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Conjugation of Ibuprofen to Poly Ethylene Glycol and *In-vitro* drug release evaluation

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ABSTRACT

Polymers have become an integral part of drug delivery systems due to their improved pharmacokinetics properties. Polymer conjugation is a well-known and widely exploited technique useful to improve therapeutic properties of peptides, proteins, small molecules and oligonucleotides. Polymer conjugated drug generally exhibit prolonged half-life, higher stability, water solubility, lower immunogenicity, antigenicity and often also to the specific targeting tissue. Polymer materials are designed to be capable of delivering active substance. to the target diseased tissues and cells. Conjugation of Ibuprofen and polyethylene glycol (PEG) helps to increase the duration of action of the parent drug. The PEG-Ibu conjugates were synthesized from ibuprofen and PEG with two different molecular weights by esterification in the presence of DCC and DMAP. The PEG-Ibu conjugation characterized by FT-IR, UV, DSC and NMR and also *in-vitro* drug release study in different buffer at different ph.

Keyword: Polymer, Conjugation, *In vitro,* prodrug, spacer molecule.

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INTRODUCTION

Improving the therapeutic index of drugs is a major impetus for innovation in many therapeutic areas such as cancer, inflammatory and infective diseases. In drug–polymer conjugates, however, the drug is covalently linked to polymers such as proteins, polysaccharides and synthetic polymers and also used a spacer molecule.

Prof. H. Ringsdorf developed a rational model (Figure 1) of polymeric prodrug for the first time in 1975. And Prof. Ringsdorf was the first scientist to recognize the huge potential of polymeric prodrug, allowing polymer chemist and biologists would work together in the field. The proposed model consists mainly of fie components: the polymeric backbone, the drug, the spacer, the targeting group and the soluble agent. The backbone could be either an inert or a biodegradable polymer. The role of spacer is to control the site and the rate of releasing the active drug from the conjugate by hydrolytic or enzymatic cleavage. The drug must be covalently bonded and must remain attached to the polymer until this macromolecule reaches the target site. So, the drug chosen must be potent and intact until it reaches the target. Besides, it should have a functional group to bind with the polymer backbone directly. The targeting moiety or homing device guides the entire polymer-drug conjugate to the targeted tissue. Now, in general, there are different kinds of the therapeutic agents could be incorporated into polymer chain, end-capped or formed a pendant group of the macromolecular chain.

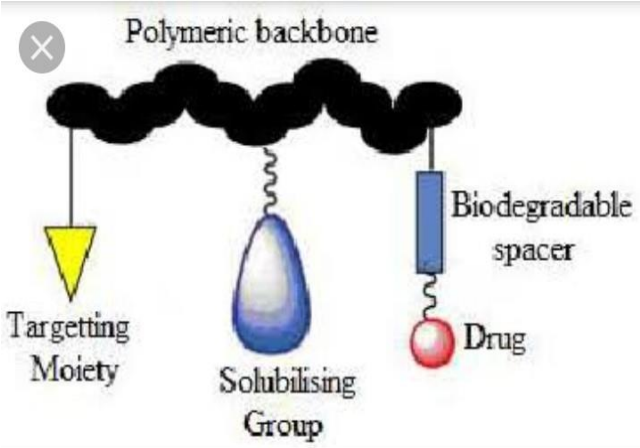


Figure 1: Prof. H. Ringsdorf model

Conjugation of ibuprofen to polyethylene glycol (PEG)

Non-steroidal anti-inflammatory drugs (NSAIDs) are usually poorly soluble in water and they frequently cause gastrointestinal side effects such as gastric ulceration, bleeding and perforation. Ibuprofen is a non-steroidal anti-inflammatory drug with well-known anti-inflammatory, antipyretic and analgesic properties. Chemically it is 2-(4-*iso*-butylphenyl)- propanoic acid and is poorly soluble in water . It is most commonly administered orally and is rapidly absorbed to reach its maximal plasma concentration with in 2 hrs. However, it has a short biological half-life of 2 hrs, which means that frequent doses are required to maintain the therapeutic efficacy over extended time periods.

EXPERIMENTAL

Materials

Poly (ethylene glycol) $_{600}$(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 was obtained from M/s. Loba Chemicals, Mumbai, and used as such. 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. Ibuptofen was obtained from M/s. Matrix Laboratories Limited, and was used after confirming its purity by its melting point and IR spectrum. All other solvents used were purified by standard techniques .

I. ***Preparation of Chloro derivative of PEG (PEG-cl$_2$)***

Poly(ethylene glycol)$_{600}$ (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.

Chloro derivative of poly-(ethylene glycol) (PEG-cl$_2$) obtained was characterized by single spot thin layer chromatography and spectral analysis. The IR spectrum was recorded in KBr pellet using Perkin-Elmer 1600 series FTIR spectrophotometer.

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 recrystallised from ethanol and purified by column chromatography using methanol and water in the ratio of 3:1. The PEG-(Glycine-COOH)$_2$ was characterized by single spot TLC and spectral analysis. The IR spectrum was recorded in nujol using Perkin -Elmer 1600 series, FTIR spectrophotometer. The H^1 and C^{13} NMR spectra were recorded using JEOL spectrometer. Similar procedures were adopted for the coupling of leucine with PEG-Cl$_2$ to obtain PEG-(Leucine-COOH)$_2$.

III. ***Synthesis of PEG-[(Glycine)-Ibuprofen]***

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). Ibuprofen (15mmol) was added in small portions to this mixture, maintained at 0^{0}C 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)-Ibuprofen] , was recrystallised twice from dichloromethane and was further purified by column chromatography using methanol and water in the ratio of 3:1 The PEG - [(glycine)-Ibuprofen] was characterized by single spot TLC and spectral analysis. The IR spectrum was recorded in nujol using perkin-Elmer 1600 series FT IR spectrophotometer. The H^1 and C^{13} NMR spectrum were recorded in JEOL Spectrometer.

Evaluation Studies

Estimation of Drug content

A stock solution of ibuprofen will be prepare 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 is being prepare using phosphate buffer of pH 7.4. The λ_{max} of the drug will determine by scanning the diluted solution between 200-400nm against a blank reagent in a double beam spectrophotometer (model UV 160 A).

In vitro Drug Release Studies

In vitro drug release testing will, therefore, carry out to assess the potential of the PEG drug conjugates synthesized. The *in vitro* drug release from the polymeric pro-drug will 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 will be 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 is stirred at 100 rpm over a period of 24h. Samples (5ml) are withdrawn at time intervals of 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 18 and 24 h. After each sample withdrawn, an equal quantity of fresh dissolution mediais replaced. The absorbance of the samples withdrawn after suitable dilution, will measured against the blank at the λ_{max} of the drug, namely, 254 nm. The amount of the drug released at different time intervals and percentage release was calculated using the formula, '

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

Stability studies

Accelerated stability studies for the synthesized PEG conjugates will carry out for three months. The pro-drugs containing equivalents of 100 mg of the parent drug (3 batches each) fills manually in hard gelatin capsules (size 1) and then packed in transparent polyvinyl chloride blisters of 0.25 mm thickness. Three different temperature and humidity conditions, prescribed by the International

Conference on Harmonization (ICH) for zone IV, were employed namely,

- 25°C with 60% relative humidity (RH).
- 40°C with 75% relative humidity (RH).
- Room temperature.

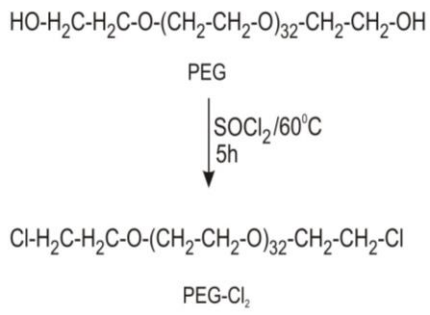
Samples were withdrawn at the end of 30, 60 and 90 days and evaluated for their physical parameters and *in vitro* drug release. The drug content in each batch will evaluate.

RESULT AND DISCUSSION

Synthesis of polymeric pro-drugs

a) 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 the Chloro derivative of PEG***

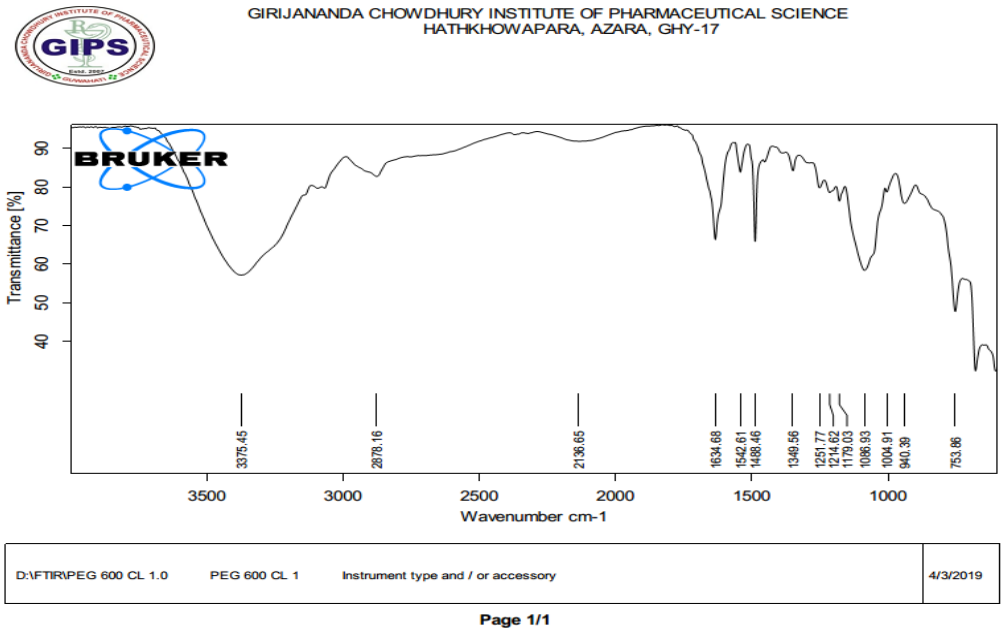


Figure 2: IR OF *PEG-Cl_2*

PEG-Cl_2 was obtained in good yield (yield 83%, mp. 41° C). The IR spectrum for PEG and . PEG exhibits a characteristic bands 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.

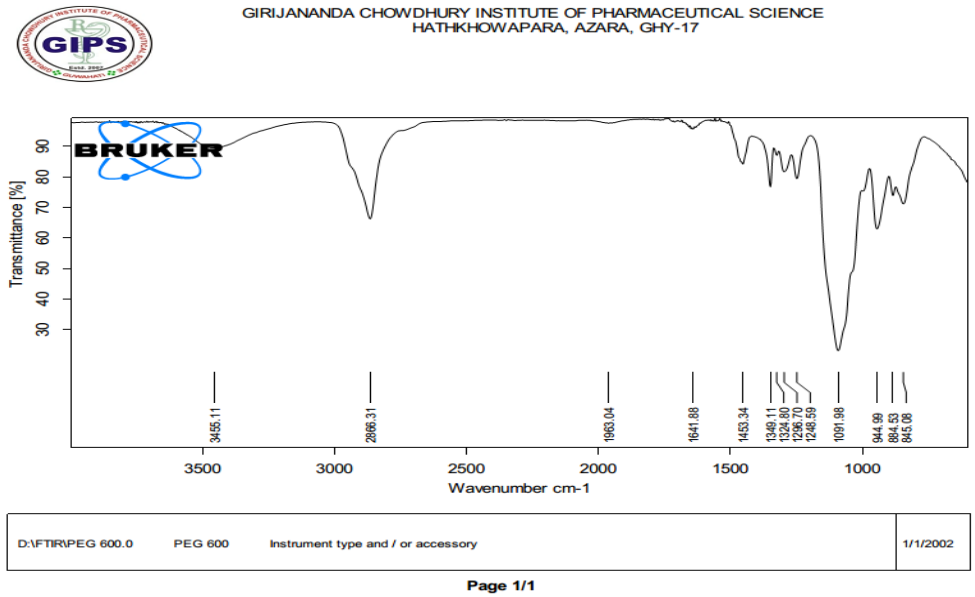
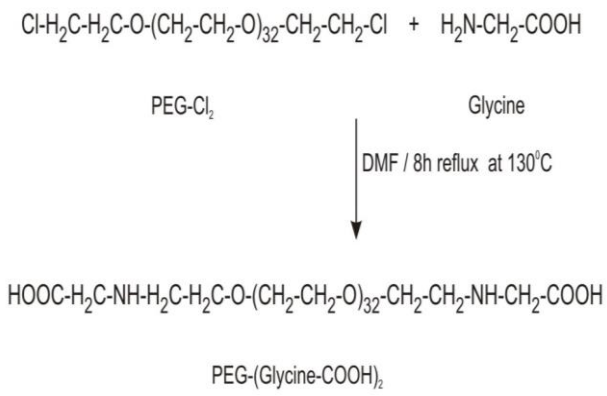


Figure 3: IR OF PEG600

b) ***Covalent binding of Glycine to PEG – Cl_2***

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



Scheme 2: Covalent binding of Glycine to PEG – Cl_2

FTIR of PEGCl2-Glycine

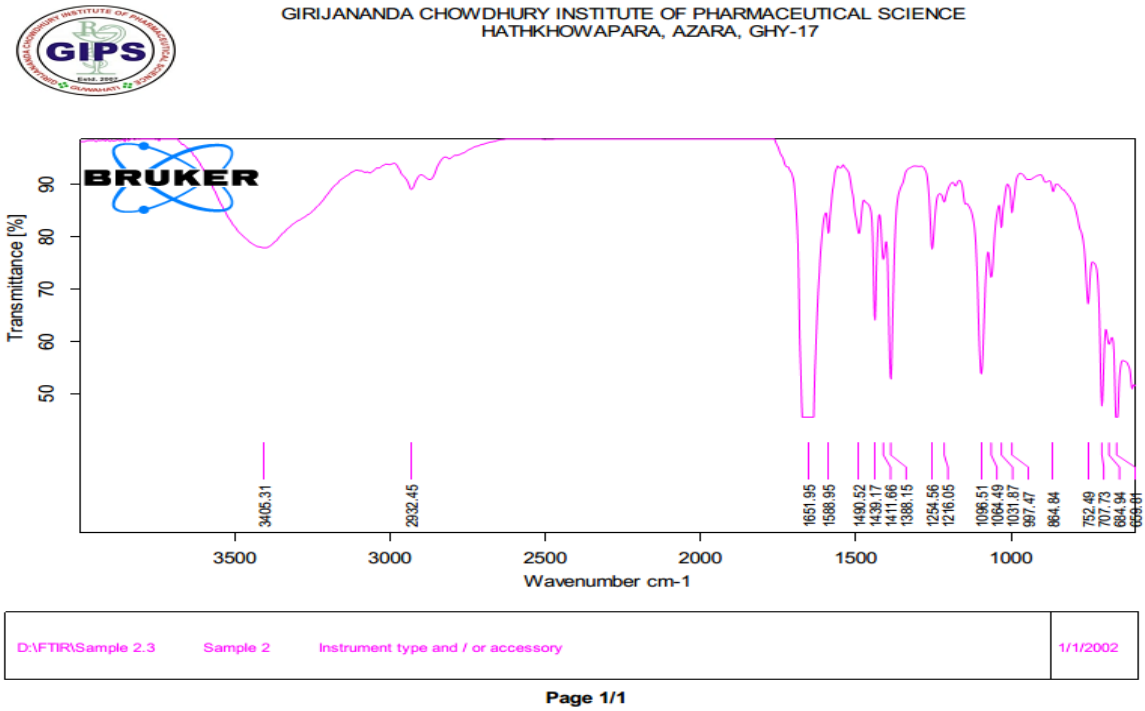
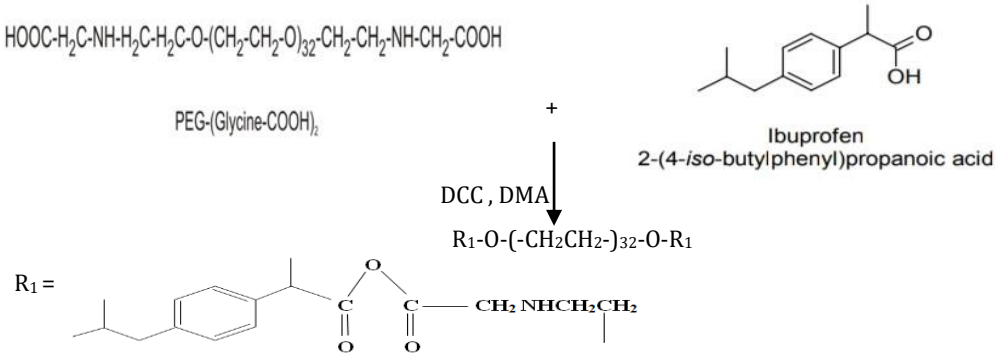


Figure 4: FTIR of PEGCl2-Glycine

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).

C) ***PEG – Cl_2*** –Glycine to Ibuprofen



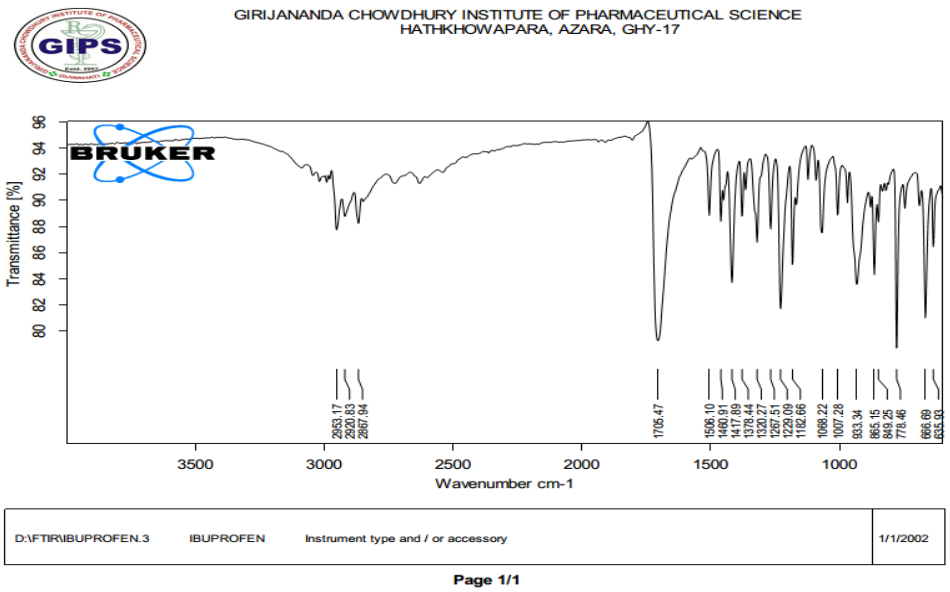


Figure 5: FTIR of Ibuprofen

IR of Ibuprofen sprectum shows broad band between 2800-2953 cm-1 (CH), 1400-1706 cm-1 (C=C) .

Estimation of Drug content

The amount of drug present in each of the pro-drugs was estimated spectrophotometerically and is given in Table 1. It was found that as the spacer length increases, the amount of drug incorporated into the polymer backbone decreases.

Table 1: Amount of drug present in each of the pro-drugs

SNo	PEG pro-drugs	Drug content/100mg of pro-drug
1	PEG [(Glycine) – Ibuprofen]	62.87mg

Table 1: Estimated drug content of PEG - pro – drugs *In-vitro* drug release from [(GLYCINE) – Ibuprofen] 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
24	22.14	56.24	62.18	66.28

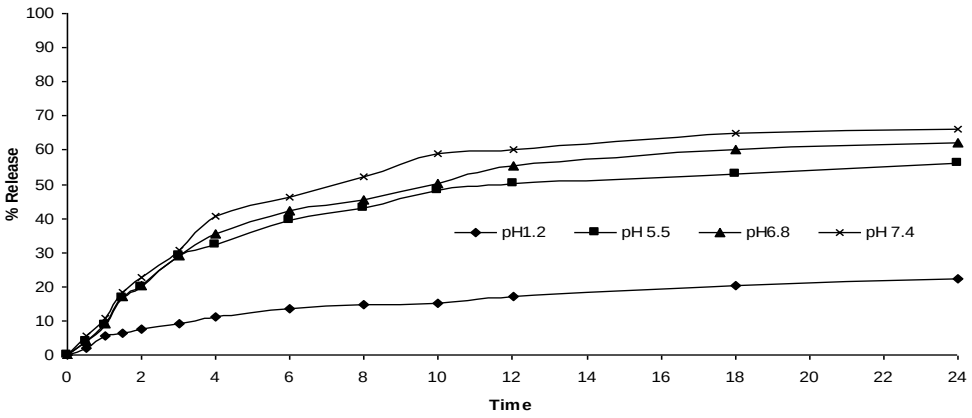


Figure 6: *In-vitro* drug release from PEG-[(Glycine) – Ibuprofen]

The *in vitro* release of acyclovir from the pro-drugs were evaluated in pH 1.2 (acidic buffer), pH 5.5(phathalate buffer) pH 6.8 and 7.4 (phosphate buffer).The *in vitro* drug release profiles of the pro-drugs, PEG-[(Glycine) – Ibuprofen].

The *in vitro* release profile of ibuprofen from the polymeric pro-drug (PEG-[(Glycine) – Ibuprofen] is given in Table and Figure. The data shows that the release of ibuprofen is sustained over a period of 24h. At pH 7.4 a maximum of 66.28% was released and the time taken for 50% of drug release (T_{50}) was 8h. At pH 6.8, a maximum of 62.18% was released with a T_{50} of 10h. At pH 5.5 a maximum of 56.24% was released with a T_{50} of 12h. Eventhough the drug release was sustained at pH 1.2, the amount of drug released over a period of 24h was much lower (22.14%) when compared to the higher Ph.

Stability Studies

Duration (in days)	PEG Cl2-Glycine (Ibuprofen)

At 25 °C / 60% RH

0	60.78
30	67.58
60	58.50
90	62.50

At 40 °C / 75% RH

0	70.75
30	68.00
60	58.50
90	58.00

At room temperature

0	67.75
30	66.00
60	68.50
90	67.25

Objective

Main objective of PEG-Ibu conjugation to improve the duration of action. The biologically active drug and a polymer conjugation is one of the numerous methods for changing and controlling the pharmacokinetics, biodistribution, and also helps to decrease the toxicity of the compound. Conjugation of PEG with ibuprofen also helps the control release of drug from the system.

Non-steroidal anti-inflammatory drugs (NSAIDs) are usually poorly soluble in water and they frequently caused gastrointestinal side effects such as gastric ulceration, bleeding and perforation, Ibuprofen is a NSAIDs with well known anti inflammatory, antipyretic and analgesic properties. Chemically it is 2-(4-iso butylphenyl)propanoic acid poorly soluble in water. It is mostly administered orally; it has a short biological half life of 2 hrs which means frequent doses are required to maintain the therapeutic efficacy over extended time periods. These problems can be overcome by preparation of polymeric prodrug which helps to the decrease the dose and also reduced toxicity.

In this study PEG is being used as the carrier polymer , because it is known to as nontoxic , non-antigenic and non teratogenic , non-immunogenic ,biocompatible ,available in variety of molecular weights, linear, uncharged, amiphilic polymer, widely soluble in water and in most organic solvents and has solubilizing properties.

CONCLUSION

This article deals with the investigations carried out by the writer on the synthesis, characterization and evaluation of some polymeric pro-drugs. This technique of polymeric prodrug conjugation is a fascinating approach for efficient drug delivery and appears to have a bright future in therapeutics. PEG-Ibu conjugation helps to increase the therapeutic activities , it helps to reduce the dose no which leads to reduce toxicity. Main objective of this experiment is that to control the release of drug and improve the duration of action.

CONFLICT OF INTEREST: The present work does not have any conflict of interest.

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