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Research Article

Synthesis, Chemical Hydrolysis and Bioavailability Evaluation of Poly(HEMA)-Ibuprofen Conjugate as Macromolecular Prodrug.

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ABSTRACT

Ibuprofen (IBU) suffers from the general side effects of NSAIDs owing to local and systemic action. The study aimed to retard the adverse effects of gastrointestinal origin. A macromolecular prodrug of IBU was synthesized by coupling the drug to 2-hydroxypropyl methacrylate (HEMA) to get a monomeric drug conjugate which was then polymerized to get polymeric prodrug. Purified synthesized prodrug was characterized by m.p, TLC, UV, FTIR, NMR and MS. The prodrug was evaluated for its *in-vitro* drug release behavior in buffers of pH 1.2 and 7.4 mimicking the upper and lower GIT respectively. The results showed that the drug release from the polymeric backbone takes place in a sustained manner over a period of 12 h and the amount of drug released was comparatively higher at pH 7.4 indicating that the drug release should takes place predominantly at the alkaline environment of the lower GIT rather than at the acidic environment of the upper GIT. The *in-vivo* release studies in rabbits after oral administration of IBU conjugate revealed sustained release characteristics within the prodrug treated animals. This study suggested that after oral administration, the drug –polymer conjugate can release IBU for prolonged periods and predominantly in the lower GIT in a site specific manner, thus reducing the direct contact of the drug to the gastric mucosa and hence the GIT complications.

Key words: NSAIDs, Polymeric prodrug, Ibuprofen, Hydroxyethyl methacrylate, Sustained release.

INTRODUCTION

Anti-inflammatory drugs (NSAIDs) are widely employed in the treatment of pain and inflammation in a large number of conditions like rheumatic fever, rheumatoid arthritis (RA) and osteoarthritis (OA)(1, 2). However, they have been reported to be associated with a number of undesirable effects, which in particular include gastrointestinal toxicity. The NSAID, IBU is used clinically in the treatment of chronic articular rheumatism, but to obtain good therapeutic effects, repeated administration is inevitable. Frequent medication of IBU, however, is well known to cause serious gastrointestinal damage, like other NSAIDs. Also, IBU has a short plasma half-life of 1-2 hours which further necessitates frequent administration to maintain therapeutic drug doses (3, 4, 5).

A possible way to solve this problem is to derivatize the carboxylic functional group of the IBU to produce prodrug with adequate stability at the acidic pH of the stomach and releasing the drug in a sustained manner predominantly at the intestinal pH in order to avoid direct contact of free carboxyl group of the drug to the gastric mucosa.

It was proposed therefore to synthesize a macromolecular prodrug of IBU in which the drug is covalently attached to poly(HEMA) through an ester linkage which would undergo hydrolysis to cleave the drug predominantly at intestinal pH and in a sustained manner and to evaluate its *in-vitro* hydrolysis studies at pH 1.2 and pH 7.4 mimicking the pH of upper and lower GIT. *In-vivo* release studies were carried out in rabbits after oral administration of the prodrug.

Poly (HEMA) was used as the drug carrier as it is known to exhibit low interfacial energies with aqueous solutions and has a weak tendency to absorb biological species such as blood cells and proteins. It is a typical hydrogel which swells in water, facilitating the easy penetration of water and release of the drug by hydrolysis. Moreover, it is not absorbed by mucosal surfaces and is excreted unchanged. Ester linkage, which is formed between the drug and the poly(HEMA) has relatively low stability and can hydrolyze easily in the physiological medium (6, 7, 8).

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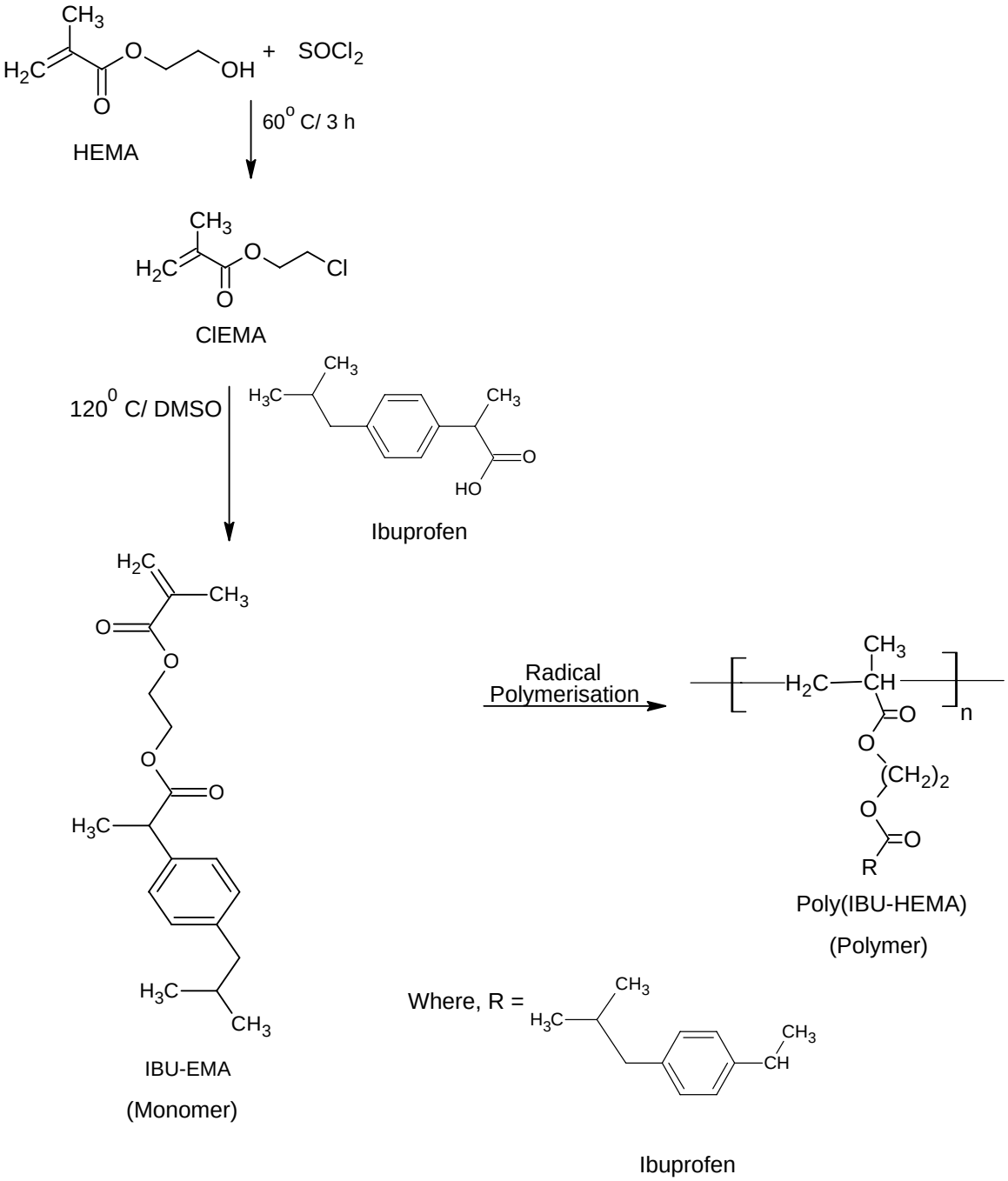
MATERIALS AND METHODS

Apparatus and materials

The melting points were determined by open capillary method. The purity of the compounds was ascertained by TLC on precoated silica gel-60 F_{254} plates (Merck, Mumbai). The IR spectra of the synthesized compounds were recorded on a Shimadzu-8400S FT-IR spectrophotometer, in KBr pellets. The ^{1}H NMR spectra were recorded in DMSO using Varian VR_x-300 ($300MH_z$) instrument. The Mass spectrum was recorded on Shimadzu-2010A mass spectrophotometer. The absorbance maxima (λmax) were determined on a



Scheme 1. Synthesis of polymeric prodrug of Ibuprofen

Shimadzu 160 A UV-Visible double beam spectrophotometer using 10 mm matched quartz cells.

2-Hydroxypropyl methacrylate (HEMA) was obtained from Fluka (Switzerland). Benzoyl peroxide was purchased from Wilson Laboratory, USA. Ibuprofen was a gift sample from Apex chemicals, Hyderabad, Chennai and was used after confirming its purity. All other solvents used were purified by standard techniques.

Preparation of Polymeric Pro-Drugs

i) Preparation of chloro derivative of HEMA

2-hydroxyethyl methacrylate (HEMA, 173.61 mmol) and thionyl chloride(220.34 mmol) were refluxed at 70^{0}C for 3 h with continuous stirring. The excess thionyl chloride was removed by distillation under pressure and the chloro ethyl methacrylate (ClEMA) was obtained as a liquid.

IR (KBr) spectrum showed bands at: 1722 cm^{-1}(C=O stretching of ester group), 1636 cm^{-1} (C=C stretching), absence of any signals in the region 3000 to 3500 cm^{-1} and the presence of characteristic band at 759 cm^{-1} (C-Cl stretching), thus indicating the replacement of the hydroxyl group by chlorine.

ii) Coupling of IBU to ClEMA (monomer)

Ibuprofen (48.47 mmol) and ClEMA (53.25 mmol) were refluxed in presence of DMSO(50 ml) at 120^{0}C for 8 h with continuous stirring to get a IBU-EMA conjugate. The reaction mixture was then poured into distilled water (200 ml) with vigorous stirring. A brown precipitate was formed which was filtered and dried to get the monomeric (IBU-EMA) drug derivative. The drug derivative was dissolved in 20 ml acetone and reprecipitated from distilled water. The monomeric drug derivative IBU-EMA conjugate obtained with a 76 % yield (w/w) and m.p 89 ºC was characterized by its FTIR, ^{1}H-NMR and Mass spectroscopy.

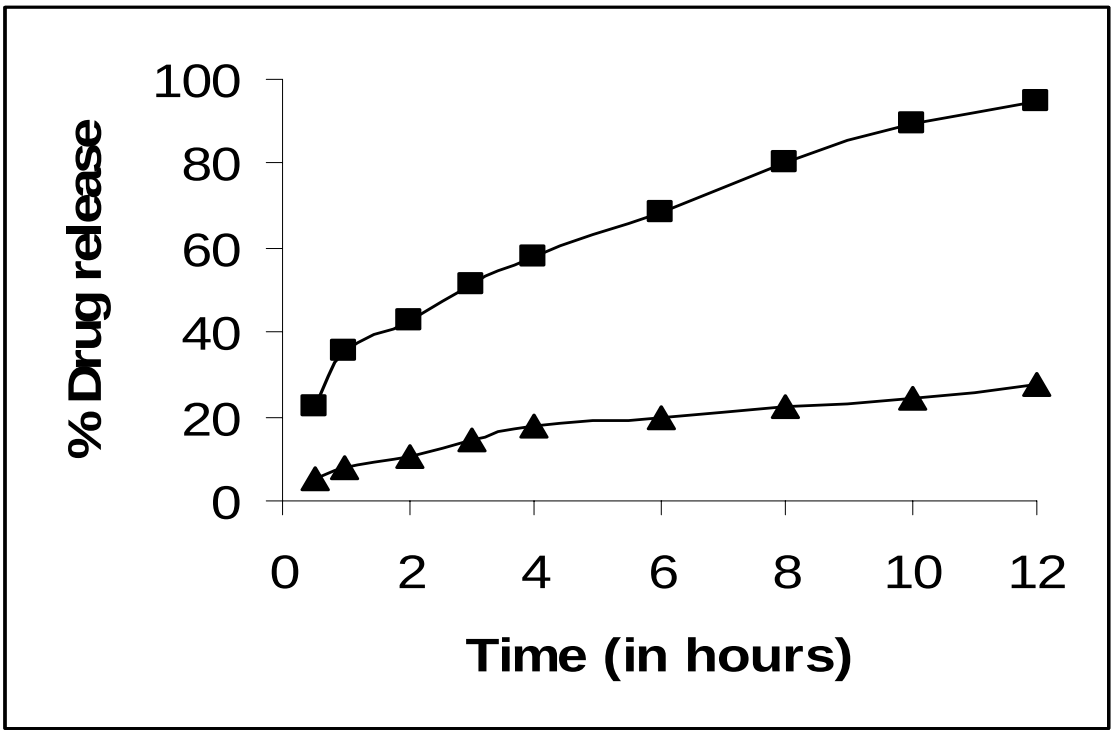


Figure 1. ***In-vitro*** **release of Ibuprofen from polymeric prodrug in buffer solutions of pH 1.2 (▲) and pH 7.4 (■) at 37±0.1 ºC. Each value is the mean ± S.D, n=3**

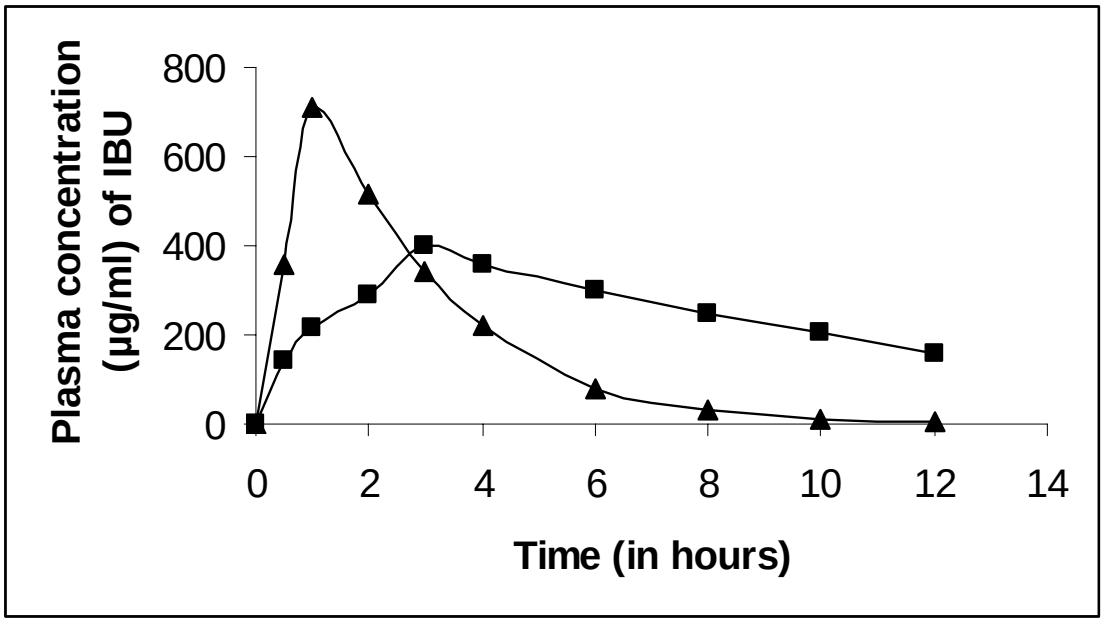


Figure 2. Plasma concentration of IBU after oral administration of (▲) IBU and (■) prodrug to rabbits. Mean ± SD, n=3

IR (KBr) spectrum showed bands at: 1743 cm^{-1}(C=O stretching of ester group), 1636cm^{-1} (C=C stretching), 3048 cm^{-1} (aromatic CH stretching), 2949(CH stretching of CH_3) 760 cm^{-1} (C-Cl stretching), thus indicating the replacement of the hydroxyl group by chlorine.

^{1}H-NMR (DMSO): ppm, 0.90 (d, 6H, C$\underline{H_3}$-CH- C$\underline{H_3}$); 1.45 (s, 3H, =C-C$\underline{H_3}$); 2.16 (s, 2H, -C$\underline{H_2}$-CH-); 2.46 (m, 1H, CH_3-C$\underline{H}$- CH_3); 3.48 (m, 4H, –COO-(C$\underline{H_2}$)$_2$); 6.8 (s, 2H, -C=C$\underline{H_2}$); 7.18 (m, 4H in the ring).

The mass spectra of IBU-EMA shows molecular ion peak at m/z 318 corresponding to the molecular formula $C_{19}H_{26}O_4$(Formula weight: 318).

iii) Polymerization of the monomeric drug derivative:

To a solution of monomer (11.88 mmol) in DMSO (50 ml) was added benzoyl peroxide (0.1 g) and ascorbic acid (0.1 g) and the contents were refluxed at 70^{0}C for 6 h with continuous stirring. The reaction mixture was then cooled and poured into distilled water to get a brown precipitate of the polymeric prodrug i.e. Poly(IBU-EMA). The precipitate was purified by dissolving it in 20 ml acetone, reprecipitated from distilled water and dried at reduced pressure to a constant weight. It was further purified by column chromatography using benzene : methanol (9:1) as the solvent. M.P- 108 ºC.

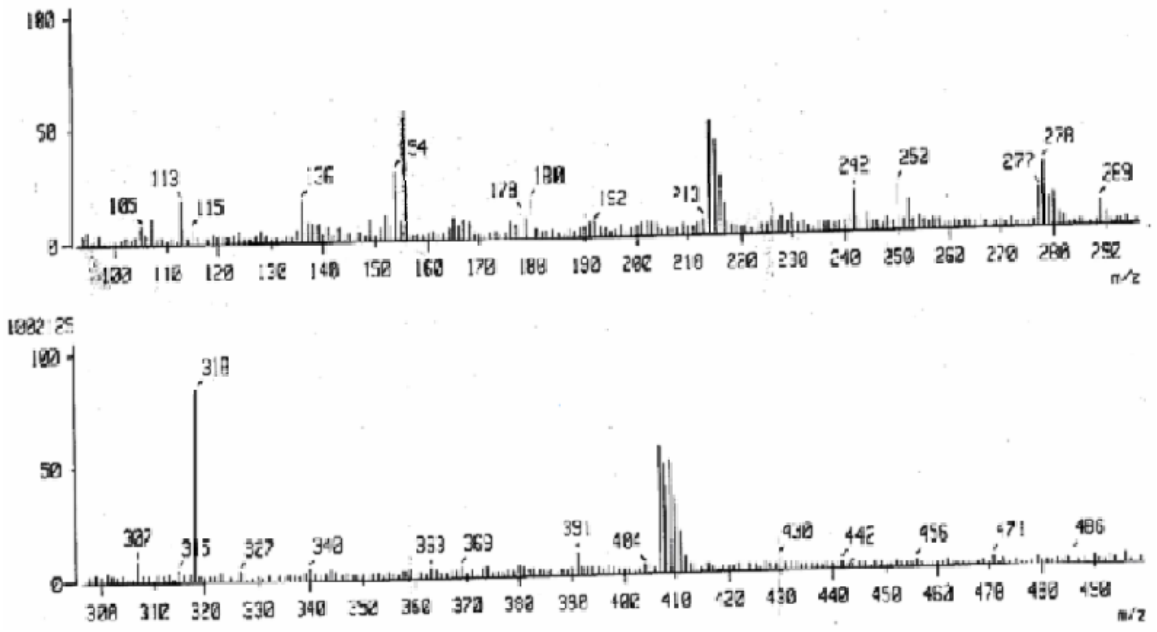


Figure 3. Mass spectrum of IBU-EMA conjugate (monomer)

FTIR (KBr) shows disappearance of peak at1636 cm^{-1} for C=C stretching indicating polymerization of the monomer. ^{1}H-NMR (DMSO) spectra shows the disappearance of olefinic protons at 6.8 ppm of the IBU-EMA conjugate and appearance of the corresponding alkyl protons at 1.81ppm(s, 2H, -C$\underline{H_2}$-C-) of the poly(IBU-EMA).

Estimation of drug content

The amount of drug attached to polymer backbone was estimated by allowing 100 mg of the prodrug to get hydrolyze completely by keeping it overnight in 1 N NaOH solution. The solution was filtered and λ_{max} of the filtrate was found to be 263 nm which was same as that of pure IBU in the same media indicating the insolubility of the polymeric backbone in the aqueous media. The absorbance of the resulting solution was measured at λ_{max} of IBU and the amount of the IBU in the pro-drug was calculated.

In-vitro drug release studies.

The *in-vitro* drug release study of the polymeric pro-drug was carried out using USP dissolution apparatus (Type-2, Paddle assembly) (9). 100 mg of the polymeric prodrug was placed separately in dissolution test apparatus containing 900 ml of dissolution media of pH 1.2 (HCl buffer) and pH 7.4 (Phosphate buffer) and stirred at 100 rpm at 37± 0.1° C over a period of 12 h. An aliquot of 5 ml of the samples were withdrawn at each time interval (0.5, 1, 2, 3, 4, 6, 8, 10 and 12 h) and replaced with equal volume of fresh dissolution medium. The samples withdrawn at each time interval were filtered and the absorbance maxima (λ_{max}) of the filtrates were determined after making appropriate dilutions using the respective buffer solution as blank. The amount of drug released from the polymeric prodrug at different time intervals and percentage release were calculated according to the method reported by M.J.N. Chandrasekar (10, 11) . Each experiment was repeated in triplicate.

In-vivo drug release studies:

Two healthy overnight fasted male rabbits (approximately 2 kg) obtained from Central Animal House, J.S.S.College of Pharmacy, Ootacamund, India were used for the experiments. The experimental protocol was approved by the Institutional Animal Ethical Committee (JSSCP/IAEC/Ph.Chem/Ph.D/01/2005-06). The rabbits were used to compare the bioavailability of the prodrug with that of IBU following oral administration.

IBU (10 mg/kg body weight) and Poly(IBU-EMA) (14.29 mg/kg body weight) were suspended in 1% carboxy methyl cellulose and the homogenous microsuspensions were administered orally using a conventional gastric delivery tube. Each drug solution was prepared immediately prior to administration. Blood (1.5 ml) was withdrawn immediately prior to drug administration from the marginal ear vein. Further 1.5 ml blood samples were withdrawn at intervals of 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 h period using a sterilized syringe.

The blood samples collected in the centrifuge tube containing the anticoagulant (0.2 ml) were centrifuged at 7000 rpm for 15 min. and the plasma were separated and stored at -20°C. The drug concentration in the plasma was analysed by using UV spectrophotometer as it is not interfered by the polymer backbone. A plasma concentration-time curve was plotted. The area under the plasma concentration-time curve ($AUC_{0\rightarrow\alpha}$) was calculated using the trapezoidal rule. Each experiment was repeated in triplicate.

RESULTS AND DISCUSSION

In order to improve the biopharmaceutics of the current NSAID, Ibuprofen, we selected the macromolecular prodrug approach in which the drug was coupled to a macromolecular carrier through an ester linkage.

The synthesis of macromolecular prodrug of IBU involved three steps (Scheme I). The first step involved the conversion of HEMA into its chloro derivative (ClEMA) by using thionyl chloride in order to make it susceptible for esterification with drug. In the second step the drug was coupled to ClEMA to get monomeric drug conjugate (IBU-EMA). Third step involved polymerization of the monomeric drug conjugate by free radical polymerization method using free radicals that are liberated during a redox reaction between a polymerization accelerator (benzoyl peroxide) and a polymerization initiator (ascorbic acid).

Free radicals induce the aperture of the double bond of a HEMA molecule and a radical is transmitted to this unstable monomeric molecule which can in turn react with a new monomer molecule [8].

The purity of the synthesized compounds were verified by TLC using benzene : methanol (9:1) as solvent system. The analytical and spectral data were in agreement with the attributed structure.

Drug Content:

The amount of IBU linked to poly(HEMA) as evaluated after keeping the prodrug for complete hydrolysis in 1N NaOH for 24 h and analysed by UV spectrophotometer was found to be 701 mg/g of the macromolecular prodrug.

In-vitro release studies:

The polymeric pro-drugs synthesized, on 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 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.

The *in-vitro* drug release studies were carried out in buffers of pH 1.2 and 7.4 using USP type 2 dissolution test apparatus. The samples withdrawn from the dissolution media at different time intervals were filtered. The filtrates showed λ_{max} equal to that of pure ibuprofen i.e 263 nm which indicates that the polymer backbone [Poly(HEMA)] is insoluble in buffer solutions but releases free drug on hydrolysis.

The *in-vitro* drug release profile of Poly(IBU-HEMA) conjugate as given in Figure 1 shows a pH dependent drug release behavior. At pH 7.4, an initial burst release of 35.23 % was observed within 1 hour followed by a sustained release over a period of 12 hours. At pH 1.2, drug release was seen to be comparatively slower. A sustained drug release with a maximum drug release of only 27.51% was observed over a period of 12 hours. The t_{50} of the drug release at pH 7.4 was about 3 hours and the maximum release was found to be 94.88%.

This confirms that the release of IBU should occur predominantly at higher pH of the intestine. This may be due to the fact that ester hydrolysis is a reversible reaction in acidic pH and in alkaline pH it is irreversible and complete (12). The predominant release of IBU from the prodrug at pH 7.4 indicates potential of the prodrug to reduce the gastric complications caused by direct contact of free carboxyl group of the drug to gastric mucosa.

***In vivo* drug release studies:**

One of the initial screening criteria for new drug candidates is their oral bioavailability. Many of the potential drug candidates are passed over because of their poor bioavailability. A comparative bioavailability study between the free drug, IBU and its prodrug was therefore carried out following a cross over study in two healthy male albino rabbits over a period of 12 h. Each drug solutions were prepared immediately prior to use and the administration was carried out at a distance of 21 days in order to guarantee the complete elimination of IBU from the body. The plasma concentration of IBU over time following oral administration of the conjugate and the free drug are shown in figure 2. IBU gives a plasma concentration of drug that increases sharply within 1 h after oral administration, but decreases very rapidly with time and remains fairly constant. The polymeric prodrug showed a sustained release of IBU after oral administration with lower drug plasma concentration initially compared to the administration of free drug and decreases very slowly with time, remaining practically constant for about 12 h.

The maximum plasma concentration of IBU (C_{max}) obtained in the case of free drug was 710.57 µg/ml within 1 h whereas for the prodrug was 397.45 µg/ml after a period of 5 h. The delay in the t_{max} is thus due to the slower rate of drug release from the conjugate and consequent absorption. Detectable concentration of drug was observed till 12 h in the case of prodrug whereas it was only 6 h for the free drug. There was no significant change in the extent of drug absorption between the free drug and the prodrug as the area under the plasma concentration-time curve for the free drug and the prodrug is almost the same with values 734.78 and 725.64 respectively. This confirms the complete release of the drug from the prodrug and its consequent absorption in the systemic circulation.

CONCLUSION

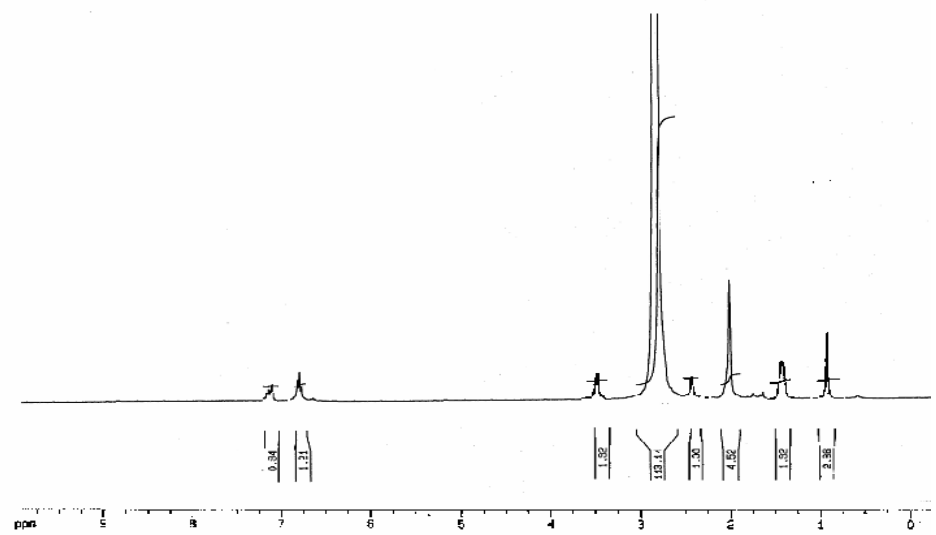


Figure 4. NMR spectrum of IBU-EMA conjugate (monomer)

The macromolecular prodrug obtained by linking the IBU molecules to Poly(HEMA) showed pH dependent and sustained drug release behavior with higher rate and amount of drug released at higher pH. This behavior revealed the potential of the polymeric prodrug to improve the pharmacokinetic parameters especially $t_{1/2}$ and to reduce the adverse effects by delivering the drug in a site specific and sustained manner, thus reducing direct contact of the drug to the gastric mucosa.

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