Poly[*N*-(2-hydroxypropyl)methacrylamide] Prodrug for Metaxalone via a Chloroacetyl Chloride Linker: Synthesis and Controlled Release Evaluation

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ABSTRACT: Poly[*N*-(2-hydroxypropyl)methacrylamide] (PHPMA) and its drug conjugates are some of the most intensively investigated drug-delivery systems. Metaxalone (Met) was covalently linked to PHPMA via a spacer, and the procedure of the chemical modification for PHPMA was conducted by a two-step protocol: (1) synthesis of PHPMA with different molecular weights and (2) synthesis of PHPMA–Met. The Met content in the conjugate could reach 18%. The controlled drug-release studies were performed in buffer solutions with pH values equal to 1.1, 7.4, and 10.0. The results demonstrate that the rate of hydrolysis for PHPMA–Met was the slowest at pH 1.1, and a greater amount of Met was detected releasing from prodrug matrices in the presence of enzyme in a buffer solution at pH 8.0. It was also found that the novel prodrug effectively improved Met's pharmacokinetics and, furthermore, markedly increased its half-life period. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 125: 1538–1543, 2012

Key words: barrier; drug delivery systems; polyamides; synthesis

INTRODUCTION

Poly[*N*-(2-hydroxypropyl) methacrylamide] is a hydrophilic, nontoxic, nonimmunogenic, and biocompatible polymer.[1,2] Poly[*N*-(2-hydroxypropyl)methacrylamide] (PHPMA) and its copolymers are among the most intensively studied polymeric drug carriers.[3–6] The conjugation of low-molecular-weight drugs to PHPMA exhibits more desirable biodistribution, elimination, and metabolism properties than free drugs.[7,8] In addition, several *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer–drug conjugates have progressed into clinical trials.[9,10] In addition to its biocompatibility and nonimmunogenicity, an advantage of PHPMA over poly(ethylene glycol) (PEG) is its multifunctionality, which allows multiple drug or targeting molecules to be conjugated to the same polymer chain.[11]

Metaxalone (Met) is a muscle relaxant used to treat pain and stiffness in muscle injuries, including strains, sprains, and muscle spasms. It works by blocking nerve impulses that are sent to the brain. It may be related to its sedative structure.[12] Met can be metabolized by the liver and excreted via urine. Its half-life period is 2–3 h,[13] but its low solubility in water restricts its wide clinical application. The side effects of Met for human beings are drowsiness, dizziness, blurred vision, and low blood pressure.[14] Therefore, it is desirable to develop a delivery system for Met that can effectively control its pharmacokinetics, enhance its therapeutic results, and at the same time, reduce its adverse effects.

The preparation of polymeric drug-delivery systems, in which a small molecular drug is covalently bonded to the backbone of a polymer that is hydrolysable in body fluid, is currently recognized as an effective way to prolong the drug's pharmacological activity.[15] The mechanism of this technology can make small molecular drugs gradually free of polymer matrices if they are subjected to the hydrolysis of body fluid.

In a previous study,[16,17] we covalently bound Met to PEG and dextran to prepared two polymeric prodrugs, which were PEG–Met and dextran–Met conjugates, respectively. However, the content of Met in the two conjugates was relatively low. Recently, we developed a new, improved synthetic method for PHPMA–Met conjugation, and Met was successfully

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conjugated to PHPMA. The Met content in this conjugate could reach 18%.

In this study, we selected PHPMA as a polymer drug carrier and Met as a small molecular drug; Met was covalently linked to PHPMA to prepare a PHPMA–Met prodrug. The weight-average molecular weights used for PHPMA were 10,000, 14,000, and 23,000, respectively. PHPMA10,000–Met, PHPMA14,000–Met, and PHPMA23,000–Met prodrugs were synthesized, and their detailed molecular structures were characterized via Fourier transform infrared spectroscopy and differential scanning calorimetry (DSC) techniques. The products obtained showed markedly improved solubilities and half-life periods. Furthermore, an *in vitro* study of drug release from the prodrug in various media demonstrated that the controlled release effect for Met was achieved in a significant manner.

EXPERIMENTAL

Materials

2,2′-Azobisisobutyronitrile (AIBN) was purchased from Shan-Pu Chemical, Ltd. (Shanghai, China). α-Chymotrypsin was purchased from Kaiyau Medicine Co.(Beijing, China). A dialtic bag (cutoff molecular weight = 5000) was obtained from Huamei Biochemical Co. (Beijing, China). HPMA was synthesized as previously described in ref. 18. Met (99.9% w/w, analyzed by gas chromatography) was synthesized according to a method reported in the literature.[19] *N*-chloroacetyl metaxalone (99.9% w/w, analyzed by gas chromatography) was also synthesized according to a method reported in the literature.[16] Ethanol, ether, dimethyl sulfoxide, and other chemicals were analytical grade and were made in China; they were used as received without further purification.

Analytical methods

Infrared spectroscopy experiments were performed on a Specode 75 model (Carl Zeiss, Jena, Germany) with KBr as the sample holder. Ultraviolet–visible spectra were recorded on a UV-1700 spectrophotometer (Beijing Byond Technology Development Co., Ltd., Beijing, China). DSC (MDSC 2910, TA Instruments, USA) was used to determine the thermal behavior of Met and the prodrugs. The scan rate was set to 20°C/min within the temperature range 30–300°C. High performance liquid chromatography (Varian 5020, USA) was used to determine the Met content in the polymer–drug conjugates.

Synthesis of PHPMA

In a three-necked, round-bottom flask equipped with a magnetic stirring bar and a reflux condenser, 16 g of HPMA, 2.86 g of AIBN, and100 mL of anhydrous ethanol were added. After bubbling with nitrogen gas for 30 min,[20] the reactant was heated to 55°C for 24 h, the solvent was evaporated *in vacuo*, the system was cooled to room temperature, and the product was obtained by precipitation into 80 mL of ether and then filtered. The product was washed three times with anhydrous ethanol, and finally, a yellow power was obtained (11.6 g). The weight-average molecular weight of PHPMA was 14,000 (PHPMA14,000):

IR (KBr, υ, cm^{-1}): 3390 (—OH), 2930 ($—CH_3$), 1639 (C=O amide).

With controlled amounts of AIBN, PHPMA with different molecular weights were synthesized. The same procedure was used to prepare PHPMA10,000 and PHPMA23,000.

Synthesis of the PHPMA–Met conjugates

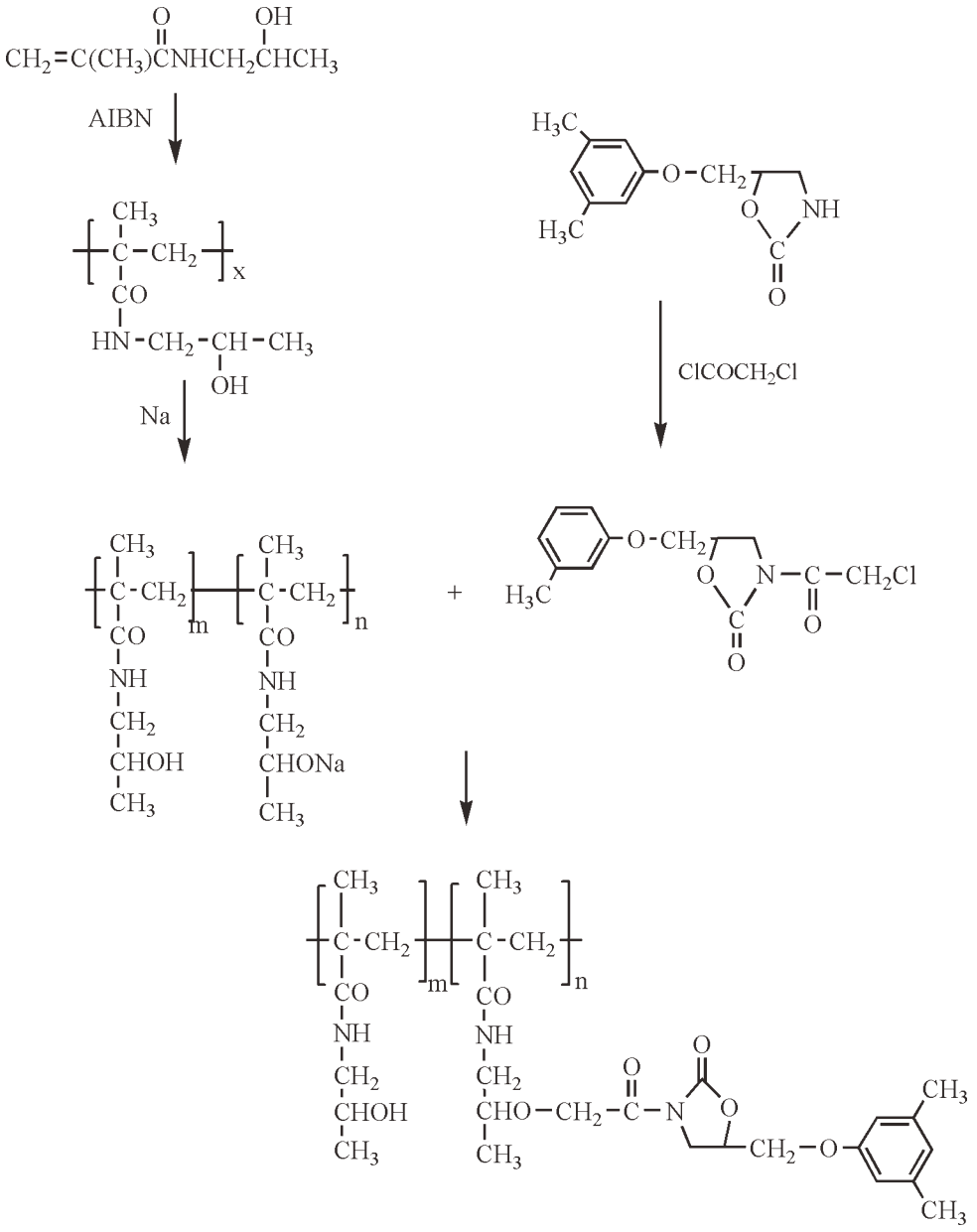
In a 250-mL, three-necked, round-bottom flask equipped with a magnetic stirring bar and a reflux condenser, PHPMA14,000 (24.0 g) dissolved in 100 mL of distilled dimethyl sulfoxide and 2.1 g of Na were added under stirring and heated to 70°C for 4 h. Then, the system was cooled down to room temperature, the excessive Na was filtered off, the product (**1**) was kept intact for the next step of the reaction.

In a three-necked, round-bottom flask containing product **1,** 8.93 g of *N*-chloroacetyl metaxalone was added under stirring and heated to 80°C for 7 h. The solvent was evaporated *in vacuo*, the system was cooled down to room temperature, and the product was obtained by precipitation into 80 mL of acetone and then filtered. The product was washed three times with anhydrous ethanol, and 21.2 g of PHPMA14,000–Met conjugate was obtained. The Met content in the conjugate was 17.5%.

IR (KBr, υ, cm^{-1}): 3390 (—OH), 2933 ($—CH_3$), 1733 (C=O ester), 1639 (C=O amide), 840–690 (Ar-1,3,5 substitute).

The same procedure was used to prepare PHPMA10,000–Met and PHPMA23,000–Met. The Met contents in the PHPMA10,000–Met and PHPMA23,000–Met conjugates were 18.9 and14.6%, respectively.

The fact that there was no free drug existing in the polymer prodrug was confirmed by thin-layer chromatography and DSC measurements. A standard curve was made by pure Met in a water solution. The content of Met in the conjugate was calculated by comparison the slopes of the standard curve of Met at a wavelength of 271.0 nm on the basis of the release of Met in alkaline media after 1 h at 60°C.[21]



Scheme 1 Schematic synthetic route of PHPMA–Met.

Drug-release tests via hydrolysis

The drug-release experiments via hydrolysis were carried out at pH 1.1 (0.002*M* glycine and 0.002*M* KCl adjusted to pH 1.1with HCl), pH 7.4 (0.005*M* Na_2HPO_4–0.001*M* KH_2PO_4 adjusted to pH 7.4 with NaOH), pH 8.0 (0.080*M* Tris and 0.100*M* $CaCl_2$ adjusted to pH 8.0 with HCl), and pH 10.0 (0.012*M* Na_2CO_3 and 0.008*M* $NaHCO_3$ adjusted to pH 10.0 with NaOH) at 37 ± 0.1°C.[22,23] PHPMA–Met was first put into a dialtic bag, sealed, and then immersed into 25 mL of a buffer solution. After a suitable time interval, 5.0 mL of the released solution was withdrawn, and another 5.0 mL of fresh buffer solution was added to maintain the system at a stable volume.[24] Standard curves were made by pure Met in various buffer solutions. The concentration of the Met released was analyzed and calculated by the comparison of the slopes of the standard curves at 271.0 nm. Each experiment was repeated three times.

Enzymatic hydrolysis study

The hydrolytic stability of the PHPMA–Met to enzyme was assessed in 0.08*M* Tris buffer and in 0.1*M* $CaCl_2$ buffer solution at pH 8.0.[22] An amount of 200 μL of a 10^{-5} *M* enzyme solution plus 0.001*M* HCl was added to 2 mL of a PHPMA–Met solution containing 7.1 mg of Met and was then put it into dialtic bag, sealed, and incubated in a buffer

solution of 40 mL at 37 ± 0.1°C. After a suitable interval time, 5.0 mL of the released solution was withdrawn, and 5.0 mL of fresh buffer solution was added to maintain the system at a stable volume. A standard curve was made of Met in a pH 8.0 buffer solution. The concentration of Met released was analyzed and calculated by the comparison of the slopes of the standard curves at 271.0 nm. Each experiment were repeated three times.

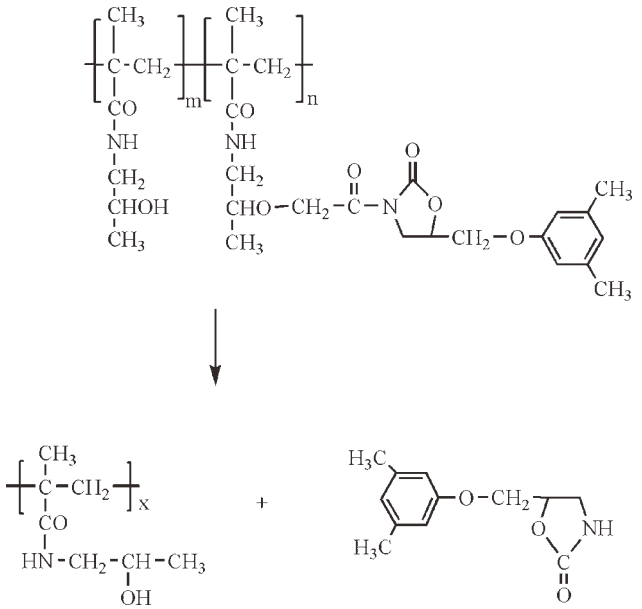
Met content in the conjugates

The Met content was estimated by both HPLC and UV spectroscopy. The UV spectroscopy data were obtained by the comparison of the absorbances of Met and the conjugate at 271.0 nm. The HPLC data were obtained by determination of the concentration of free Met after total hydrolysis of the polymer–drug conjugates. Both measurement results were in good accordance.

RESULTS AND DISCUSSION

Synthesis of the PHPMA–Met conjugates

PHPMA was first obtained by the polymerization of HPMA with AIBN as the initiator. PHPMA could chemically react with Met by using a chloroacetyl chloride linkage. To perform this synthesis, a preliminary reaction of Met with chloroacetyl chloride to obtain an intermediate of *N*-chloroacetyl metaxalone was necessary. Then, the PHPMA–Met conjugate was synthesized by the reaction of the intermediate with sodium PHPMA, which was synthesized by the treatment of PHPMA with metal sodium. The detailed chemical route is shown in Scheme 1.



Scheme 2 Schematic hydrolyzed or enzymically hydrolyzed route of PHPMA–Met.

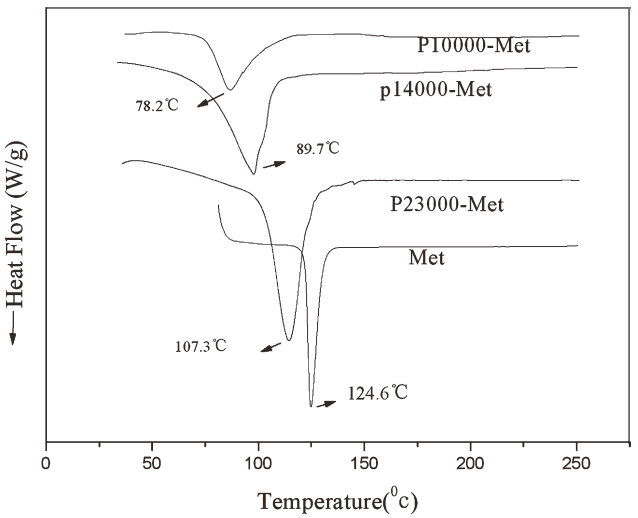


Figure 1 DSC thermograms of Met, PHPMA10,000–Met (P10000–Met), PHPMA14,000–Met (P14000–Met), and PHPMA23,000–Met (P23000–Met).

The amide bond in the PHPMA–Met conjugate was hydrolyzed or enzymically hydrolyzed via human body fluid. This was the fundamental mechanism for realizing controlled drug release for Met with pharmacological activity. The detailed release process is shown in Scheme 2.

Additionally, thin-layer chromatography and DSC were used to determine free Met in the polymer prodrug, and the results are shown in Figure 1. The pure Met's melting point was at 124.6°C on its DSC thermogram; however, no peak was visible near Met's melting point for the three polymer prodrugs. It was also clear that the melting points were 78.2°C

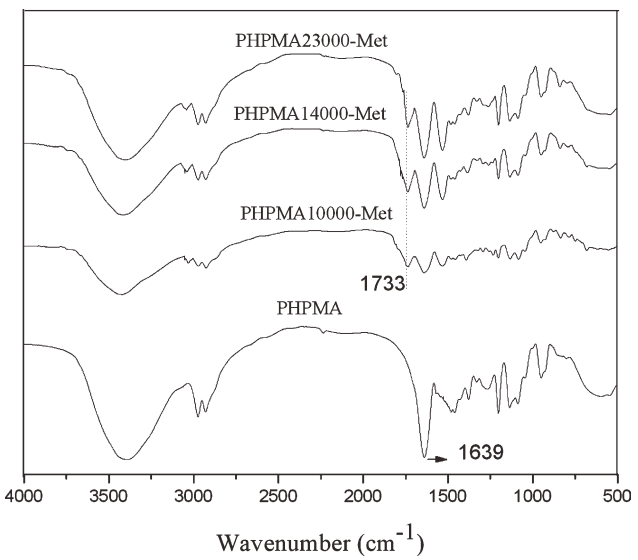


Figure 2 IR spectra for PHPMA, PHPMA10,000–Met, PHPMA14,000–Met, and PHPMA23,000–Met.

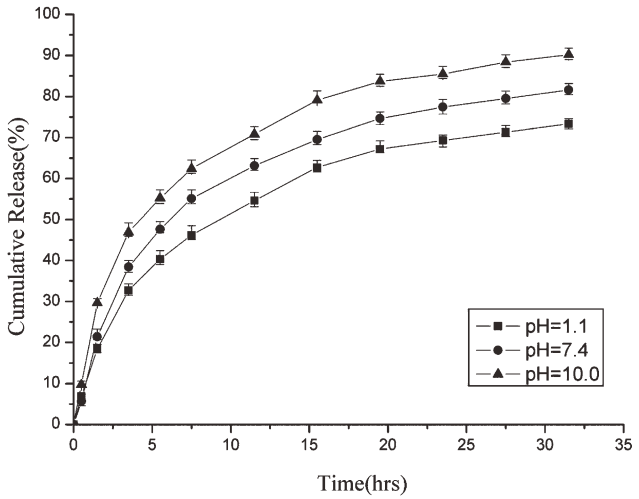


Figure 3 Release of Met in buffer solution from PHPMA10,000–Met.

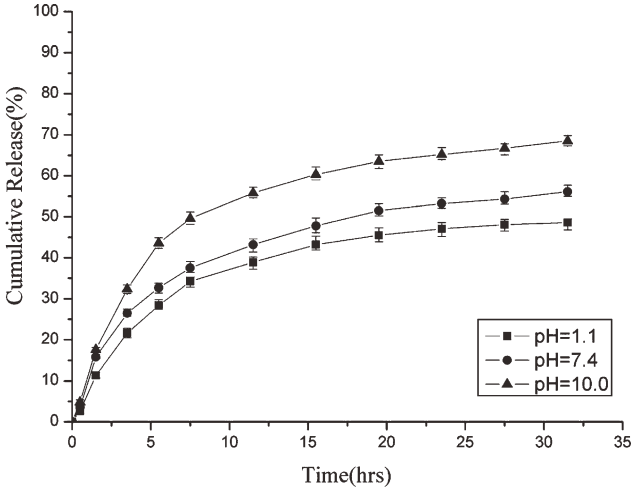


Figure 5 Release of Met in buffer solution from PHPMA23,000–Met.

for PHPMA10,000–Met, 89.7°C for PHPMA14,000–Met, and 107.3°C for PHPMA23,000–Met.

In the Fourier transform infrared spectrum of PHPMA–Met was shown (Fig. 2) a peak at 1733 cm^{-1} belonging to the ester carbonyl stretching band (C=O). The peak at 1639 cm^{-1} corresponded to the amide bond, whereas the stretching vibrations of Ar-1,3,5 substitute were found at 840–690 cm^{-1}. It was assumed that Met was successfully grafted onto PHPMA. The products obtained showed markedly improved solubility of the drug. Met was almost insoluble in water, but the solubility of the PHPMA10,000–Met prodrug in water was 11.2 g/L at 20°C.

Controlled release *in vitro*

To obtain some preliminary information about the potential use of PHPMA–Met as a drug-delivery system for prolonged release, the hydrolysis process for the prodrug *in vitro* was studied in buffer solutions at pH 1.1, 7.4, and 10.0 and at pH 8.0 in the presence and absence of enzyme.

Figure 3 presents the hydrolysis rates of PHPMA10,000–Met at three pH levels. As can be seen, PHPMA10,000–Met was subjected to hydrolyzation to 69.6% at pH 1.1 and to 77.8% at pH 7.4. After 24 h, the hydrolyzation percentage reached 85.9% at pH 10.0.

Figure 4 depicts the hydrolytic behavior for PHPMA14,000–Met. Clearly, at pH 1.1, the hydrolyzation percentage reached 58.9%, and at pH 7.4 and 10.0, the hydrolyzation percentages reached 70.8 and 76.7%, respectively.

Figure 5 depicts the same hydrolytic behavior for PHPMA23,000–Met. The hydrolyzation percentages

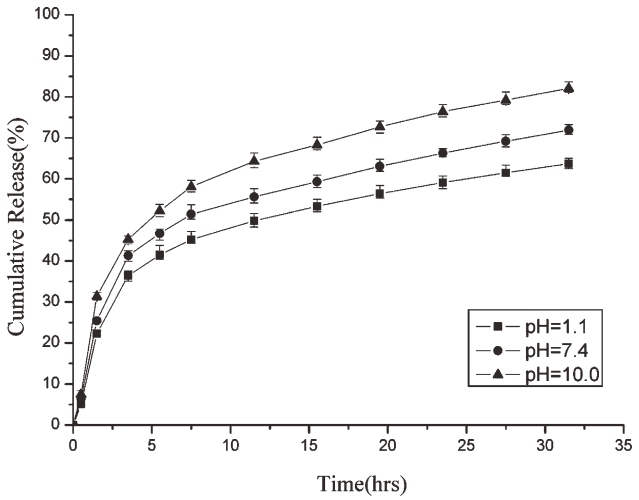


Figure 4 Release of Met in buffer solution from PHPMA14,000–Met.

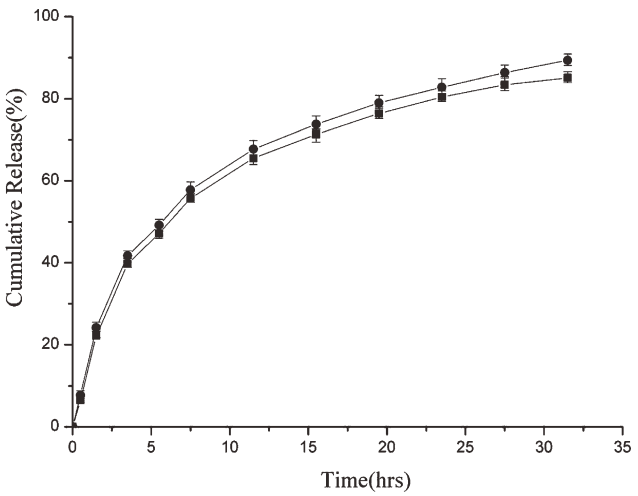


Figure 6 Release of Met from PHPMA10,000–Met in 0.08*M* Tris butter at pH 8.0 in the (▲) presence or (■) absence of α-chymotrypsin.

were 47.5% at pH 1.1, 53.4% at pH 7.4, and 62.3% at pH 10.0.

The hydrolysis rates of Met from the three prodrugs were all relatively faster within 8 h. The hydrolysis rates of Met from PHPMA10,000–Met, PHPMA14,000–Met, and PHPMA23,000–Met were calculated to be 6.03, 5.89, and 4.33%/h, respectively, at pH 1.1 and 7.4. The hydrolysis rates were calculated to be 8.9, 7.9, and 6.2%, respectively, at pH 10.0. The hydrolysis rate of Met from PHPMA 23,000–Met was the slowest among the three prodrugs under the same conditions.

Also, to understand the effect of α-chymotrypsin, the release of Met from the PHPMA–Met prodrugs in the presence and absence of α-chymotrypsin at pH 8.0 was studied to determine the release rates.

As can be seen from Figure 6, 82.9% of Met was released from PHPMA10,000–Met after 24 h in the presence of α-chymotrypsin, and 80.5% of Met was released in the absence of α-chymotrypsin.

An amount of 74.8% of Met was released from PHPMA14,000–Met in the presence of α-chymotrypsin, and 71.3% was released in the absence of α-chymotrypsin after 24 h. An amount of 59.2% of Met was released from PHPMA23,000–Met in the presence of α-chymotrypsin, and 53.8% was released in the absence of α-chymotrypsin after 24 h.

On the basis of the experimental results, more Met was released in the presence of α-chymotrypsin than in its absence under the same testing conditions. However, there existed a small difference between the release rates of Met from the three prodrugs with and without α-chymotrypsin.

CONCLUSIONS

Three novel polymer prodrugs were prepared via the linkage of Met to PHPMA; they possessed different molecular weights and had chloroacetyl chloride as a spacer. The Met content in the conjugates could reach 18%. The PHPMA-based prodrugs exhibited effective increases in the solubility of Met, whereas the Met molecular bonding via an amide bond guaranteed the drug release. Three prodrugs were found to be relatively stable in different pH solutions by inspection of their release behaviors *in vitro*, and they slowly released the active drug molecules. The hydrolysis rate for PHPMA23,000–Met was the slowest among the three polymer prodrugs under the same conditions. Thus, PHPMA10,000–Met was shown to be more susceptible to the pH value. In the same buffer solution and for the same time period, more Met was released in the presence of α-chymotrypsin than in its absence. These three prodrugs markedly improved the pharmacokinetics of Met, particularly, in increasing its half-life.

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