Synthesis of Dextran-Metaxalone Conjugates and Study on Their Control Drug Release Behaviors

Juan Zhang,[1] Yi-Feng Liu,[1] Bo Lan,[1] Xiao-Dong Fan[2]

[1]*Applied Chemical Institute, Northwest University, Xian, People's Republic of China 710069*
[2]*Department of Applied Chemistry, School of Science, Northwestern Polytechnic University, Xian, People's Republic of China 710072*

Received 8 December 2005; accepted 26 June 2007
DOI 10.1002/app.27379
Published online 30 November 2007 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Metaxalone (Met), a drug for treatment of pain and stiffness due to muscle injuries, was covalently linked to dextran via a chloroacetyl chloride spacer. The average molecular weights of dextran are 20,000 (D_{20000}) and 40,000 (D_{40000}), respectively, and the procedure of chemical modification for dextrans was conducted by a two-step protocol: (1) synthesis of *N*-chloroacetyl-metaxalone; (2) synthesis of D_{20000}-Met and D_{40000}-Met. The controlled drug release studies were performed in buffer solutions with pH values of 1.1, 7.4, and 10.0. The results demonstrate that, under the same condition, the rate of release for D_{20000}-Met is slower than that of D_{40000}-Met, and more amount of Met can be detected releasing from polymer-drug conjugate at the presence of α-chymotrypsin in a buffer solution with pH = 8.0. It was also found that these novel polymer-drug conjugates can effectively improve the Met's pharmacokinetics, and can increase its half-life period. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 3386–3392, 2008

Key words: metaxalone; dextran; polymer-drug conjugates; *N*-chloroacetyl-metaxalone; controlled release

INTRODUCTION

Metaxalone (Met), also known as Skelaxin with a chemical name of 5-[(3,5-dimethylphenoxy) methyl]-oxazolidin-2-one, is a muscle relaxant, used to treat pain and stiffness of muscle injuries, including strains, sprains and muscle spasms. It works by blocking nerve impulses (or pain sensations) that are sent to brain. It may be related to its sedative structure.[1] Met can be metabolized by liver and excreted via urine. Its half-life period is 2–3 h,[2] but the low solubility in water restricts its clinical applications. The side effects of Met for human being are presented with drowsiness, dizziness, blurred and low blood pressure.[3] Therefore, it is desirable to develop a delivery system for Met, which can effectively control its pharmacokinetics, enhance its therapeutic result, and at the same time, reduce its adverse effects. To date, many novel approaches have been proposed to overcome these restrictions for effective utilization of this small molecular drugs.[4]

Water-soluble polymers can be model compounds for studying the influence of macromolecules on the cell metabolism and also are suitable for being used as drug delivery vectors. Polymer drug delivery systems can often compensate some shortcomings of small molecular drugs such as side effects, limited water solubility, poor biocompatibility, bio-stability, immunogenicity and so on.[5]

Dextran is a biologically inert water-soluble macromolecule carrying numerous reactive hydroxyl groups, which can be used for drug fixation. Among many macromolecules, dextran seems to be one of the most suitable candidates for the drug carrier,[6,7] primarily to increase the effect of therapeutic agent in the circulation. This is achieved mainly through relatively longer blood half-life of dextran-based conjugate, compared with the intact drug. Dextran has even been used as a plasma expander, for targeting delivery of drugs to specific sites in human body. In other aspect, dextran has been used to increase stability and decrease the immunogenicity of proteins or enzymes.[8,9]

Considering these properties and advantages, in this research, we selected dextran as a drug carrier and Met as a low molecular drug, which can be covalently linked to dextran for preparing D-Met conjugate. By using dextrans with different molecular weights, several polymer-drug conjugates were synthesized, and their detailed molecular structures were characterized via NMR and FTIR techniques. The products showed markedly improved solubility and half-life period. Furthermore, study *in vitro* on drug release from these polymer-drug conjugates in

Correspondence to: J. Zhang (zjuannn@163.com).
Contract grant sponsor: National Nature Science Foundation of China; contract grant number: 20674060.

various media demonstrated that controlled release for Met has been achieved successfully.

EXPERIMENTAL

Materials

Met (99.9%w/w, analysed by GC) was synthesized according to the report in the literature.[10] Dextrans with average molecular weight of about 20,000 and 40,000, were analytic grade made in Tian-Tai Fine Chemical (China); α-chymotrypsin was purchased from Kaiyau Medicine (Beijing, China). Dialitic bag (cut-off molecular weight 10,000) was obtained from Huamei Biochemical (Beijing, China). Chloroacetyl chloride was a chemical grade from Guoyao Chemical Co. (Shan-Xi, China); Triethylamine, toluene, petroleum ether and dimethyl sulfoxide were analytical grade and were made in Xian Chemical (Xian, China); they were used as received without further purification.

Analytical methods

^{1}H NMR measurements were conducted on Varian INOVA-400 spectrometer at room temperature with $CDCl_3$, d6-DMSO as a solvent. Infrared spectra were recorded on a Specode 75 model (Carl Zeiss, Jena, Germany) using KBr as the sample holder. Elemental analyses were carried out on a Vario EL III instrument. Ultraviolet–visible spectra were recorded on a UV-1700 spectrophotometer (Beijing, China). DSC (MDSC 2910, TA Instruments) measurements were used to determine the thermal properties of Met and polymer-drug conjugates. The scan rate was 20°C/min within the temperature range of 30–300°C. HPLC (Varian 5020, USA) were used to determine the Met content in the polymer-drug conjugate.

Synthesis of chloroacetyl-metaxalone

In a three-neck round bottomed flask equipped with a magnetic stirring bar and reflux condenser,[11] 0.5 g of Met and 0.3 g of triethylamine dissolved in 15 mL of toluene together with 0.41 g chloroacetyl chloride were added under stirring. The system was maintained at 50°C for 30 min, and then, heated to reflux for 3 h. After completing the reaction, the system was cooled down to room temperature. The excessive chloroacetyl chloride was washed off by water, the organic layer was separated. The solvent was removed under reduced pressure. The white product was precipitated and filtered. The product was recrystallized twice with toluene. Yield: 0.63 g (92.6%w/w). Rf = 0.72 (1:10 ethyl acetate: dichloromethane).

Synthesis of sodium dextranate

In a 100-mL three-neck round bottomed flask equipped with a magnetic stirring bar and reflux condenser, 2.0 g of D_{20000} dissolved in 10 mL dimethylsulfoxide together with 0.01 g of Na were added under stirring heated to 100°C for 4 h. And then, the system was cooled down to room temperature, the excessive Na was filtered off. Sodium dextranate was kept under the vacuum for next step reaction.

Synthesis of D_{20000}-Met conjugate

In a 100-mL three-neck round bottomed flask equipped with a magnetic stirring bar and reflux condenser containing sodium dextranate above, 0.15 g of *N*-chloroacetyl-Met was added under stirring and heated to 90°C for 15 h, the solvent was evaporated under vacuum, the system was cooled down to room temperature and the product was obtained by precipitating it into 20 mL of anhydrous ethanol, and then filtered. The product was washed three times with anhydrous ethanol, and finally, a white power was obtained and dried under vacuum.

The D_{20000}-Met conjugate with the yield of 92.0% was inspected by thin layer chromatography and DSC measurements where no free Met was identified in polymer-drug conjugate.

The same procedure was used to prepare D_{40000}-Met as described above. In this case, 2.0 g of D_{40000} was dissolved in 10 mL of dimethylsulfoxide, and 0.01 g of Na, 0.15 g of *N*-chloroacetyl-Met was used. The product's yield is 88.0%.

Hydrolysis studies

The hydrolysis experiments were carried out at pH 1.1 (KCl, HCl, and glycine), 7.4 (Na_2HPO_4/KH_2PO_4), and 10.0 ($Na_2CO_3/NaHCO_3$),[12] at 37 ± 0.1°C, respectively. D-Met was first put into dialitic bag and sealed, then was immersed in 40.0 mL of a buffer solution.[13,14] After a suitable interval time, 5.0 mL of the solution released was withdrawn and 5.0 mL of fresh buffer solution was added for maintaining a constant volume of the system. Each experiment was repeated three times.

Enzymatic hydrolysis studies

The hydrolytic stability of D-Met was assessed to α-chymotrypsin in 0.08*M* Tris buffer, 0.1*M* $CaCl_2$ at pH 8.0 buffer solution. Two hundred microliters of solution of α-chymotrypsin $10^{-5}M$ in 0.001*M* HCl was added to 2.0 mL samples of D-Met solution containing 7.1 mg of Met, then was put into dialitic bag and sealed, incubated in a buffer solution of 40 mL at 37 ± 0.1°C. After a suitable interval time, 5.0 mL

of solution released was withdrawn and 5.0 mL of fresh buffer solution was added for maintaining a constant volume to 40 mL.[15] Each experiment was repeated three times.

Metaxalone content in the conjugates

The Met content was estimated by both HPLC and UV spectroscopy. The amount of 0.025 g D_{20000}-Met conjugate was dissolved in 50 mL of buffer solution with pH = 10.0. The reaction mixture was maintained at 60°C for 72 h after which it was neutralized with hydrochloric acid. The amount of Met released from the conjugate was determined by HPLC. The experimental procedure was conducted in triplicate. The drug loading in the conjugate was calculated to be 5.0%.

The UV spectroscopy data were obtained by comparing the absorbance of Met and the conjugate at 271.0 nm. The drug loading in the conjugates was calculated to be 5.0%. Both measurement results were in good accordance.

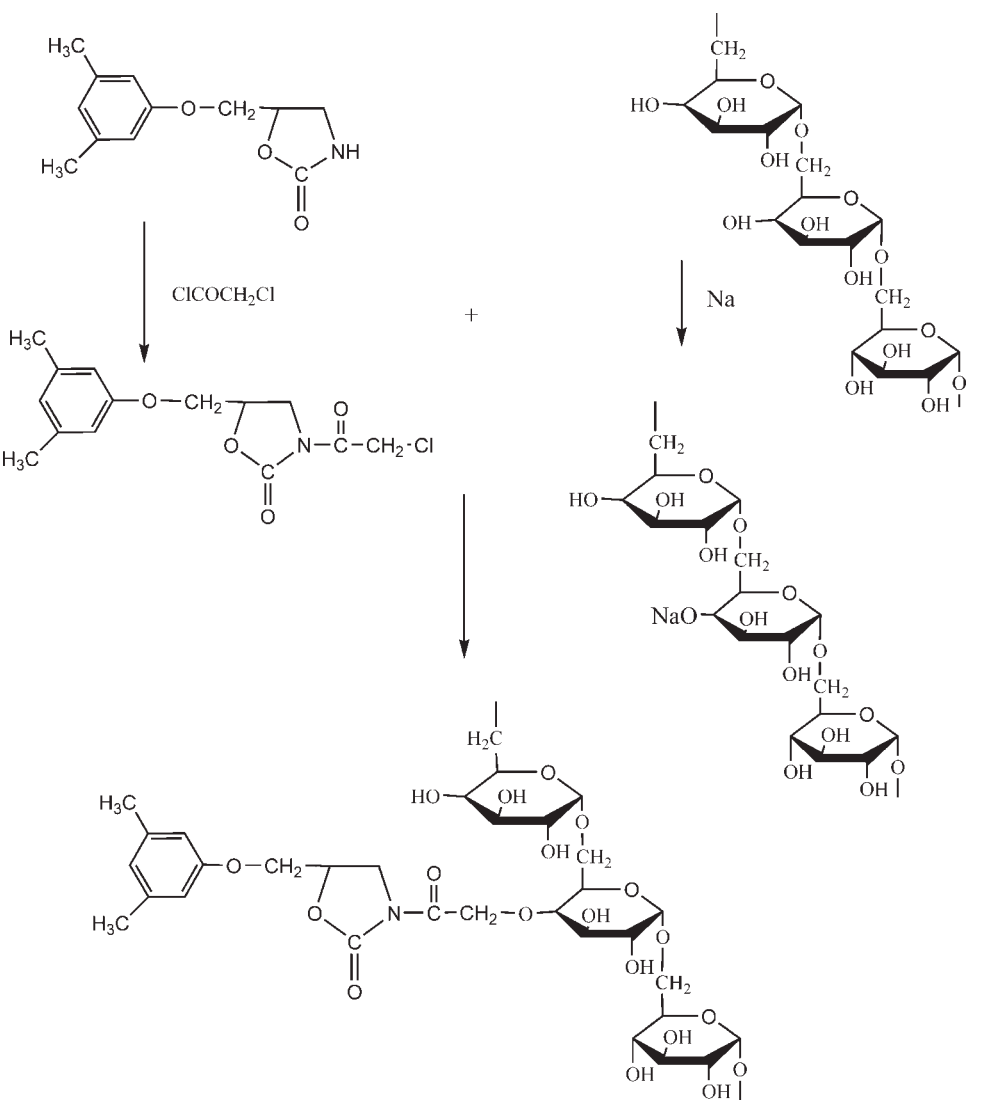
RESULTS AND DISCUSSION

Synthesis of Met-D conjugate

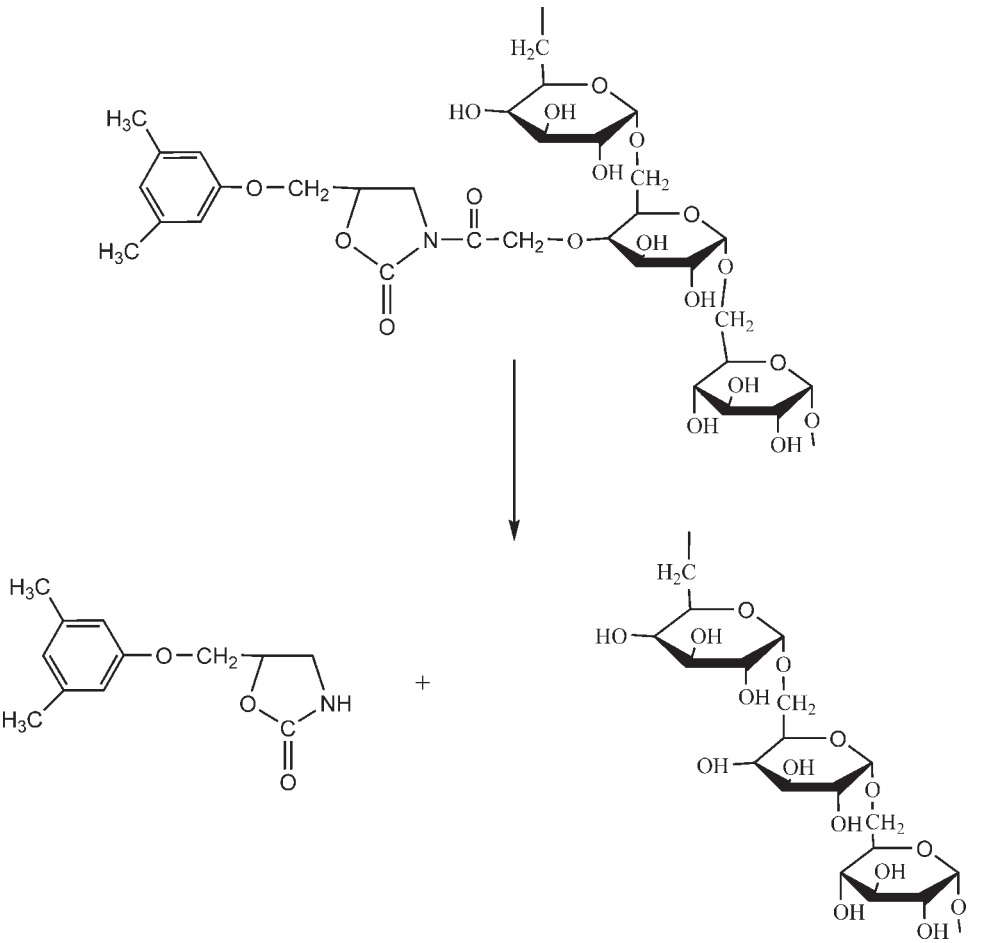
The Met can chemically react with dextran, but need to utilize a chloroacetyl chloride linkage. To fulfill the goal, firstly, the reaction of Met with chloroacetyl chloride for obtaining an intermediate of *N*-chloroacetyl-Met is necessary. *N*-chloroacetyl-Met was characterized by elemental analysis and IR and NMR spectra.

Anal.Calc.for $C_{14}H_{15}NO_4Cl$: C, 56.32; N, 4.85; H, 5.54. MW. 296.7. Found: C, 56.67; N, 4.72; H, 5.10%.

IR (KBr): 1774 (O=C, ester), 1713 (O=C amide), 1232 (C—O—C=O, ester), 1072 (Ar—O—C, ether) cm^{-1}. ^1H NMR(δ, ppm): 2.29 [s,6H,$(CH3)_2$—Ar], 4.12



Scheme 1 Schematic synthetic route of dextran-metaxalone.



Scheme 2 Schematic hydrolyzed, or enzymically hydrolyzed route of dextran-metaxalone.

(m,2H,CH_2—N), 4.22 (m,2H,Ar—O—CH_2), 4.76 (s,2H, CH2—Cl), 4.98 (m,1H,CH2—CH—O), 6.51 (s,2H, H—Ar), 6.66 (s,1H,H—Ar). Melting point: 152–153°C.

D-Met conjugate is synthesized by reaction of the intermediate with sodium dextranate, which can be synthesized by treatment of dextran with metal sodium (Scheme 1).

The amide bond in D-Met conjugate can be hydrolyzed, or enzymically hydrolyzed via human body fluid. This is the fundamental mechanism to realize the controlled drug release for Met with pharmacological activity. The detailed release process is shown in Scheme 2.

The reaction conditions and the Met content in the polymer-drug conjugate are presented in the Table I. The data, presented in Table I, show that the Met content in the polymer-drug conjugate increase as the increase in reaction temperature and the molar ratio between the dextran and the *N*-chloroacetyl-Met. Met content in D_{20000}-Met is 5.0% when molar ratio of dextran and *N*-chloroacetyl-Met reached 1:5 at 90°C for 15 h. With further increase in reaction temperature, the color of the polymer-drug conjugate becomes fuscous, and the separation of polymer-drug conjugate faces difficulty because of more byproducts.

TABLE I
Effect of the Reaction Condition on the Met Content in the Conjugate

Sample	Type of prodrug	Dextran: *N*-chloroacetyl-Met (molar ratio)	Temperature (°C)	Met content of conjugate (%)
a	D_{20000}-Met	1 : 5	60	2.1
b	D_{20000}-Met	1 : 5	70	2.9
c	D_{20000}-Met	1 : 5	80	4.0
d	D_{20000}-Met	1 : 5	90	5.0
f	D_{40000}-Met	1 : 5	90	2.2

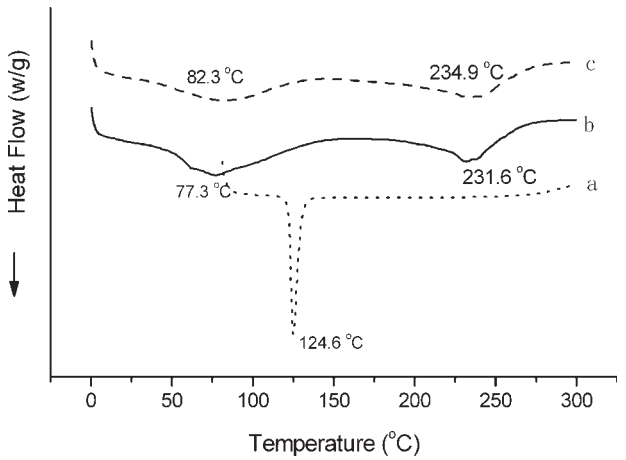


Figure 1 DSC-thermograms of metaxalone (a), D_{20000}-Met (b), and D_{40000}-Met (c).

Thin layer chromatography and DSC measurements can be used for inspecting if there exists free Met in polymer-drug conjugates, and the results are showed in Figure 1. As can be seen, Met's melting point (a) is at 124.6°C, however, no peak is visible near the Met's melting point for two polymer-drug conjugates. It can also be clearly observed that the endothermic peaks for D_{20000}-Met are 77.3 and 231.6°C, endothermic peaks for D_{40000}-Met are 82.3 and 234.9°C, respectively. A carbonyl CO stretch with an intense strong band near 1745 cm^{-1} can be seen in infrared spectrum for two D-Met based conjugates (Fig. 2). And chemical shifts of aromatic hydrogen showed up from 6.34–6.71 ppm in NMR spectroscopy for two polymer-drug conjugates. These results indicate that two polymer-drug conjugates had already formed. Dextran's conjugation increase Met's solubility. Solubility of Met in water is 5%, compared with Met, and Solubility of conjugate, which is 20% at 20°C.

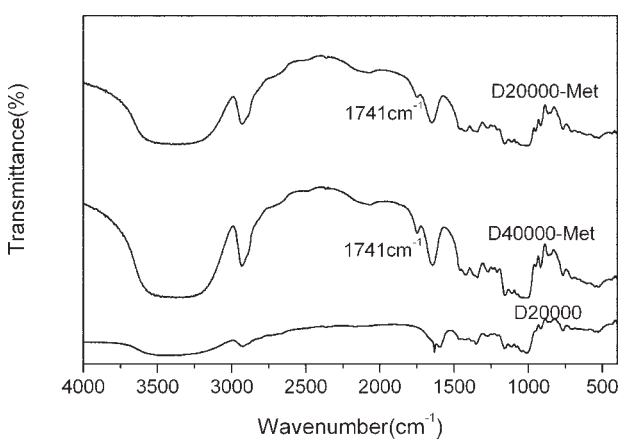


Figure 2 IR spectra for D20000-Met and D40000-Met.

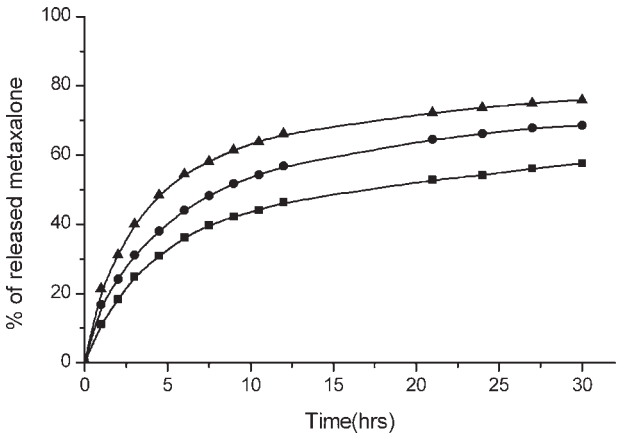


Figure 3 Release of metaxalone in buffer solution, pH 1.1 (■), pH 7.4 (●), pH 10.0 (▲), at 37°C from D_{20000}-Met.

Controlled release *in vitro*

To obtain some preliminary information about the potential use of D-Met as a drug carrier for a prolonged release, hydrolysis process for two polymer-drug conjugates *in vitro* is studied in buffer solution at pH 1.1, 7.4, 10.0, and 8.0 in both presence and in the absence of α-chymotrypsin.

Figure 3 presents the release rates of D_{20000}-Met at three pH values. As can be seen, after 24 h, 54.2% of Met is released from D_{20000}-Met at pH 1.1, and at pH 7.4, 10.0, the Met released from D_{20000}-Met are 66.1 and 73.7%, respectively.

Figure 4 depicts the same release behavior for D_{40000}-Met. After 24 h, 66.1% of Met is released at pH 1.1, and at pH 7.4, 10.0, the Met released from D_{40000}-Met are 76.6 and 97.8% released, respectively.

The release rates of Met from D_{20000}-Met and D_{40000}-Met are relatively faster within 6 h. The release rates of Met from the two polymeric conjugates were calculated to be 6.0 and 6.6% per hour at

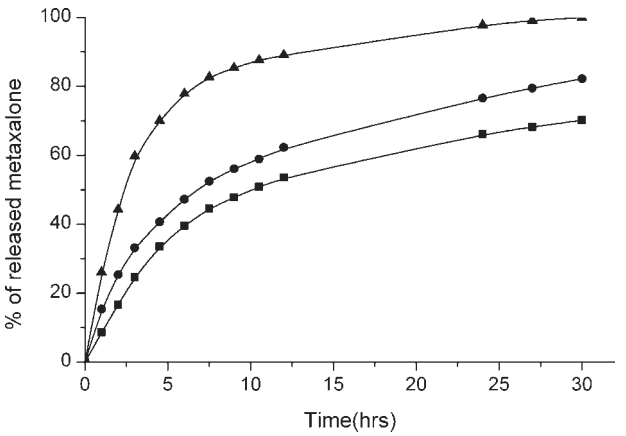


Figure 4 Release of metaxalone in buffer solution, pH 1.1 (■), pH 7.4 (●), pH 10.0 (▲), at 37°C from D_{40000}-Met.

pH 1.1, and at pH 7.4, the release rates were calculated to be 7.4 and 7.9% per hour. And at pH 10.0, the rates were calculated to be 9.1 and 13.0% per hour, respectively. The release rates of Met from D_{20000}-Met are slower than that of Met from D_{40000}-Met in the same condition. The phenomenon may be related to the different surroundings of Met molecule in polymer-drug conjugate. The calculating results indicate that the number of hydroxyl groups around Met in D_{40000}-Met molecule is about twice compared with D_{20000}-Met. The existence of more hydroxyl groups is able to make the conjugates with strong solvation effect. Molecular weight of dextran is larger, the interval space of random arranging drugs functional groups in polymer-drug conjugates molecules is bigger. This can be beneficial to water solvation for polymer-drug conjugates. Therefore, the release rate of Met from D_{40000}-Met is faster.

α-chymotrypsin, one of the digestive enzyme secreted by the pancreas, belong to a family of enzymes that cleave proteins into small peptides. It is also well known for it to be able to catalyze the hydrolysis of ester bonds and amide bonds.[16,17] To understand the effect of α-chymotrypsin, the releases of Met from D_{20000}-Met and D_{40000}-Met conjugates in the presence and in the absence of α-chymotrypsin at pH 8.0 are studied for determining the release rates.

The release rates of Met from D_{20000}-Met with and without α-chymotrypsin are shown in Figure 5 at same conditions, the release rates of Met from D_{40000}-Met are shown on Figure 6.

As can be seen in Figure 5, 72.6% of Met is released from D_{20000}-Met after 30 h in the presence of α-chymotrypsin, and 69.6% of Met is released in the absence of α-chymotrypsin. The release rates of Met from D_{20000}-Met are calculated to be 7.5 and 7.0% per hour at pH 8.0 with and without

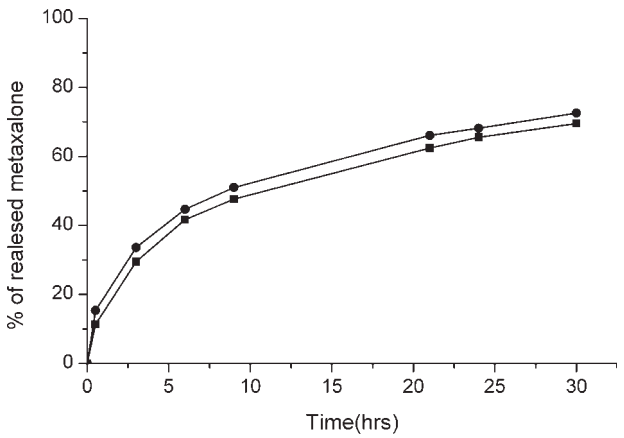


Figure 5 Release of metaxalone from D_{20000}-Met in 0.08*M* Tris buffer at pH 8.0 in presence (●) and in absence (▲) of α-chymotrypsin.

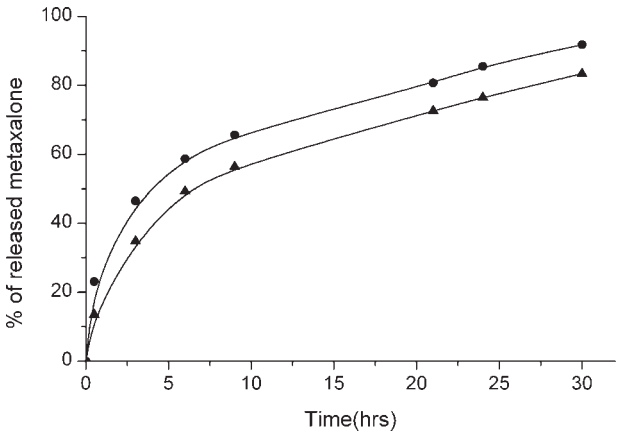


Figure 6 Release of metaxalone from D_{40000}-Met in 0.08*M* Tris buffer at pH 8.0 in presence (●) and in absence (▲) of α-chymotrypsin.

α-chymotrypsin within 6 h. There is a slight difference for the release rate of Met from D_{20000}-Met with and without α-chymotrypsin.

In Figure 6, it can be seen that 91.8% of Met is released from the polymeric conjugate D_{40000}-Met in the presence of α-chymotrypsin, and 83.4% of Met are released in the absence of α-chymotrypsin after 30 h. The release rates of Met from the two polymeric conjugate are calculated to be 9.8 and 8.2% per hour at pH 8.0 with and without α-chymotrypsin within 6 h. There also exists a slight difference between the release rates of Met from D_{40000}-Met with and without α-chymotrypsin.

As can be seen in both cases, more of the Met is released in the presence of α-chymotrypsin compared with the absence of α-chymotrypsin in the same condition. D_{20000}-Met is more stable in various buffer solutions and in the presence or in the absence of α-chymotrypsin, these results suggest the amide bond between Met and D_{40000} may be more susceptible to this enzymatic hydrolysis than the amide bond between Met and D_{20000}.

CONCLUSIONS

Two novel polymer-drug conjugates are prepared via chemically reacting Met to the dextran, which possesses different molecular weights using chloroacetyl chloride as a spacer. Met contents in the D_{20000}-Met and D_{40000}-Met can reach to 5.0 and 2.2%, respectively, if the reaction temperature is at 90°C, and molar ratio of dextrans and *N*-chloroacetyl-Met is 1 : 5. Actually, dextran's conjugation increases Met's solubility, while the drug binding via an amide bond can promote the drug release. *In vitro*, release studies show all products are stable at different pH buffer solutions and can slowly release the free, active drug. The release rate of D_{20000}-Met

is slower than that of D_{40000}-Met in the same condition. D_{40000}-Met shows more susceptible to pH values. In the same buffer solution and time period, more of the Met is released in the presence of α-chymotrypsin than in its absence. Two polymer-drug conjugates can improve the pharmacokinetics of Met, particularly by increasing its half-life period.

References

1. Harden, N. R.; Argoff, C. J Back Musculoskelet Rehabil 2000, 15, 63.
2. Moore, K. A.; Levine, B.; Fowler, D. Forensic Sci Int 2005, 149, 49.
3. Toth, P. P.; Urtis, J. Clin Ther 2004, 26, 1355.
4. Cavallaro, G.; Licciardi, M.; Caliceti, P.; Salmaso, S.; Giammona, G. Eur J Pharm Biopharm 2004, 58, 151.
5. Zacchigna, M.; Luca, G. D.; Cateni, F.; Zorzet, S.; Maurich, V. Farmaco 2003, 58, 1307.
6. Yura, H.; Yoshimura, N.; Hamashima, T.; Akamatsu, K.; Nishikawa, M.; Takakura, Y.; Hashida, M. J Controlled Release 1999, 57, 87.
7. Nomura, T.; Saikawa, A.; Morita, S.; Sakaeda, T.; Yamashita, F.; Honda, K.; Takakura, Y.; Hashida, M. J Controlled Release 1998, 52, 239.
8. Mehvar, R. J Controlled Release 2002, 69, 1.
9. Ouchi, T.; Ohya, Y. Prog Polym Sci 1995, 20, 211.
10. Lunsfurd, C. D.; Mays, R. P.; Richman, J. A.; Murphey, R. S. J Am Chem Soc 1962, 82, 1166.
11. Mocanu, G.; Airinei, A.; Carpov, A. J Controlled Release 1996, 40, 1.
12. Liu, Y. Y.; Fan, X. D. J Appl Polym Sci 2003, 89, 361.
13. Zacchigna, M.; Luca, G. D.; Maurich, V.; Boccu, E. Farmaco 2002, 57, 207.
14. Zhu, L.; Kumar, V.; Banker, G. S. Int J pharm 2001, 223, 35.
15. Giammona, G.; Cavallaro, G.; Fontana, G.; Pitarresi, G.; Carlish, B. J Controlled Release 1998, 54, 321.
16. Harada, M.; Sakakibara, H.; Yano, T.; Syzuki, T.; Okuno, S. J Controlled Release 2000, 69, 399.
17. Fersht, A. R. J Am Chem Soc 1972, 94, 293.