduction to controlled drug delivery systems, passive drug targeting and specific tissue targeting, cellular uptake of polymers, site specific drug release, polymer conjugates, incorporation of spacers in pro-drug conjugates and PEG chemistry.

The use of simple synthetic procedures and the quite general applicability of the drug anchoring strategy render the entire approach suitable for the synthesis of similar conjugates, varying the structure of the NSAID and the polymer length. It is noticeable that, in all cases, the drug-polymer conjugates were obtained with a high degree of purity, avoiding expensive and time consuming purification procedures.

ACKNOWLEDGMENT

The author expresses his gratitude towards the head of the department Chemistry, Girijananda Chowdhury Institute of Pharmaceutical Science, Guwahati for the facilities provided to carry out this research work.

CONFLICT OF INTEREST

There is no conflict of interest related to the present work.

REFERENCE

1. B.K Satheeshababu, I. Mohamed, "Synthesis and characterization of sodium alginate conjugate and study of effect of conjugation on drug release from matrix tablet". *Indian J. Pharm Sci*. 77(5),2015, 579-585.
2. O. Zhukova, S. Bulgakova, "Preparation of a Doxorubicin Conjugat with (Co) polymer of Methacrylic acid for targeted tumor therapy", *J. Appl.Pharm. Sci,* 6 (7), 2016,20-26.
3. G. Pasut, F.M. Veronese "*Polymer–drug conjugation, recent achievements and general strategies*" Prog. Polym. Sci. 32 (2007) 933–961.
4. Ruth Duncan "*Development of HPMA copolymer–anticancer conjugates: Clinical experience and lessons learnt"* journal homepage: www.elsevier.com/locate/ addr, Advanced Drug Delivery Reviews 61 (2009) 1131–1148.
5. Haag R, Kratz F. "Polymer *therapeutics: concepts and applications*." Angew. Chem. Int. Ed. 2006; 45:1198-1215.
6. Duncan R, Vicent MJ. "*Polymer therapeutics-prospects for 21st century: the end of the beginning"*. Adv. Drug. Del. Rev. 2013; 65:60-70.
7. R. Duncan, L. Izzo, Dendrimer biocompatibility and toxicity, *Adv. Drug Delivery Rev.* 2005**,** 57, 2215–2237.
8. R. Duncan N-(2-Hydroxypropyl) methacrylamide copolymer conjugates. *In Polymeric Drug Delivery Systems, (Ed.: G. S. Kwon), Marcel Dekker,* New York, 2005, 1–92.
9. J.J Khandare, M.G Kulkarni, Polymerizable macromers and preparation thereof, US patent (Council of Scientific and Industrial Research, New Delhi, IN 6.822, 064.) 2004.
10. R. Haag, Dendritic Polyglycerol Sulfates as New Heparin Analogues and Potent Inhibitors of the Complement System, *Angew. Chem.* 2004, 116, 280–284.
11. K Ashim. Mitra, S Banmeet. Anand, M James. Hill, Surajit Dey, Koicbi Maruyamma, S. Partha Bhattacharjee, E. Marvin. Myles, and E. Yasser Nasbed, In vivo Antiviral Efficacy of a Dipeptide Acyclovir pro-drug, val-val-acyclovir, against HSV-1 Epithelial and stromal keratitis in the Rabbit eye model, *Inves. Ophthalmol.visual sci.* 2003**,** 44, 6.