

MAGNOLOL ENTRAPPED ULTRA-FINE FIBROUS MATS ELECTROSPUN FROM POLY(ETHYLENE GLYCOL)-*b*-POLY(L-LACTIDE) AND *IN VITRO* RELEASE*

Hao Wang^{a, d}, Hong-rui Song^b, Yong Cui^c, Ying-jie Deng^{a**} and Xue-si Chen^{d**}

^a School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China

^b School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, China

^c School of Life Science and Pharmacology, Shenyang Pharmaceutical University, Shenyang 110016, China

^d State Key Laboratory of Polymer Chemistry and Physics, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China

Abstract Ultra-fine fibrous mats with magnolol entrapped have been prepared by electrospinning biodegradable copolymer poly(ethylene glycol) blocked poly(L-lactide). Drug entrapment was perfect which was confirmed by scanning electron microscopy and differential scanning calorimetry. According to *in vitro* drug release investigation by high performance liquid chromatography, it was found that fibers with 10%, 20% and 30% drug entrapped respect to polymer (mass ratio) presented dramatically different drug release behavior and degradation behavior under the effect of proteinase K. The reason may be that fibers with 10% drug entrapped was more easily affected by enzyme while, to some degree, magnolol in fibers with 20% and 30% entrapped prevented polymer from being degraded by enzyme.

Keywords: Medicated fiber; Electrospinning; Drug release; Poly(ethylene glycol); Polylactide; Magnolol; Proteinase K.

INTRODUCTION

Electrospinning is a novel technique for fabricating fibers of micro or nano-scale by using polymers as drug loading matrix materials^[1, 2]. By adopting proper polymer materials and preparing process, drugs entrapped in fibers could be released in a controlled pattern favoring more reasonable clinical treatment. Last decade, the release behavior of fibers loaded with various kinds of drugs and related pharmacological activity of these fibers were investigated^[3–5]. It was found that polymer selection, polymer composition and drug-polymer pair had effects on the drug release profile. Magnolol (MNL, chemical structure shown in Fig. 1a) is a pharmacological compound extracted from traditional Chinese medicine. The drug shows lipophilic character in organic solutions due to its two propenyl groups connected with biphenyl but is also soluble in pH > 7 aqueous solutions because of ionizable phenolic hydroxyl groups which exist in inner part of the molecule. Now, we successfully entrap MNL into electrospun fibers of a biodegradable block copolymer of poly(ethylene glycol) (MePEG2000) and poly(L-lactic acid) (PLLA) (MePEG-*b*-PLLA, chemical structure shown in Fig. 1b)^[6–9], and find that drug loading amount is a crucial factor affecting the degradation of fiber mats and consequently affecting the release behavior of the drug.

* This work was financially supported by the National Natural Science Foundation of China (Nos. 20274048, 50373043) and by Chinese Academy of Sciences (No. KJCX2-SW-H07).

** Corresponding author: Ying-jie Deng (邓英杰), E-mail: dengyingjie45@yahoo.com.cn

Xue-si Chen (陈学思), E-mail: xschen@ciac.jl.cn

Received February 5, 2010; Revised May 4, 2010; Accepted May 27, 2010

doi: 10.1007/s10118-011-1024-z

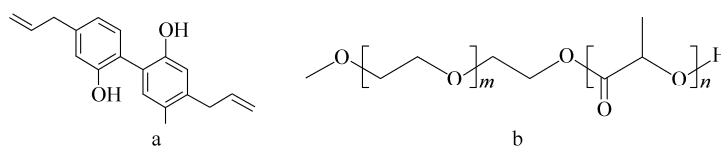


Fig. 1 Chemical structure of magnolol (a) and MePEG-*b*-PLLA (b)

EXPERIMENTAL

Materials

Copolymer MePEG-*b*-PLLA (short for PEG-PLLA) was synthesized as described in Ref. [9] with MePEG2000 as initiator and Sn(Oct)₂ as catalyst. Molecular weight of the copolymer obtained finally was 80400 with polydispersity of 1.22 determined by gel permeation chromatography (GPC, USA Waters combined with Wyatt DAWN EOS (MALLS) and DSP (RI)) by using chloroform as mobile phase.

Preparation and Characterization of Medicated Fibrous Mats

Medicated fibers were prepared as described in Refs. [9, 10]. For preparing polymer solutions for electrospinning, 6, 12 and 18 mg/mL (10 wt%, 20 wt% and 30 wt% mass ratio respect to polymer materials) of the model drug Magnolol (MNL, 98.2% purity) were added in polymer solutions, respectively. Triethyl benzyl ammonium chloride (TEBAC, Sigma Aldrich) of 3 mg/mL (5% mass ratio respect to polymer materials) was added in polymer solutions as electroconductive substance which is necessary for polymer solution electrospinning.

The electrospinning set-up was the same as reported by Zeng *et al.*^[3] and Xu *et al.*^[9]. Every copolymer chloroform solution with MNL added was transferred to a syringe with a right angle-shaped and 0.4 mm-inner diameter needle. A steady flow of the solution was maintained from the needle tip at 1.5–2.0 mL/h. The electric field applied for spinning was 4.5 kV and the distance from the needle tip to harvesting plate was 50 cm. All electrospinning experiments were carried out at about 30°C in air. Final fibrous mat product was obtained by removing residual chloroform in fibers under vacuum at room temperature.

An environmental scanning electron microscope (ESEM, Model XL 30ESEM FEG from Micro FEI Philips) was used to observe the morphology of all fibrous mats which were vacuum-coated with an aurum layer prior to examination. The surface crystalline structures of fibrous mats were analyzed by wide angle X-ray diffraction (WAXD, Rigaku, D/max 2500V PC) from 5° to 60° at a scanning rate of 2 (°)/min. Fibrous mats were also detected by differential scanning calorimetry (DSC, Perkin) from 25°C to 300°C.

In vitro Drug Release and Statistical Analysis

Release behavior of MNL entrapped in PEG-*b*-PLLA fibrous mats with 10 wt%, 20 wt% and 30 wt% MNL loading respectively was investigated for 3 times represented by $n = 3$ in Fig. 4. Experiments were carried out by incubating pieces of drug loaded fibrous mats of about (25 ± 2) mg in 25 mL phosphate buffer solutions (PBS, prepared according to the Pharmacopeia of China by dissolving 6.8 g KH₂PO₄ and 1.58 g NaOH into deionized water which was metered to 1000 mL) of pH 7.4 in a thermostated shaker of 37°C under oscillation at 100 r/min. For comparing the enzyme degradation effect on polymer fibers, three kinds of fibrous mats were investigated in PBS with and without proteinase K (Amesco, biotechnical grade, supplied as lyophilized powder) which was widely used for polyester degradation. 2.5, 5 and 10 µg/mL proteinase K PBS was selected for investigation. At predetermined time interval the fibrous mats were taken out of the release media and immersed into corresponding new release media. 20 µL release media was injected into high performance liquid chromatography (HPLC) system and analysed.

The HPLC system was conducted by using a Waters HPLC system equipped with a column (Dikma Diamond C18, $\phi 4.5 \times 150$ mm, Dikma), a UV-detector (Shimadzu, UV-975) and a PU-980 pump (Shimadzu). The mobile phase was composed of an 80/20 mixture (*V/V*) of methanol and deionized water. The pH of the mobile phase was adjusted to pH 2.7 by phosphoric acid before use. The flow rate was 1.0 mL/min, and the detection wavelength was 294 nm.

RESULTS AND DISCUSSION

Characterization of Fibrous Mats

PEG-*b*-PLLA fibers with MNL loaded were successfully prepared. Soft and continuous fibrous mats were obtained and the fibrous mats with 10% MNL loaded presented light yellow color and those with 20% and 30% MNL loaded present dark yellow color. All fibrous mats obtained presented a certain extent of tenacity after they were accumulated to 0.5 mm thickness, suitable for clinical application as implantable or stuffing drug delivery device.

ESEM photos of fibrous mats obtained are shown in Figs. 2(a, b, c). The products showed fiber networks which looked uniform and the average diameters of most of fibers were from about 500 nm to about 1 μ m for all the three samples. Increasing of the amount of MNL did not obviously increase the diameters of fibers. Fiber surface was smooth and there were no drug crystals on fibers surface. WAXD measurements show obviously that pure MNL was crystalline, while PEG-*b*-PLLA fibers with or without MNL loaded were amorphous. No phase separation took place during the rapid evaporation of chloroform in the electrospinning process suggesting that MNL existed in the fibers or on the surface of fibers as amorphous form showing that MNL could be entrapped into the PEG-*b*-PLLA fibers finely.

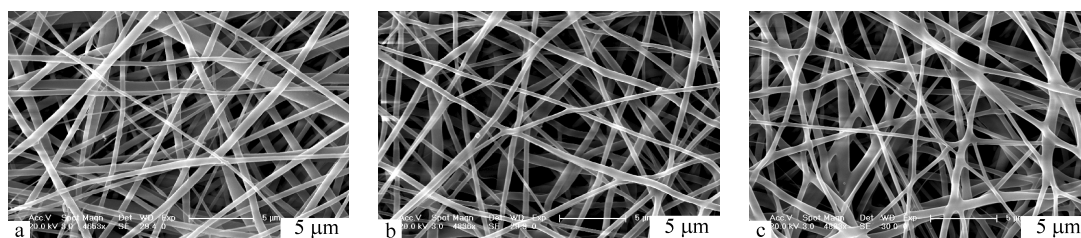


Fig. 2 ESEM photographs of medicated fibrous mats loaded with 10% (a), 20% (b) and 30% (c) MNL, respectively

It could be also found from ESEM that fibers were fused or bonded together at their contacting sites leading to a three-dimensional network of fibers which would improve significantly the mechanical strength of the fibrous mats favoring local application. And as exhibited from (a) to (c) in Fig. 2, it could be found that the more amount MNL was loaded in fibers, the more fusions (connections) appeared.

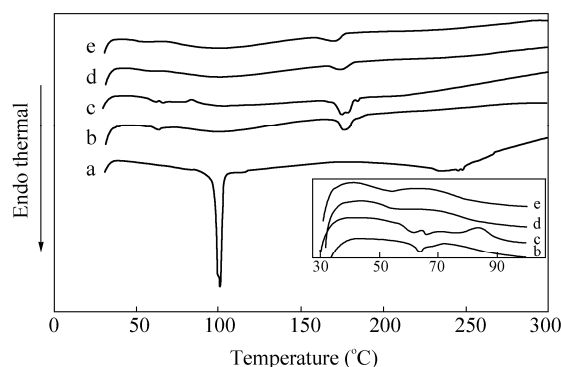


Fig. 3 DSC detection of MNL powder (a), PEG-*b*-PLLA fibrous mats without MNL (b), PEG-*b*-PLLA fibrous mats with 10 wt% MNL (c), PEG-PLLA fibrous mats with 20 wt% MNL (d) and PEG-*b*-PLLA fibrous mats with 30 wt% MNL (e)

Physical state of MNL in the fibers, pure MNL powder and four kinds of fibrous mats with MNL loaded were characterized by DSC shown in Fig. 3, DSC determination indicated that T_m of MNL is at about 100°C and T_g of PEG-*b*-PLLA fibrous mats with no drug loaded was at about 63°C. After MNL had been loaded, the

fibrous mats' endothermal peaks were all shifted to low temperatures due to the plasticizing effect of MNL to polymer after electrospinning which was also discovered by Xu *et al.* This phenomenon is also likely to be an evidence of desirable MNL entrapment into PEG-*b*-PLLA fibers.

***In vitro* Drug Release**

Drug release experiments were carried out for 2 weeks. From the results shown in Figs. 4(a) and 4(b), it can be seen that MNL entrapped in fibrous mats presented generally sustained release character and no burst release was found for all fibrous mats. This is mainly due to the small molecule diffusion mechanism of drug release and the polymer degradation effect.

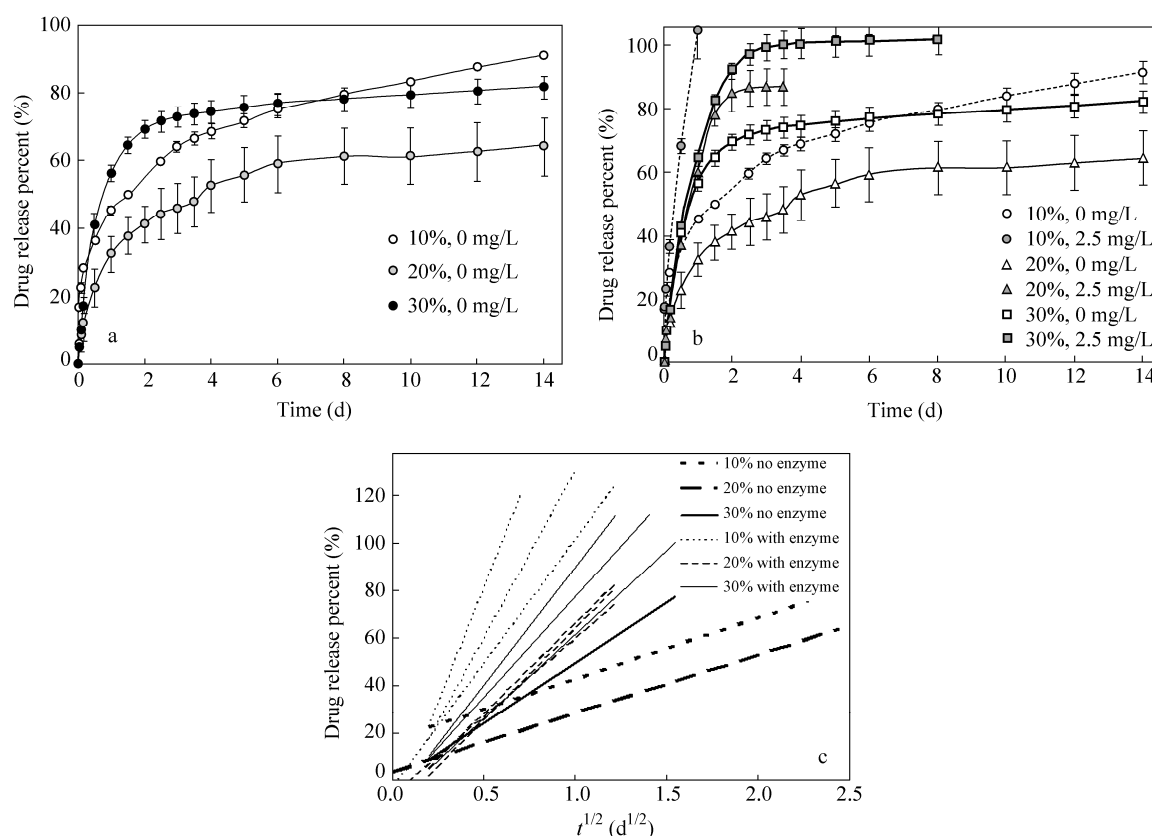


Fig. 4 (a) MNL release profile from fibrous mats in PBS without proteinase K added ($n = 3$); (b) Comparison of MNL release profile from PBS without and with 2.5 mg/L proteinase K added ($n = 3$); (c) Linear fitting of accumulated drug release (%) and square root of time $t^{1/2}$ for fibrous mats with MNL loaded ($n = 3$)

For fibrous mats with 10 wt% MNL loaded, 100% (respect to totally loaded MNL in fibrous mats) entrapped MNL was released within 1 day in PBS of pH 7.4 with 2.5, 5 and 10 $\mu\text{g/mL}$ proteinase K added, while in PBS without proteinase K the entrapped drug was released with 1 day less than 50% and after 17 days more than 90%. Enzyme in release media dramatically facilitates the release of MNL in fibers due to the degradation effect to ester bonds of PLLA. What's more, fibrous mats maintained integrity after 17 days in PBS, while in PBS with proteinase K fibrous mats broke down into tiny pieces and dissolved in release media after only 3 days.

For fibrous mats with 20 wt% and 30 wt% MNL loaded, after 2 weeks, about 100% entrapped MNL was released from fibrous mats when the concentration of proteinase K in PBS release media was 2.5, 5 and 10 $\mu\text{g/mL}$, while the release percentage was only about 60% and about 80% respectively when there was no proteinase K added in release media. Addition of proteinase K in release media significantly facilitated the

degradation of polymer thus resulting the quick release of drug entrapped, and higher concentration of proteinase K made polymer being degraded more quickly.

It could be known from the result that concentration of proteinase K presented significant effect on the degradation of fibers and consequently affected MNL release significantly. Nevertheless, MNL being located in fibers also play a key role for drug release profile.

PEG blocks presented partly on the fibers surface after electrospinning which were contacting to the PBS release media would facilitate the H₂O molecules saturating the surface of the fibers and helped proteinase K close to PLLA block and contact to the ester bond which led to the degradation of the fibers. However, because MNL show water insoluble character when being entrapped in PLLA fiber though it could be dissolved in pH 7.4 PBS (48.8 µg/mL, 37°C) due to aqueous protonizable phenol, more lipophilic MNL molecules would shelter the hydrophilic PEG block from being hydrated, and resulting the poor contact of proteinase K to PLLA blocks. What's more, the fusion at contact points between neighbouring fibers results in the formation of scaffolds with three dimensional crosslinking structure, and this compact structure would also prevent the degradation of PEG-*b*-PLLA polymer and cause the drug release profile as shown in Fig. 4.

In our experiment, fibrous mats could be considered as solid dispersion DDS (drug delivery system) with polymer as matrix, so the linear fitting could be obtained according to Higuchi's equation:

$$\alpha = \left[\frac{D\varepsilon}{T} \times (2A - \varepsilon C_s) \times C_s t \right]^{1/2}$$

which could be simplified as $\alpha = Kt^{1/2}$. α — drug release amount per unit area, mg/cm²; D — diffusion coefficient of drug in release media, cm²/s; ε — porosity of matrix material; T — tortuosity of the matrix; C_s — solubility of drug in release media, mg/cm³; A — drug amount per unit volume solid dispersion, mg/cm³.

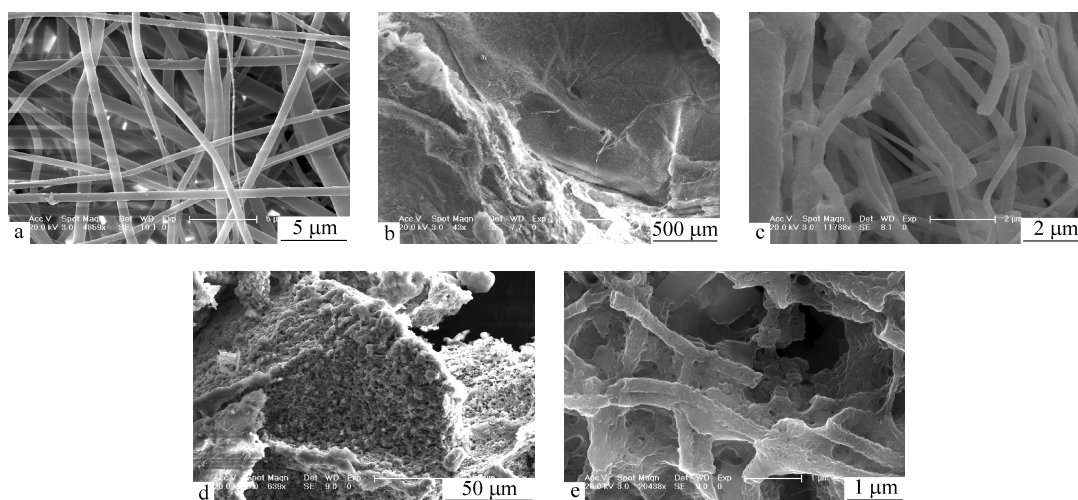
This equation was established under the assumption that drug was dispersed in matrix composed of water-insoluble indegradable polymer. However, By degradation of PEG-*b*-PLLA step by step, parameter ε became bigger and T became smaller which would lift the K value and favor the release of MNL from fibers. In the experiment, MePEG-PLLA would be degraded by enzyme, so ε and T would change little by little resulting in increasing K value in defined drug release period for the total fibrous mat matrix as long as proteinase K had effect on the copolymer. MNL release from poly(lactic acid) matrix followed a combined mechanism of MNL diffusion through the matrix and enzymatic degradation corrosion of the matrix especially there was proteinase K added in release media which led to experiment data of comprehensive result.

For every fibrous mats with MNL loaded, there was obvious linear relationship between the accumulated drug release percent (%) and square root of time $t^{1/2}$ shown in Fig. 4(c) and data of linear fitting were shown in Table 1 with slope rate and linear regression (r^2). We could find that release rate of MNL from fibrous mats increased as the enzyme concentration in PBS increased, and more desirable fitting result could be obtained for fibrous mats in PBS with proteinase K added compared with that without proteinase K added.

Compared with MNL release in PBS with no proteinase K added, MNL released more fast in release media with proteinase K added. Due to the degradation of PLLA by enzyme breaking down polymer matrix structure and facilitating drug diffusion, the higher concentration of proteinase K in release media, the faster release of entrapped MNL from fibers which could be known from Fig. 4(c). However, as the drug loading amount was lift to 20% and 30%, although more proteinase K was added in release media, the fibrous mats still maintained integrity for about 3 days and more than 5 days respectively. After being washed by pure water and freeze-dried, the residue of fibrous mats with 10% and 30% MNL loaded was observed by ESEM, it could be found that fibrous mats with 30% MNL remained integrity and smooth surface in release media without proteinase K added (Fig. 5a). As to fibers with 10% MNL loaded (Fig. 5d and 5e), proteinase K degraded the fibers into small segments and there were many cavities on fiber surface. However, as to fibers with 30% MNL loaded (Fig. 5b and 5c), proteinase K could not degrade the fibers into pieces but leaving only unsmooth surface and little cavities less than the case in fibers with 10% MNL loaded.

Table 1. Regressive equation of accumulated drug release (%) and square root of time $t^{1/2}$

MNL mass ratio in fibrous mats (%)	Enzyme concentration in release media (mg/L)	Slope from regressive equation	R^2
10%	0	25.722	0.9809
	2.5	104.91	0.9965
	5	141.21	0.9933
	10	195.31	0.9883
20%	0	24.502	0.9791
	2.5	66.065	0.9793
	5	77.852	0.9882
	10	76.294	0.9825
30%	0	51.075	0.9652
	2.5	71.088	0.992
	5	84.470	0.995
	10	99.373	0.9923

**Fig. 5** ESEM of fibrous mats after drug release without proteinase K degradation (a fibers with 30% MNL entrapped) and being degraded by proteinase K (b and c fibers with 30% MNL entrapped; d and e fibers with 10% MNL entrapped)

There was no burst release for all fibrous mats and in PBS release media with different concentrations of proteinase K. The 5 min release percentage of small molecules in all the fibrous mats could demonstrate the status of MNL existing in the fibers, *i.e.* the drug release behavior is relevant to drug distribution status in matrix, the results revealed that MNL molecules combined tightly with PLLA blocks which could be easily understood that both PLLA polymer and MNL are lipophilic. And fibers with 20% and 30% MNL entrapped show less burst release than fibers with 10% MNL entrapped which supported the conclusion that high loading amount of MNL shielded the PEG block which would provide hydrophilic channel leading to drug burst release.

So by comprehending the results of ESEM, WAXD and MNL release profile, it could be supposed that MNL was perfectly entrapped in the fibers composed of PLLA with PEG blocked, and the MNL loading amount has significant effect on the release behavior of the drug.

There have been some experimental results supporting the suggestion that PEG blocks facilitate the release of compound 2-(*p*-isobutylphenyl) propionic acid (ibuprofen) from PEG-*b*-PLLA fibers and cause burst release of the compound^[11, 12]. What's more, when ever there was PEG block in PLLA or not, it was found that as entrapped ibuprofen amount lifted, drug burst release percent became bigger and drug release speed became fast. Nevertheless, as to fibers with MNL entrapped, lifting of drug loading amount to 20% and 30% did not facilitate

the drug burst release any more than the fibers with 10% loading amount for the much more lipophilicity and water insolubility character of MNL. On the contrary, MNL release rate of fibrous mat with 10 wt% MNL loaded was faster than that with 20 wt% and 30 wt% MNL loaded in the first 6 h whether or not there was proteinase K added in PBS which showed that un-sheltering to PEG block led to drug hydration and dissolving to aqueous solution.

Addition of other kinds of polymers or small molecules into main matrix polymer is a kind of method regulating the drug delivery system property and performance^[13-15], such as recently Wang *et al.* reported addition of PEG blocked polyester into PEG-*b*-PLLA fibers and found that the polymer additive had prohibited PLLA block from degradation and sustained ibuprofen release. Now, it is discovered that small molecule loading amount variation could also regulate the release of the compound from fiber matrix and prohibit the polyester matrix from enzyme degradation.

REFERENCES

- 1 Zeng, J., Xu, X., Chen, X., Liang, Q., Bian, X., Yang, L. and Jing, X., *J. Control. Release*, 2003, 92(3): 227
- 2 Kenawy, E., Bowlin, G.L., Mansfield, K., Layman, J., Simpson, D.G., Sanders, E.H. and Wnek, G.E., *J. Control. Release*, 2002, 81(1-2): 57
- 3 Zeng, J., Yang, L., Liang, Q., Zhang, X., Guan, H., Xu, X., Chen, X. and Jing, X., *J. Control. Release*, 2005, 105(1-2): 43
- 4 Kim, K., Luu, Y.K., Chang, C., Fang, D., Hsiao, B.S., Chu, B. and Hadjiargyrou, M., *J. Control. Release*, 2004, 98(1): 47
- 5 Luu, Y.K., Kim, K., Hsiao, B.S., Chu, B. and Hadjiargyrou, M., *J. Control. Release*, 2003, 89(2): 341
- 6 Zhu, K.J., Lin, X.Z. and Yang, S.L., *J. Appl. Polym. Sci.*, 1990, 39(1): 1
- 7 Cerral, P., Tricoli, M., Lelli, L. and Guerra, G.D., *J. Mater. Sci., Mater. Med.*, 1994, 5(6-7): 308
- 8 Bae, Y.H., Huh, K.M., Kim, Y. and Park, K.H., *J. Control. Release*, 2000, 64(1-3): 3
- 9 Xu, X., Yang, L., Xu, X., Wang, X., Chen, X., Liang, Q., Zeng, J. and Jing, X., *J. Control. Release*, 2005, 108(1): 33
- 10 Xu, X., Chen, X., Xu, X., Lu, T., Wang, X., Yang, L. and Jing, X., *J. Control. Release*, 2006, 114(3): 307
- 11 Wang, H., Song, H., Chen, X. and Deng, Y., *Chinese J. Polym. Sci.*, 2010, 28(3): 417
- 12 Wang, H., “Drug release from poly(L-lactic acid) electrospun fibers and hollow microsphere under enzyme degradation”, Thesis, Shenyang Pharmaceutical University, 2010
- 13 Cui, W., Qi, M., Li, X., Huang, S., Zhou, S. and Weng, J., *Int. J. Pharm.*, 2008, 361(1-2): 47
- 14 Urata, T., Arimori, K. and Nakano, M., *J. Control. Release*, 1999, 58(2): 133
- 15 Luu, Y.K., Kim, K., Hsiao, B.S., Chu, B. and Hadjiargyrou, M., *J. Control. Release*, 2003, 89(2): 341