In Vitro Controlled Release of Polymeric Drug-Saccharide Conjugates with Ketoprofen, Ibuprofen, and Naproxen Pendants

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ABSTRACT: Polymerizable monomers of glucose and three types of nonsteroidal anti-inflammatory drugs were prepared. Free radical polymerization of the resulting monomers was carried out in DMF. The polymers with drug pendent were characterized using FTIR, NMR, and GPC spectroscopy. Following hydrolysis of polymeric drug-saccharide conjugates at 37°C, the cumulative released of naproxen, ibuprofen, and ketoprofen were 70, 55, and 30% after 10 days, respectively. Half-lives ($t_{1/2}$) of the copolymers were calculated through the first 12 h and these were found to be 4.2, 5.3, and 7.8 h, suggesting a slow and sustained drug release mechanism. The drug release profiles were also carried out at different pH, temperature, and ionic strength and solution varieties. It was concluded that different release profiles occurred and were dependent on temperature and pH. It was apparent that the polymeric drug-saccharide conjugate had the potential to be developed as a system that enhances drug delivery. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 121: 1654–1660, 2011

Key words: copolymerization; copolymers; controlled release; degradation

INTRODUCTION

Understanding of the controlled release and hydrolysis characteristics is essential in determining the effectiveness of drug delivery systems, especially in polymeric prodrugs.[1,2] Dissolution/release tests are generally not only used in the quality control of the drug formulation, but also in predicting their behavior *in vivo*.[3,4] Based on the physical or chemical characteristics of polymeric prodrugs, drug release mechanism from a polymer system also is significant and many systems exhibit various release mechanism under different conditions.[5–7]

The drug-saccharide polymer (DSP) system has been attempted using some prodrugs, which provide many advantages including prolonged drug release, changed biodistribution, reduced toxicity, and increased patient acceptance.[8–11] Moreover, biological activities, environmental safety, and nontoxic of saccharide [12,13] are also provided. Therefore, polymeric prodrugs with saccharide have been recently investigated as a potential strategy for merging the relative advantages of saccharide and macromolecules into a single system.[9–14] This system can be useful to both increase drug stability and avoid the rapid release. Thus it is worthy of interest to evaluate sugar as a potential carrier for improving drug dissolution behavior. However, research on the influence of parameters that relate to *in vitro* drug release is required as this would provide available information to further investigate the therapeutic benefit of drugs and their derivatives. Unfortunately, there are few reports that relate to factors linked to polymeric drug-saccharide conjugates when applied as a single system.

In this work, ketoprofen, ibuprofen, and naproxen, which are widely used for alleviation of pain and inflammation associated with tissue injury,[15,16] have been used as the model drugs. DSPs were produced using an efficient chemical-enzyme combined method and the structures of the conjugates were confirmed by FTIR, NMR, and GPC. The copolymers were hydrolyzed in aqueous buffer solutions under physiological conditions, and the effects of different variables (pH, ionic strength, temperature, and medium variation) on drug release were investigated.

Additional Supporting Information may be found in the online version of this article.

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EXPERIMENTAL

Materials

Succinic acid (for synthesis) was purchased from Sinopharm Chemical Reagent (Shanghai, China). Alkaline protease, EC 3.4.21.62, was from Wuxi Xue Mei Technological (Wuxi, China). Lipase immobilized from *Mucor miehei*, EC 3.1.1.1 was bought from Fluka. Nonsteroidal anti-inflammatory drug (NSAIDs, ketoprofen, ibuprofen, and naproxen) was supported by Zhejiang Jiuzhou Pharmaceutical (Taizhou, P R China). 2,2′-Azo-bis-iso-butyronitrile (AIBN) was purified by recrystallization with methanol. Glucose was purchased from Sinopharm Chemical Reagent (Shanghai, China). All other chemicals used were analytical grade and were first dried over 4 Å molecular sieves for 24 h prior to use.

Methods of preparation and characterization

Synthesis of polymerizable monomers having NSAIDs or glucose

Drug vinyl ester and Succinic acid vinyl ester were synthesized as described by Cai et al.[15] Racemic drugs or Succinic acid (5.0 g) and mercuric acetate (0.25 g) were dissolved in 70 mL of vinyl acetate. After stirring the mixture for 30 min at room temperature, 0.2 mL of concentrated sulfuric acid was added, and the solution was refluxed for 3 h at 77°C. Then the mixture was allowed to cool to room temperature, and sodium acetate (1.5 g) was added to quench the catalyst. After the solution immobile, it was filtered and concentrated. The crude products were purified by silica gel column chromatography with the mobile phase of petroleum ether/ethyl acetate (9 : 1, v/v). After the work, three kind of drug vinyl ester were produced, ketoprofen vinyl ester (KVE), ibuprofen vinyl ester (IVE), naproxen vinyl ester (NVE), and Succinic acid vinyl ester. The yield was 89.7, 90, 85, and 43.3%, respectively.

Polymerizable glucose derivatives (6-*O*-vinylsuccinoyl-D-glucose, VSG) were synthesized using alkaline protease as catalyst. Glucose and Succinic acid vinyl ester (1 : 3, mol/mol), and alkaline protease (2.0 g) were mixed with 100 mL anhydrous pyridine in the cone-shaped bottle (250 mL). Then the mixture reacted at 50°C under 250 rpm for 4 days. After the reaction, the mixture was filtered and concentrated. The crude products were purified by silica gel column chromatography with the mobile phase of ethyl acetate/methanol (17 : 2, v/v). The yield was 41.9%.

Polymerization of prodrugs with NSAIDs pendent

A mixture containing drug vinyl ester and VSG (1 : 1, mol/mol), AIBN (2%, w/w), using *N*, *N*-Dimethylformamide (DMF) as solution, was placed in a 10-mL sealed polymerization tube, stirred at 70°C under N_2. The resulting product was repeatedly precipitated in methanol and then dried under reduced pressure. The products were poly(KVE-*co*-VSG) **(a)**, poly(IVE-*co*-VSG) **(b)**, and poly(NVE-*co*-VSG) **(c)**.

Characterization of polymeric prodrugs

The polymeric prodrugs were characterized by ^{1}H-NMR, ^{13}C-NMR (300 MHz spectrometer, Bruker Avane, Germany), FTIR (Perkin Elmer) and GPC (mobile phase: DMF; run time: 50 min; column temperature: 50°C).

Drug release from polymeric prodrugs

In vitro release studies of the polymeric prodrugs were carried out by placing dried and loaded samples in definite volume of releasing medium. The products (5 mg) were placed in a dialysis membrane (MWCO = 3500 Da) adding with 1 mL medium, then dialyzed in a 25 mL bottle with 10 mL corresponding solution for 10 days stirring throughout (100 rpm). The 10 mL medium was replaced with the same volume of fresh solution at predetermined times. The released drug was determined by HPLC (mobile phase: methanol/water, 80/20, v/v; wavelength: 254 nm; flow velocity: 1 mL/min), and drug concentration was monitored by UV–vis spectrophotometer at 254 nm. For release studies, different pH buffer (1.2, 5.8, 7.4, 8.0, 10.0), different media (0.5*M* NaOH, 0.5*N* HCl, 0.1*M* PBS, distilled water), different temperature (25, 37, and 50°C), and different ionic strength (0.5*M* NaCl, 0.2*M* NaCl, distilled water) were used.

RESULTS AND DISCUSSION

Preparation and characterization of polymeric drug-saccharide conjugates

In preparing of pharmaceutically active polymer monomers, it is necessary to design synthetic conditions mild enough to allow attachment of agents that have no adverse effect on the physiological activity of the drug.[17,18] In this study, we chose to synthesize such prodrugs using a protocol that involved polymerization of drug molecules containing vinyl functional groups and a functional spacer arm was located between the drug and vinyl moiety. The polymeric prodrugs with NSAIDs and glucose pendent were prepared by a two step process, using 2,2′-azobisisobutyronitrile (AIBN) as initiator and anhydrous DMF as solvent at 70°C.

The structures of the products were confirmed by FTIR and NMR. Taking poly(KVE-*co*-VSG) (**a**) as an

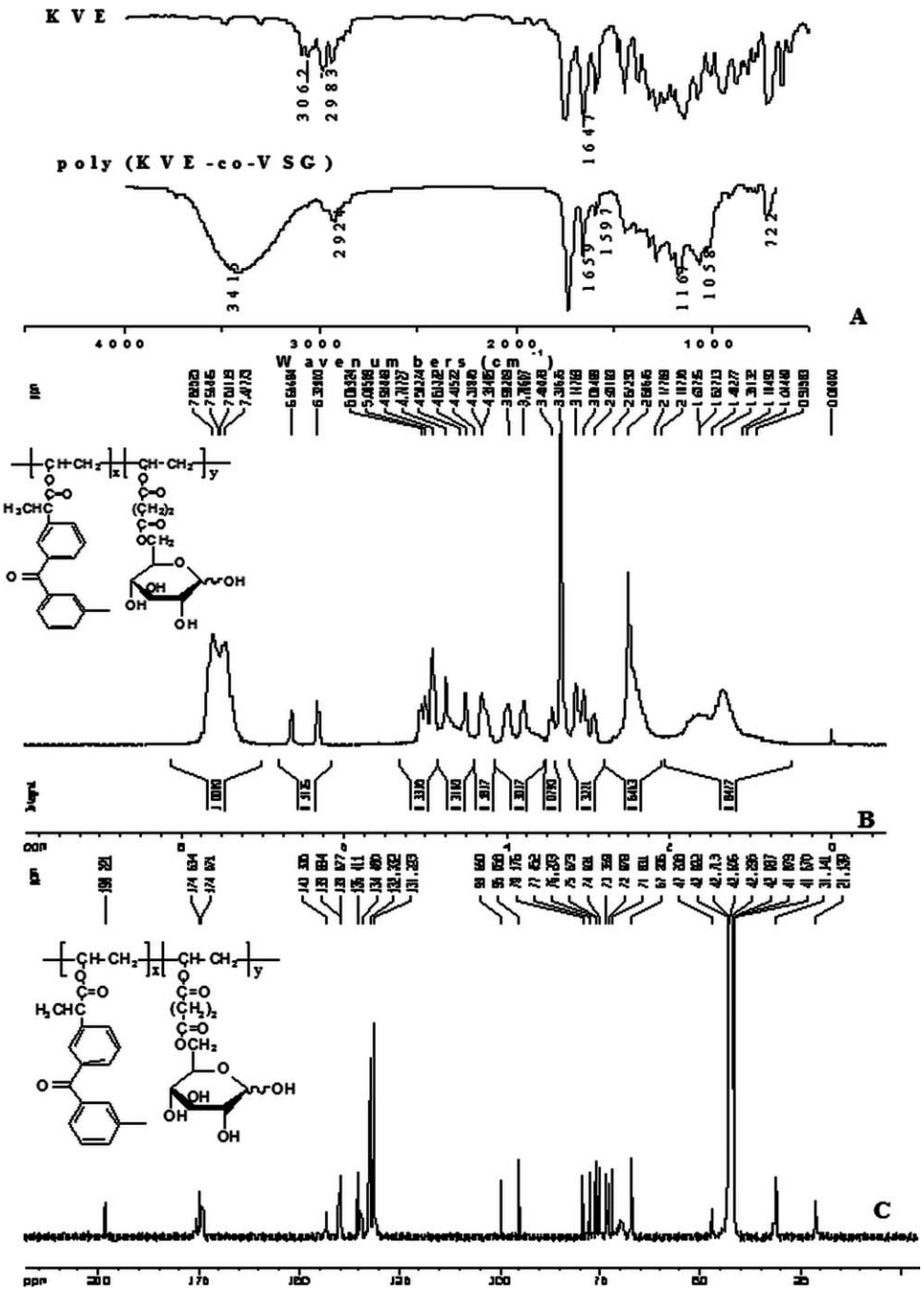

Figure 1 IR and NMR spectra of poly (KVE-co-VSG) **(a).**

example, the IR spectrum showed that the vinyl group absorption in the KVE monomer disappeared in the corresponding polymer [Fig. 1(A)] and the characteristic absorption assigned to D-glucose moieties and aromatic ring were now evident. The results confirmed the structure of the synthesized polymers. NMR data also revealed the disappearance of vinyl group with the presence of ketoprofen, glucose group, and poly(vinyl alcohol) main chain. Thus results from IR and NMR spectra clearly demonstrated that the conjugate products were formed. Moreover it was estimated from NMR spectra that the ratio of drug vinyl ester to VSG in copolymer was 0.72 : 1, 0.75 : 1, and 0.73 : 1 and drug loading capacities were 41.07 wt % **a**, 38.13 wt % **b,** and 34.97 wt % **c**, respectively (Table I).

One parameter in characterization of polymeric prodrugs was determination of molecular weight. We used gel permeation chromatography (GPC) for the determination of average molecular weights of the polymeric prodrugs. The values were collected in Table I. From the table, we found that copolymers were obtained in the molecular weight range of M_n 20,000–40,000 Da with low polydispersity index.

TABLE I
The Molecular Weight of Polymeric Prodrugs

Prodrug	$M_n \times 10^{-4}$	M_w/M_n	Drug loading in prodrugs[a] (w/w %)	Release half-life ($t_{1/2}$, day)
poly (KVE-co-VSG) **(a)**	2.964	1.724	41.07	7.8
poly (IVE-co-VSG) **(b)**	2.126	2.033	38.13	5.3
poly (NVE-co-VSG) **(c)**	2.743	2.157	34.97	4.2

[a] Determined by the integration ratio of 1H NMR spectra.

In vitro drug release

It has been widely demonstrated that side chain hydrolysis of drug pendent polymers depends on the strength and chemical nature of the drug-polymer bonding, the structure of the polymer and the surrounding condition.[18,19] The *in vitro* hydrolysis behavior of drug-polymer adduct was studied under physiological conditions.

Drug (ketoprofen, ibuprofen, and naproxen) release profiles from polymeric prodrugs were studied in PBS solution at pH 7.4 (Fig. 2). Under these experimental conditions the polymers hydrolyzed to release the parent drug as evidenced by HPLC analysis. Drug release was found to be consistently retarded and not more than 50% was released from all the polymeric prodrugs over a 5 days. This could be due to relative stability of the ester bonds between drug and polymer chain.

It was apparent that the release of naproxen reached about 70% after 10 days, whereas about 55% and 30% of ibuprofen and ketoprofen respectively were released over the same time interval. Interestingly this is despite of the similar technique used to prepare drug loaded material. It was interesting to note that the polymeric prodrugs provided a release with an initial burst effect up to 12 h and then this was drastically reduced after 24 h (Fig. 2). An initial burst of drug followed by a constant release profile was normally regarded as being appropriate system for therapeutic studies that could account for compensating for metabolic losses.

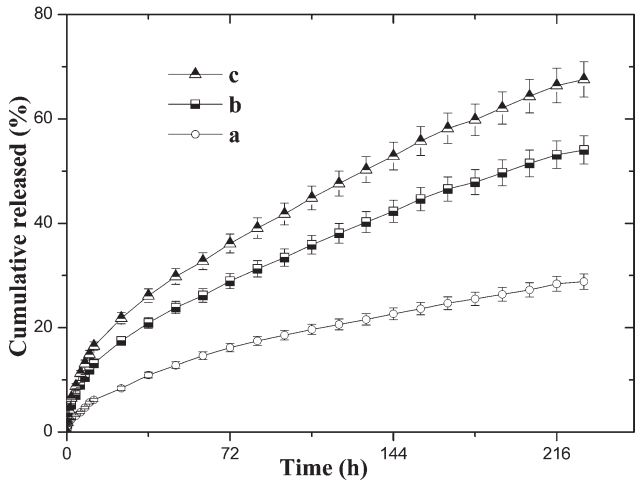

Figure 2 Drug release profiles of NSAIDs in buffer solution from polymeric prodrugs at pH 7.4, 37°C.

The half-life of drug release from polymeric prodrug was calculated using the release data and the data was represented in Table I. The $t_{1/2}$ of **a**, **b**, and **c** was found to be 4.2, 5.3, and 7.8 h, suggesting a slow and sustained release and therefore effectiveness for a longer duration. Overall, release studies in buffer indicated that these types of polymeric prodrugs could effectively prolonged the release of the parent drug and the drug release pattern could be manipulated to achieve clinical requirements.

Influence parameters on the drug release

Drug release from polymeric prodrug strongly depends on the type of functional groups. This not only depends on the type of bonding, monomer structures, molecule weight, but also on the pH and ions present in the degradation medium. These factors all influence hydrolysis rates.[20]

To investigate potential influence factors to the *in vitro* release retarding performance of the polymeric drug-saccharide conjugates, poly(KVE-*co*-VSG) was taken as an example. The effects of pH of dissolution medium, ionic strength, buffer solution variation, and temperature on the release profiles were studied.

Effect of pH on drug release

For many pharmaceutically relevant polymers, the pH of the environment is important in influencing the released characteristics of the agent. Hydrolysis rates can vary at different pH values.[21] This is of particular importance since the degradation products used in pharmaceutical applications are generally acidic in nature.

To examine the degradation of the prodrugs at pH values corresponding to the environment of the stomach [21], different pH were selected. NSAIDs were not recommended to be taken in the fasting state.

The effect of pH on the cumulative release of ketoprofen is shown in Figure 3. Generally, pH has a more marked effect in the near neutral and alkaline pH ranges. As shown in Figure 3(A), the release rate

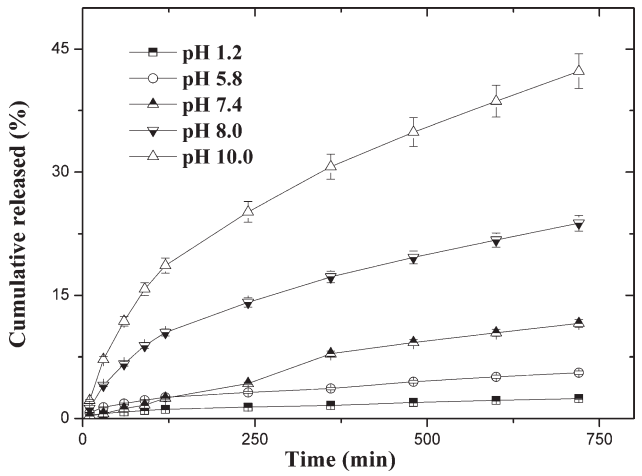

Figure 3 Effects of pH on ketoprofen release characteristics from poly(KVE-*co*-VSG) at 37°C.

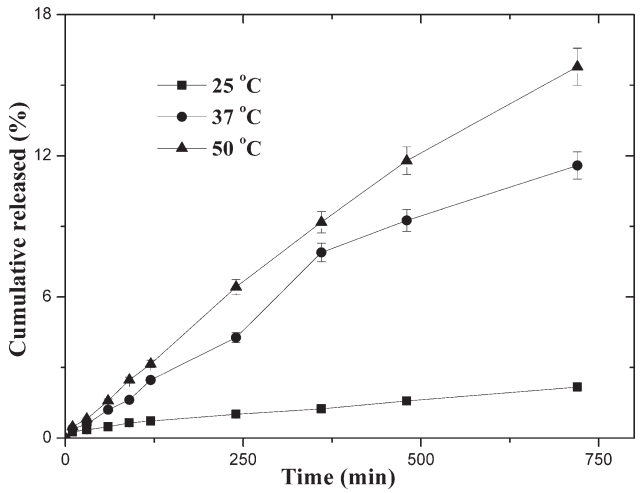

Figure 4 Effect of temperature on ketoprofen release characteristics from poly(KVE-*co*-VSG) in pH 7.4 media.

of drug from polymeric drug-saccharide conjugate at alkaline medium was higher than the release rate of drugs when subjected to acidic condition. This can be explained on the basis of faster hydroxyl ions induce rapid hydrolysis of the ester bond between the drug and polymer chain. Another reason might be that carboxyl drugs tended to exist in a form of salt in alkaline solution, which is beneficial for the drug release. For example, the cumulative release of ketoprofen from **a** reached 42.3% after 12 h at pH 10.0, while it only reached 2.5% under the same condition at pH 1.2. These observations of higher release rates found at the alkali pH values were consistent with results reported by others.[22] It seemed that the drug-saccharide conjugates had a low degree of swelling in the acidic medium and the drug was protected against hydrolytic attack. [23–25] Indeed, it was likely that alkaline conditions favor swelling that in turn might facilitate drug release.

Effect of temperature on the drug release

It is generally known that increase of temperature invariably results in an increase in diffusion rate of molecules either in liquids or solids.[25] The drug release behavior was studied at different temperatures (25, 37, and 50°C) to determine the effect of temperature on the drug dissolution process. The release profiles of ketoprofen from poly(KVE-*co*-VSG), pH 7.4 at different temperature are shown (Fig. 4). Under these conditions the cumulative release of ketoprofen from poly(KVE-*co*-VSG) reached 15.8% after 12 h at 50°C, while only 2.2% was detected in the media under the same condition at 25°C (Fig. 4). These results indicated that the drug release rate of ketoprofen from polymeric drug-saccharide conjugate increased with temperature. This phenomenon might be attributed to increase in kinetic energy effects of the solvent molecule and so this increases in rate of diffusion at temperatures, enhancing release through hydrolysis of the drug from the ester bond that is present between the drug and polymer chain. Therefore, the rate of the hydrolysis reaction is also sensitive to temperature.

Effect of medium on the drug release

The drug release profiles from polymeric prodrug were found to be highly influenced by the dissolution medium composition (Fig. 5). Results from Figure 5 demonstrated marked variability between NaOH media and other buffer solutions. This result was likely linked to the pH values rather than to the chemical nature of the different media. It implied that the level of hydroxide ions stimulate drug release.

Drug release study in water has shown a similar trend to 0.5*N* HCl, although the rate appeared

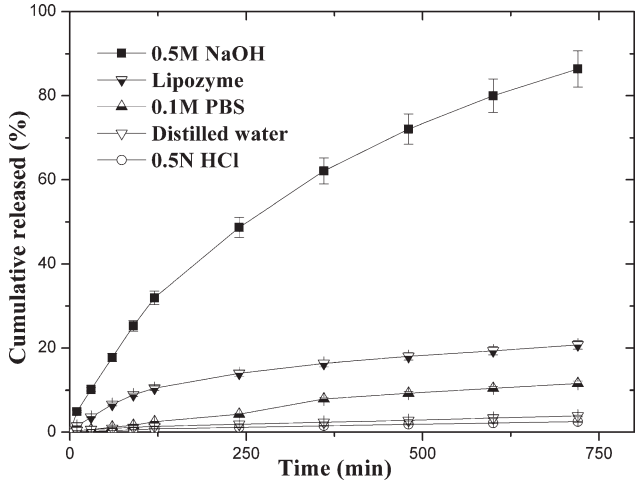

Figure 5 Cumulative drug released from poly(KVE-*co*-VSG) in different dissolution medium at 37°C.

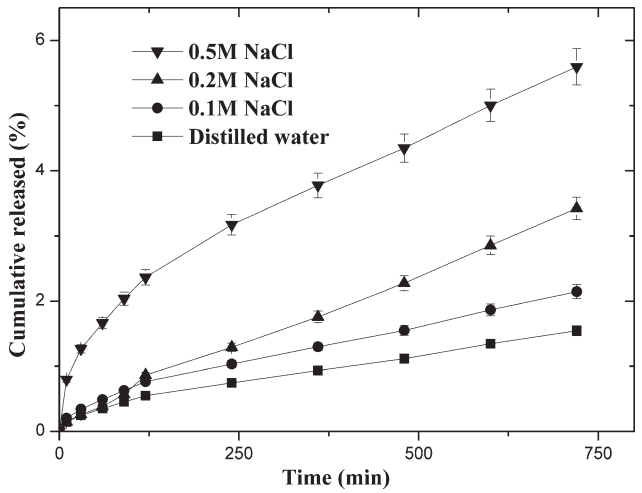

Figure 6 Ketoprofen release from poly(KVE-*co*-VSG) in different ionic strength at 37°C.

faster. The effect of increased hydrolysis could be attributed to the difference in their ionization nature at different pH values. Ketoprofen had a relatively high solubility at alkaline pH and thus the release from copolymer was faster under more alkaline conditions so contributing to the increased release rates.[26]

Drug release catalyzed by lipase, which also hydrolyzed prodrugs *in vivo* was investigated. From Figure 5, the rate of hydrolysis rate catalyzed by lipase (immobilized from *Mucor miehei*, 10 mg/mL) was higher when the same prodrug was treated in 0.1*M* PBS. About 21% was released after 12 h, compared to 12% in PBS solution. It seemed to be desirable to reach a therapeutic concentration rapidly through a disintegration catalyzed by enzyme *in vivo* and maintain the level by compensating for metabolic loss thereafter.[21]

Effect of ionic strength on drug release

To estimate the drug release behavior from polymeric prodrug in different ionic strength, release studies were carried out in water and buffers containing varying concentration over the range 0.2–0.5*M* at constant pH (7.0). Increase in buffer concentration showed a weak difference in the drug release characteristics from the copolymer (Fig. 6). Cumulative released of ketoprofen in water was found to be 1.5% after 12 h, but increased to 5.6% in 0.5*M* NaCl, indicating that the observed effect of drug release was in part a result of increasing salt ion. This was possibly due to the increased susceptibility of the ester bond linking the drug and polymer chain. However, due to poor solubility of ketoprofen in water, it was less likely to dissolve in the solvent and so diffuse through the pores and channels as this process could be rate limiting step for drug release.[27] Therefore it could be postulated that diffusion and dissolution properties of loaded drug along with polymer chain played a significant role in determining the release characteristics of drug.

Ionic strength of the medium appears to have only a minor effect on drug release from polymeric prodrug containing glucose and higher ionic strength induced a faster drug release. This observation might be rationalized on the basis of the increased ionic strength initially causing disruption of the system leading to increased drug release.

Although the ionic strengths of the solutions used in this study exceeded physiological conditions found in the human gastrointestinal tract, they were used in an attempt to determine the behavior of NSAIDs at low and moderated ionic strength. The objective was to take the polymer through a complete continuum of physical changes. In this way, the drug release characteristics and polymer behavior could be observed under all these conditions.

The resulting conjugates could be regarded as polymeric prodrugs since the drug could be released by hydrolysis under mild condition similar to those in biological systems. Furthermore, this type of monomer could easily be copolymerized with various vinyl monomers to improve the hydrolytic behavior by introducing hydrophilic units along the polymer chain.

Considering the results of dissolution studies in all the media used, it was apparent that both temperature and pH had a direct effect on performance. Differences in release could be due to the effect of these factors on the solubility of drugs or on the physicochemical properties of the polymer that regulated the drug release.

CONCLUSIONS

Synthesis of high molecular weight polymeric prodrugs having NSAIDs and glucose groups were achieved which is highly desirable for possible medical applications. Copolymers were obtained in the molecular weight range of M_n 20,000–40,000 Da with low polydispersity index. The products were characterized by FTIR, NMR, and GPC. Studies relating to hydrolysis were carried out in media that simulated physiological conditions. NSAIDs release from polymeric prodrugs was found to be affected by the pH, temperature, and buffer concentration of the release medium. These parameters play a significant role in describing the *in vitro* drug release characteristics. The outcomes suggest saccharide can be used for developing an effective flexible drug release system.

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