Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmaceutics and Biopharmaceutics 70 (2008) 165–170
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

# The release behavior of doxorubicin hydrochloride from medicated fibers prepared by emulsion-electrospinning

Xiuling Xu [a,d], Xuesi Chen [a], Ping'an Ma [b], Xinri Wang [c], Xiabin Jing [a,*]

[a] State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Changchun, China
[b] School of Life Science, Northeast Normal University, Changchun, China
[c] The Key Laboratory of Zoonosis of Ministry of Education, Jilin University, Changchun, China
[d] Graduate School of Chinese Academy of Sciences, Beijing, China

Received 22 October 2007; accepted in revised form 18 March 2008
Available online 28 March 2008

**Abstract**

The release behavior of a water-soluble small molecule drug from the drug-loaded nanofibers prepared by emulsion-electrospinning was investigated. Doxorubicin hydrochloride (Dox), a water-soluble anticancer agent, was used as the model drug. The laser scanning confocal microscopic images indicated that the drug was well incorporated into amphiphilic poly(ethylene glycol)–poly(L-lactic acid) (PEG–PLA) diblock copolymer nanofibers, forming "core-sheath" structured drug-loaded nanofibers. The drug release behavior of this drug-loaded system showed a three-stage diffusion-controlled mechanism, in which the release rate of the first stage was slower than that of the second stage, but both obeyed Fick's second law. Based on these results, it is concluded that the Dox-loaded fibers prepared by emulsion-electrospinning represent a reservoir-type delivery system in which the Dox release rate decreases with the increasing Dox content in the fibers.


*Keywords:* Emulsion; Electrospinning; Nanofiber; Drug delivery; Diffusion

## 1. Introduction

The principle of controlled release drug therapy involves the delivery of a pre-determined amount of drug, over a specified period of time, in a predictable manner. The aim of all controlled release systems is to improve the effectiveness of drug therapy [1,2]. This improvement can take the form of increasing the therapeutic activity compared to the intensity of side effects, reducing the number of drug administrations required during the treatment, or eliminating the need for specialized drug administration (e.g., repeated injections) [3,4].

Today, there are numerous controlled release systems. Among them, polymeric drug delivery systems, especially the drug delivery devices using biodegradable polymers such as poly(lactic acid)-type polymers, have been investigated extensively [5–10]. Various nanotechnologies focusing on formulating therapeutic agents in these biodegradable matrices, such as nanoparticles, nanocapsules, micellar systems, and conjugates have been well developed [4,11–14]. Very recently, with the development of electrospinning, the use of biodegradable polymer electrospun nanofibers as drug carriers seems to be a promising method for delivering anticancer drugs, especially in post-operative local chemotherapy.

Generally, drugs loaded in these polymeric delivery systems have been formulated in two basic designs: matrices or reservoirs [15–17], as illustrated in Fig. 1. In the matrix-type structure (Fig. 1A), a therapeutic agent is homogeneously dispersed throughout a polymer matrix. The rate of drug release by diffusion through the polymer matrix normally decreases with time, since the agent has a progressively longer distance to travel and therefore

* Corresponding author. State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, 5625 Renmin Street, Changchun, Jilin 130022, China. Tel./fax: +86 431 85262775.
*E-mail address:* xbjing@ciac.jl.cn (X. Jing).

0939-6411/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2008.03.010

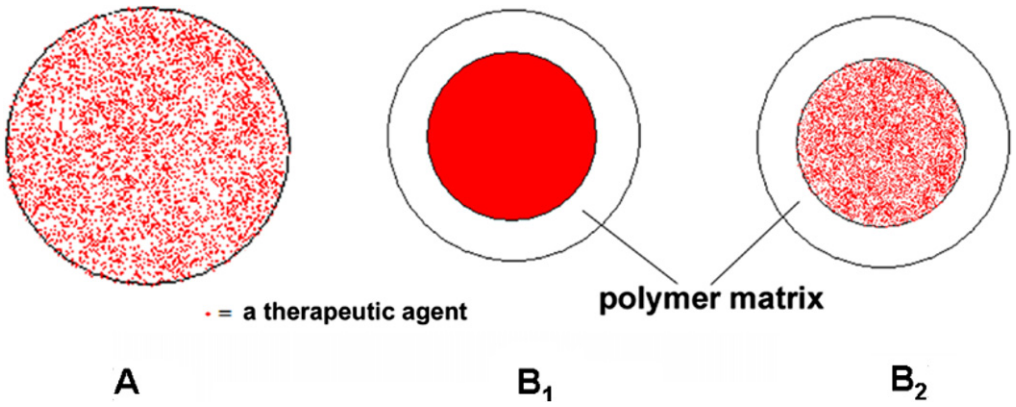


Fig. 1. Two drug distribution geometries: matrix-type (A) and reservoir-type (B). The reservoir-type may have two different structures: the core part is composed of pure drug or drug solution ($B_1$) or of a dispersion of the drug in a polymer matrix ($B_2$).

requires a longer diffusion time to release. On the other hand, in reservoir-type structure (Fig. 1B), the drug is enclosed in the polymer matrix, forming the so-called "core-sheath" structure. There are two possible structures classified by the core composition: the pure drug in solid or in solution state serves as the core surrounded by a pure continuous polymer sheath; or the drug molecules are dispersed in a polymer matrix and the whole dispersion system serves as the core. In the first case (Fig. 1$B_1$), if the polymer sheath is essentially uniform and thick enough compared to the core, the diffusion rate of the therapeutic agent will be fairly stable throughout the life of the delivery system. However, in the second case (Fig. 1$B_2$), the release behavior of the drug is more complicated, and will be discussed in detail later in this paper.

Previous studies [18] have reported that a water-soluble anticancer agent, doxorubicin hydrochloride (Dox), could be incorporated into amphiphilic poly(ethylene glycol)-block-poly(L-lactic acid) (PEG–PLA) diblock copolymer nanofibers, using a novel "emulsion-electrospinning" method. The drug could be released from the drug-loaded fibers in a sustained manner. However, the release mechanism was not investigated in detail. Moreover, to the best of our knowledge, there are few research groups investigating the release kinetics of drugs incorporated into a fiber produced by emulsion-electrospinning.

In this study, PEG–PLA nanofibers with different Dox loadings were obtained by emulsion-electrospinning. The release behaviors from the fibers were examined. The release profiles of Dox from this system consisted of three sequential diffusion-controlled stages in which the release rate decreased with the increasing Dox content in the fibers.

## 2. Materials and methods

### 2.1. Materials

Doxorubicin hydrochloride (Dox) was a gift from Zhejiang Hisun Pharmaceutical Co. Ltd. Sodium dodecyl sulfate (SDS) was obtained from Sigma and was used without further purification.

Diblock copolymer PEG–PLA (prepared from PEG750 and lactide) was synthesized in our laboratory. Its molecular weight (Mn) and polydispersity (PD) determined by GPC were 84,800 and 3.41, respectively.

### 2.2. Preparation of water-in-oil emulsions containing Dox

The method to prepare water-in-oil (W/O) emulsions was described in detail by Xu et al. [18]. In the present study, 2 ml water solutions containing 0.5, 1.0, 2.0 and 3.0 wt% of Dox (with respect to PEG–PLA) were emulsified in 25 ml of 6 wt% chloroform solutions of PEG–PLA, respectively. The rotation rate of homogenizer and the homogenization time were approximately 6300 r/min and 20 min, respectively. In order to obtain stable and homogeneous W/O emulsions, 5 wt% of SDS (with respect to PEG–PLA) was added to the oily phase prior to emulsification as a surfactant to lower the surface tension. As a result, the aqueous emulsion droplets were highly distributed in the oily phase, forming homogeneous W/O emulsions.

### 2.3. Electrospinning

The stable and homogenous W/O emulsions were electrospun using a conventional electrospinning set-up as described by Zeng et al. [19]. In the present study, typical electrospinning parameters were as follows: electric field strength: 1.8–2.0 kV/cm; air gap distance: 24 cm; inner diameter of spinneret: 0.4 mm; and flow rate of solution: 60–90 μl/min. In our experience, the optimal concentration of the PEG–PLA/chloroform solution was 6 wt%. Therefore, this concentration was fixed for all the electrospinning in the present study. In addition, all the electrospinning parameters were kept constant, and all the experiments were conducted at room temperature in air. In order to remove the residual chloroform, the fiber mats collected

were freeze-dried for about 48 h at 50 °C under a vacuum of 10 Pa.

### 2.4. Characterization of Dox-loaded electrospun fibers

An environmental scanning electron microscope (ESEM, Model XL 30 ESEM FEG from Micro FEI Philips) was used to observe the surface morphology and size distribution of the electrospun fibers. Its accelerating voltage was 20 kV. Samples were mounted on metal stubs using a double-sided adhesive tape and vacuum-coated with a platinum layer prior to examination.

It is known that Dox is a fluorescent molecule that can emit red fluorescence. Therefore, a laser scanning confocal microscope (LSCM, FV 1000, Olympus) was used to evaluate the distribution of Dox in the electrospun composite nanofibers.

### 2.5. In vitro drug release studies

The released Dox in the buffer solution was monitored by a UV–vis spectrophotometer at the wavelength of 483.5 nm. The drug-loaded fiber sample (20–30 mg, 2 cm × 2 cm × 0.20 mm) was incubated at 37 °C in 20 ml of phosphate buffered saline (PBS, pH 7.4). At the required incubation time, the sample was transferred to 20 ml of fresh buffer solution, and the released Dox in the original buffer solution was determined. The detected UV absorbance of Dox was converted to its concentration according to the calibration curve of Dox in the same buffer. Then, the accumulative weight and the relative percentage of the released Dox were calculated as a function of incubation time.

The total content of Dox in the fibers was determined as follows. The three original Dox-loaded fiber mats were placed into separate vials filled with 20 ml of 0.05 mol/l Tris–HCl buffer solution (pH 8.6) containing 50 μg/ml of proteinase K at 37 °C. After several hours, the fiber mats had all degraded into small chippings, indicating that the Dox had been completely released into the buffer solution. The resultant solutions were monitored at the wavelength of 483.5 nm. The concentrations of Dox in the release solutions were determined according to the calibration curve of Dox in the same buffer. The total content of Dox in the fibers was easily calculated from the average of the three fiber mats.

## 3. Results and discussion

The morphology of the electrospun Dox-loaded PEG–PLA nanofibers with 0.5, 1.0, 2.0 and 3.0 wt% of Dox loadings is shown in Fig. 2. They appeared uniform. The surfaces were smooth and no drug crystals were detected, indicating that the drug was finely incorporated into the electrospun fibers. Moreover, it seemed that there were no significant differences in either the morphology or the average diameter of the composite fibers containing differ-

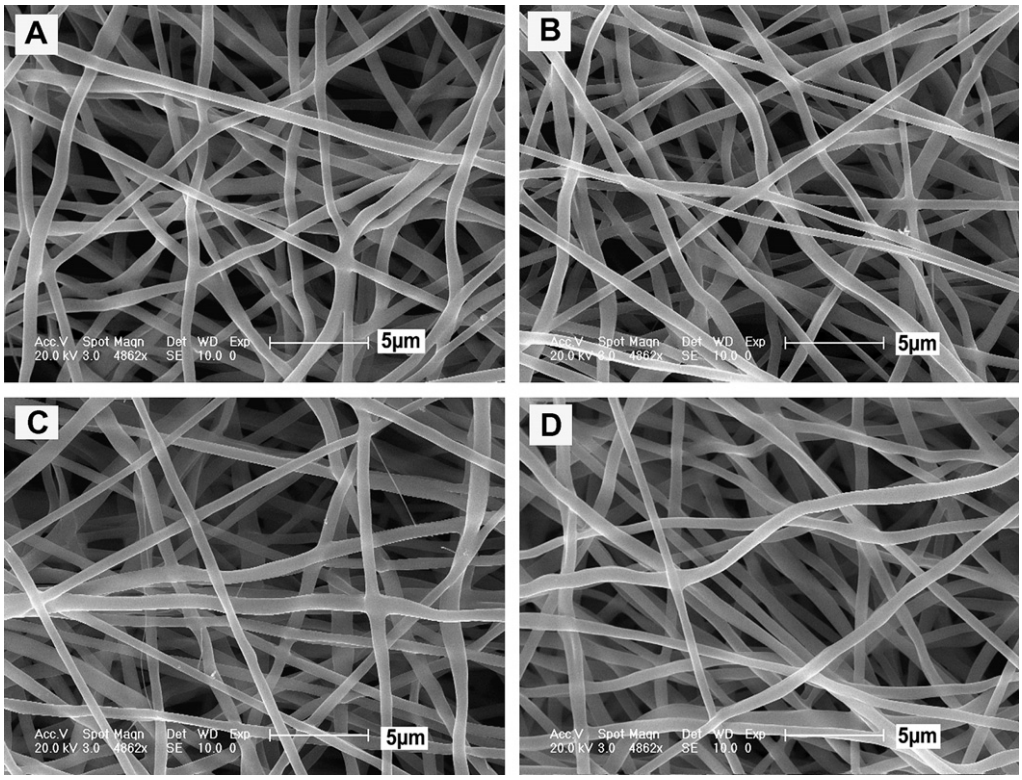


Fig. 2. ESEM photographs of Dox-loaded PEG–PLA fibers containing (A) 0.5, (B) 1.0, (C) 2.0 and (D) 3.0 wt% of Dox.

ent amounts of Dox. Their average diameters were all about 570 nm.

To gain further information on the distribution of Dox in the fibers, fluorescent microscopy observations were carried out (Fig. 3). The fibers exhibited a "core-sheath" structure having Dox enclosed in the core. As described by Xu et al. [20], the core actually consisted of fluorescent Dox aggregates dispersed in a PEG–PLA matrix. The sheath consisted of pure PEG–PLA polymer which did not show red fluorescence. In other words, Dox was incorporated into PEG–PLA fibers forming a reservoir-type drug-loaded system. As explained by Xu et al., this core-sheath structure was formed during the electrospinning of a W/O emulsion due to "evaporation and stretching induced de-emulsification" [20]. As Dox content in the spinning emulsion increased, the core part of the fibers seemed to become thinner (Fig. 3). The ratio of core to sheath diameter was about 0.81, 0.53, 0.44 and 0.31 for 0.5, 1.0, 2.0 and 3.0 wt% Dox-loaded PEG–PLA fibers, respectively. This dependence of the core diameter on the Dox content implies that the inward movement and the degree of de-emulsification (mergence) of the aqueous droplets were related to the Dox content in the emulsion droplets. This is because the electrospinning emulsions contained equal volume ratios of aqueous droplets but Dox contents in the aqueous droplets were different from one sample to another. Probably, the evaporation rate of water from the droplets is dependent on the Dox concentration in the late stage of evaporation because of the solubility of Dox in water: higher Dox content results in slower evaporation. Consequently, higher Dox content in the aqueous droplets leads to more viscosity difference between the aqueous droplets and the surrounding polymer solution matrix, and thus leads to higher degree of de-emulsification of the aqueous droplets, i.e., they move farther towards the fiber axis and concentrate there, resulting in thinner apparent "Dox core".

Fig. 4A shows the release profiles of Dox from 0.5, 1.0, 2.0 and 3.0 wt% Dox-loaded fibers, respectively. The drug release behaviors were similar, i.e., after a relatively fast release for 6–15 h, the release rate of Dox leveled off. Interestingly, the release rate of Dox decreased as Dox content in the fibers increased during the whole drug release time. For example, the release percentages were about 73.1%, 60.5%, 47.7% and 36.8% at 10 h, respectively, for the four samples examined. These results indicate that the Dox release behaviors are closely related to the distribution status of the drug. As shown in the previous paragraph and in

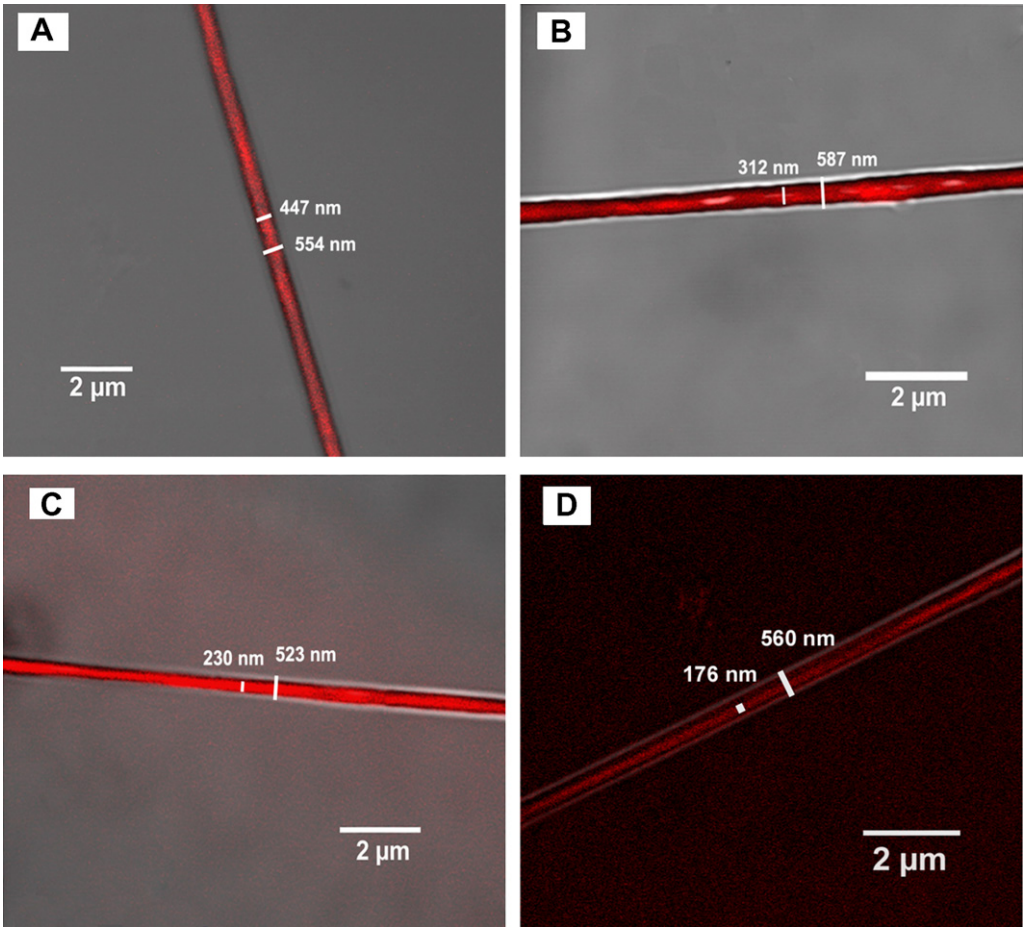


Fig. 3. LSCM images of Dox-loaded electrospun nanofibers prepared from W/O emulsions. The contents of Dox in the composite fibers were (A) 0.5 wt%, (B) 1.0 wt%, (C) 2.0 wt% and (D) 3.0 wt%, respectively.

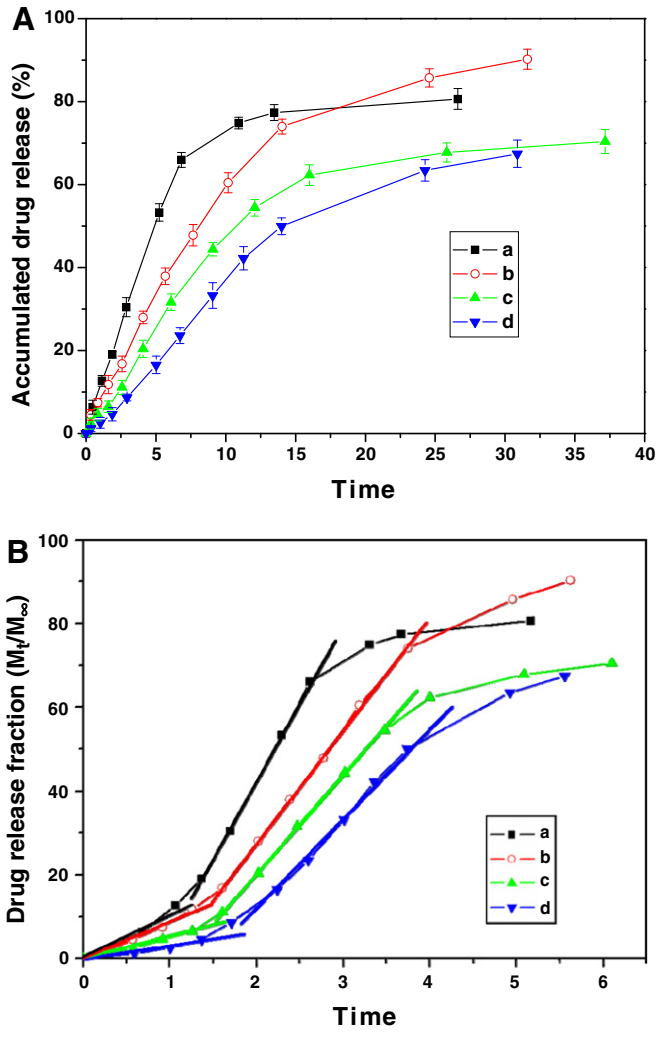


Fig. 4. (A) The release profiles of Dox from the Dox-loaded electrospun fibers in PBS at 37 °C. Dox content in the fibers: (a) 0.5 wt%, (b) 1.0 wt%, (c) 2.0 wt%, (d) 2.0 wt%. (B) The curves in (A) re-plotted against square root of time.

Fig. 3, a greater amount of Dox in the electrospinning emulsion resulted in a thinner core and a thicker sheath of fibers. Therefore, as a reservoir-type drug release system, the drug diffused more slowly through the relatively thicker PEG–PLA sheath before being released from the fibers.

To further assess the mechanism of drug release, the released fraction of Dox ($M_t/M_\infty$) was plotted against the square root of time (Fig. 4B). On the basis of a theoretical analysis [15,21–23], if a drug release is controlled by a diffusion mechanism, this plot should be a straight line. As shown in Fig. 4B, all the drug release profiles consisted of three sequential stages. Approximately, linear relationships ($R^2 > 0.963$) between $M_t/M_\infty$ and $t^{1/2}$ were obtained for both the first and the second stages. The slope of the straight lines for the second stage was much steeper than that for the first stage, indicating that the Dox release process was diffusion-controlled and the diffusion rate in the second stage was faster than in the first stage. This release kinetic agrees with the reservoir-type drug release system.

As shown in Fig. 5A, the slower first stage is related to the diffusion of the drug through the pure PEG–PLA sheath surrounding the Dox-containing core. When more Dox molecules enter and occupy the sheath layer, and the Dox concentration in the sheath layer is comparable to that in the core region, the Dox release enters the second stage. The whole drug-loaded system behaves as a matrix system (Figs. 5B and 1A), and the Dox molecules diffuse in such a way that a molecule behind always follows in the path of the one in front. Therefore, the release rate of Dox becomes faster from the first stage to the second. In the third stage, the release rate of Dox becomes slower, and the plot of the released fraction of Dox vs. $t^{1/2}$ does not fit a linear relationship. Interpretation could be as follows. Firstly, the total content of Dox in the fibers becomes less and less over the release time. Secondly, the drug molecules located in the center of the fibers have a longer distance through which to diffuse. These two possible

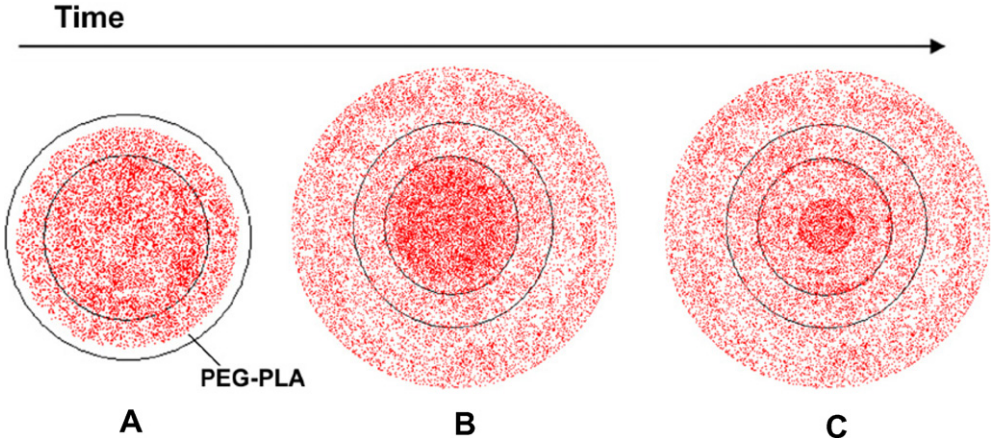


Fig. 5. The release steps of Dox from the Dox-loaded fibers prepared by emulsion-electrospinning. (A) The first stage: Dox diffused through the pure PEG–PLA sheath surrounding the Dox-containing core; (B) the second stage: the whole Dox-loaded system behaves as a matrix system; (C) the third stage: the total content of Dox in the fibers becomes less and less over the release time, and the diffusion distance become longer for the drug molecules located in the center of the fibers.

scenarios are shown in Fig. 5C. Another possible reason, as pointed out by Xu et al. [24], is the increased crystallinity of PEG–PLA as a result of their incubation in PBS at 37 °C. It is known that a small molecule diffuses more slowly through a crystalline polymer than through an amorphous polymer of the same kind [25], so the release rate of Dox becomes slower as the release time becomes longer.

In Fig. 4B, it is also seen that, irrespective of the first stage or the second stage, the slopes of the plots depend on the Dox content in the fibers. Among the four samples examined, the 0.5 wt% Dox-loaded fibers show the fastest release rates, while the 3.0 wt% fibers exhibit the slowest release rates. This result may be explained by the thicker sheath of the 3.0 wt% Dox-loaded fibers compared to the 0.5 wt% fibers, as shown in Fig. 3.

## 4. Conclusions

In this study, PEG–PLA nanofibers with different Dox loadings were prepared by emulsion-electrospinning. The Dox was molecularly distributed in the center of the fibers, forming a reservoir-type drug-loaded system. As the Dox content in the fibers increased, Dox preferred to concentrate in the center of the fibers. During the whole release time, the rate of Dox release decreased as the Dox content in the fibers increased. The release of Dox from the system consisted of three sequential stages that were all diffusion-controlled. The release rate of Dox at the first stage was slower than the rate at the second stage due to the reservoir-type structure of the fibers formed, and both obeyed Fick's second law.

## Acknowledgements

This project was financially supported by the National Natural Science Foundation of China (Project Nos.: 20274048 and 50373043), by the National Fund for the Distinguished Young Scholars (No.: 50425309), and by the Chinese Academy of Sciences (Project No.: KJCX2-SW-H07).

## References

[1] R. Langer, Drug delivery and targeting, Nature 392 (1998) 5–10.
[2] J.R.B.J. Brouwers, Advanced and controlled drug delivery systems in clinical disease management, Pharm. World Sci. 18 (1996) 153–162.
[3] K.E. Uhrich, S.M. Cannizzaro, R.S. Langer, K.M. Shakesheff, Polymeric systems for controlled drug release, Chem. Rev. 99 (1999) 3181–3198.
[4] P. Jayanth, L. Vinod, Biodegradable nanoparticles for drug and gene delivery to cells and tissue, Adv. Drug Deliv. Rev. 55 (2003) 329–347.
[5] M. Miyajima, A. Kiko, J. Okada, M. Ikeda, Mechanism of drug release from poly(L-lactic acid) matrix containing acidic or neutral drugs, J. Control. Release 60 (1999) 199–209.
[6] E.R. Kenawy, G.L. Bowlin, G.E. Wnek, Release of tetracycline hydrochloride from electrospun poly(ethylene-co-vinylacetate), poly(lactic acid) and a blend, J. Control. Release 81 (2002) 57–64.
[7] M. Miyajima, A. Kiko, J. Okada, M. Ikeda, Factors influencing the diffusion-controlled release of papaverine from poly(L-lactic acid) matrix, J. Control. Release 56 (1998) 85–94.
[8] X.H. Zong, K. Kim, C. Benjamin, Structure and process relationship of electrospun bioabsorbable nanofiber membranes, Polymer 43 (2002) 4403–4412.
[9] K.J. Zhu, L. Xiangzhou, Y. Shilin, Preparation, characterization, and properties of polylactide (PLA)–poly(ethylene glycol) (PEG) copolymers: a potential drug carrier, J. Appl. Polym. Sci. 39 (1990) 1–9.
[10] Y.H. Bae, K.M. Huh, Y. Kim, K.H. Park, Biodegradable amphiphilic multiblock copolymers and their implications for biomedical applications, J. Control. Release 64 (2000) 3–13.
[11] S. Kakinoki, C. Yasuda, I. Kaetsu, K. Uchida, K. Yukutake, M. Nakayama, S. Fujiie, D. Kuroda, M. Kato, H. Ohyanagi, Preparation of poly-lactic acid microsphere containing the angiogenesis inhibitor TNP-470 with medium-chain triglyceride and the in vitro evaluation of release profiles, Eur. J. Pharm. Biopharm. 55 (2003) 155–160.
[12] S. Freiberg, X.X. Zhu, Polymer microsphere for controlled drug release, Int. J. Pharm. 282 (2004) 1–18.
[13] R.H. Müller, K. Mäder, S. Gohla, Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art, Eur. J. Pharm. Biopharm. 50 (2000) 161–177.
[14] C.B. Packhaeuser, J. Schnieders, C.G. Oster, T. Kissel, In situ forming parenteral drug delivery systems: an overview, Eur. J. Pharm. Biopharm. 58 (2004) 445–455.
[15] M.S. Romero-Cano, B. Vincent, Controlled release of 4-nitroanisole from poly(lactic acid) nanoparticles, J. Control. Release 82 (2002) 127–135.
[16] R. Langer, N.A. Peppas, Advances in biomaterials, drug delivery, and bionanotechnology, Bioeng. Food Nat. Prod. 49 (2003) 2990–3006.
[17] L. Brannon-Peppas, Polymers in controlled drug delivery, Med. Plast. Biomater. 4 (1997) 34–44.
[18] X.L. Xu, L.X. Yang, X.Y. Xu, X. Wang, X.S. Chen, Q.Z. Liang, X.B. Jing, J. Zeng, Ultrafine medicated fibers electrospun from W/O emulsions, J. Control. Release 108 (2005) 33–42.
[19] J. Zeng, X.S. Chen, X.B. Jing, Ultrafine fibers electrospun from biodegradable polymers, J. Appl. Polym. Sci. 89 (2003) 1085–1092.
[20] X.L. Xu, X.L. Zhuang, X.S. Chen, X.R. Wang, L.X. Yang, X.B. Jing, Preparation of core-sheath composite nanofibers by emulsion-electrospinning, Macromol. Rapid Commun. 27 (2006) 1637–1642.
[21] G. Verreck, I. Chun, J. Rosenblatt, J. Peeters, A.V. Dijck, J. Mensch, M. Noppe, M.E. Brewster, Incorporation of drugs in an amorphous state into electrospun nanofibers composed of a water-insoluble, nonbiodegradable polymer, J. Control. Release 92 (2003) 349–360.
[22] T. Higuchi, Mechanism of sustained-action medication, theoretical analysis of rate of release of solid drugs dispersed in solid matrices, Pharm. Sci. 52 (1963) 1145–1149.
[23] R. Falk, T.W. Randolph, J.D. Meyer, R.M. Kelly, M.C. Manning, Controlled release of ionic compounds from poly(L-lactide) microspheres produced by precipitation with a compressed antisolvent, J. Control. Release 44 (1997) 77–85.
[24] X.L. Xu, X.S. Chen, X.Y. Xu, T.C. Lu, X. Wang, L.X. Yang, X.B. Jing, BCNU-loaded PEG–PLLA ultrafine fibers and their in vitro antitumor activity against Glioma C6 cells, J. Control. Release 114 (2006) 307–316.
[25] M. Miyajima, A. Koshika, J. Okada, M. Ikeda, K. Nishimura, Effect of polymer crystallinity on papaverine release from poly(L-lactic acid) matrix, J. Control. Release 49 (1997) 207–215.