Electrospun Hydroxypropyl Methyl Cellulose Phthalate (HPMCP)/Erythromycin Fibers for Targeted Release in Intestine

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ABSTRACT: Ultrafine fiber mats of hydroxypropyl methyl cellulose phthalate (HPMCP) were successfully electrospun and explored as drug delivery vehicles using erythromycin as a model drug. The morphology of the electrospun fiber and the drug release process in the artificial gastric juice and in the artificial intestinal juice were investigated. With the same drug-to-matrix ratio (HPMCP/erythromycin = 9/1), all the fibers were electrospun into a tape-like or ribbon shape and the average fiber diameter (AFD) was increased with the HPMCP concentration. Because of the pH-sensitive property of HPMCP, erythromycin was released from the erythromycin-containing electrospun HPMCP fiber mats by a slowly diffusion process in the artificial gastric juice, while it was released in nearly first-order kinetics in the artificial intestinal juice because of the first-order kinetics dissolution of the HPMCP fibers in the artificial intestinal juice. And the rate of erythromycin released in the artificial intestinal juice was about more than 2.5 times faster than that in the artificial gastric juice. The diameter of the fibers plays an important role on the rate and the total amount of the drug released both in stomach and in intestine, the rate and the total amount of the drug released decreasing with increasing AFD. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 106: 2177–2184, 2007

Key words: electrospinning; fibers; hydroxypropyl methylcellulose phthalate; composites; drug delivery systems

INTRODUCTION

Electrospinning, a straightforward, cheap, and efficient technique for fabricating ultrafine fibers from various polymers, has gained much attention in the last decade. Because of the high surface-to-volume and length-to-diameter ratios, the ultrafine polymer fibers are potentially attractive for a number of applications.[1,2] In the field of biomedical usage, *tissue scaffolds*[3–6] and *drug delivery*[7–13] are the two major areas of the established results and on-going researches on electrospinning biopolymers fibers. Kenawy et al.[7] have explored electrospun mats of poly(lactic acid) (PLA), poly(ethylene-*co*-vinyl acetate) (PEVA) and their blends for drug delivery systems. It was found that the drug-loaded electrospun mats gave relatively smooth release of tetracycline hydrochloride, the model drug. And due to the much lower surface area, the total percentage of drug released from the electrospun fiber mats was higher than that from the as-cast films. Since tetracycline hydrochloride is a water-soluble drug, the *burst release* of drug in first several hours was observed. Similar findings were reported from a number of researches of electrospun polymer/hydrophilic or water-soluble drug composite fiber mats, such as the composite fiber mats of poly(D,L-lactic acid) (PDLA) and poly(L-lactic acid) (PLLA)/Mefoxin® (an antibiotic drug),[8] poly(lactide-*co*-glycolide) (PLGA) and poly(D,L-lactide)–poly(ethylene glycol) (PLA–PEG) block copolymers/DNA,[9] PLLA/doxorubicin hydrochloride,[11] PLGA/Mefoxin®,[12] and poly(vinyl alcohol) (PVA)/sodium salicylate.[13] Contrastively, the burst release of drug was rarely observed in the drug delivery system of electrospun polymer/hydrophobic or poor water-soluble drug composite fiber mats, e.g. PLLA/rifampin (an antibiotic drug),[10] PLLA/paclitaxel (a promising antitumor agent),[11] and PVA/diclofenac sodium, naproxen and indomethacin.[13]

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Although different types (hydrophilic and hydrophobic) of drugs were used in the above electrospun polymer/drug composite fiber mats, only the category of water-insoluble polymer[7–12] was used in those studies except for the electrospun PVA/drug mats,[13] which used PVA, a hydrogel polymer, as the drug carrier. Because of the pH-sensitive swelling property of the hydrogel polymers, they have widely used for pH-controlled drug delivery system,[14–16] e.g. some targeted-release capsules for stomach or intestine disease therapy. However, only a few researches were focussed on the electrospun pH-sensitive polymer fiber as a drug carrier. Therefore, the main objective of the present study is to fabricate pH-sensitive polymer/drug composite fiber mats and to investigate its drug release process in different pH environments.

Hydroxypropyl methylcellulose phthalate (HPMCP), one kind of cellulose derivates, is a pH-sensitive polymer and can be dissolved in the environment of pH greater than or equal to 5.5. This is attributable to the chemical structure of the polymer; the phthalyl substituents are the cause of pH-dependent hydration and coacervate formation.[17] HMPCP has been reported in many researches for use as various forms of drug carriers (such as microcapsules,[17] nanoparticles,[18] tablets[19] and enteric drugs.[20,21] To our knowledge, electrospinning of HPMCP fiber mat for drug delivery system has not yet been reported. Erythromycin is an antibiotic drug used to treat certain infections caused by bacteria. Since erythromycin is less active at low pHs and may cause some side effects, it sometimes comes as enteric-coated tablets[22,23] to protect the contents from the inactivating effects of gastric acidity and to permit efficient absorption of the antibiotic in the small intestine. Thus, it is quite suitable to be selected as a model drug for drug-loaded electrospun HPMCP fiber mats. In the present contribution, the HPMCP/erythromycin composite fiber mats were fabricated by electrospinning; the morphology and fiber diameter of neat (without drug) and drug-loaded electrospun HPMCP fiber mats, and release characteristics of the drug from the mats in the artificial gastric juice and in the artificial intestinal juice were investigated.

EXPERIMENTAL

Materials

Hydroxypropyl methylcellulose phthalate (HPMCP, $\overline{M}_n = 7.4 \times 10^4$) was supplied from Taian Ruitai Cellulose Company (Taian, China). Erythromycin (99.9%) was purchased from Sigma (St. Louis, MO). Pepsin (99.9%) and trypsin (tissue culture grade) were supplied by ACROS (Fair Lawn, NJ) and AMRESCO, respectively, without further purification to prepare buffer solutions. Ethanol and acetone were of analytical purity and used as received without further purification.

Electrospinning and characterization of drug-loaded HPMCP fiber mats

An appropriate amount HPMCP was dissolved in a mixed solvent of ethanol and acetone (v/v = 1 : 1) with various concentrations (10–14 wt %). The model drug, erythromycin was added into the HPMCP solution with the same drug-to-matrix ratio (HPMCP/erythromycin = 9/1, w/w) under constant stirring for 2 h. Prior to electrospinning, the as-prepared solutions were measured for their viscosity, surface tension, and conductivity using a viscometer (REOTEST II, Volkseignener, Germany), a surface tension apparatus (BZY-1, Shanghai Apparatus, Shanghai, China), and a conductivity meter (DDA-11A, Shanghai Dapu Instrument, China), respectively. All the tests were carried out at 25°C and an average value for each solution was calculated from at least three measurements (as shown in Table I). Then, the homogeneous mixed polymer/drug solution was placed in a 10-mL syringe and was fed by a syringe pump (TS2-60, Baoding Changjing Pump, China) at a rate of 5 mL/h. The metallic needle (0.8 mm diameter) was connected to a high voltage supply (BPS-20, Beijing Electrostatic Facility, China), and a piece of aluminum foil was placed 10 cm below the tip of the needle to collect the fibers. All electrospinning were performed at 25°C in air, the applied voltage was fixed at 30 kV. The electrospun fiber mats were dried in a vacuum oven (ZKF-1, Shanghai Apparatus, Shanghai, China) at room temperature for 24 h to remove the residual solvent. The fiber mats were subsequently gold-coated and the morphology was studied using a scanning electron microscope (SEM) (XL-30, Philips, Dutch) operating at 20 kV. The average fiber diameter (AFD) of the electrospun fibers from each HPMCP concentration solution were obtained by using an *UTHSCSA Image Tool Program* to measure from at least five SEM images for each concentration, as shown in Table I. To study the mechanical properties, the electrospun fiber mats were tested on a fiber tensile tester (YG001A, Changzhou Textile Instrument, China) with the extension rate of 2 mm/min. The size of the samples was 20 mm length, 2 mm width, 10 mm distance between two clamps. The average value of the breaking stress and breaking strain of the selected neat and drug-loaded electrospun HPMCP fiber mats was calculated from at least five measurements. On the basis of the data summarized in Table I, the breaking stress of the neat and the erythromycin-containing electrospun HPMCP fiber mats (from the neat and the erythromycin-containing 11 wt % HPMCP solutions,

TABLE I
Some Properties of the Neat and the Erythromycin-containing HMPCP Solutions, and Relative Mechanical Properties and the AFD of the Neat and the Erythromycin-containing Electrospun HPMCP Fiber Mats

	Solution				Fiber		
Sample	HPMCP concentration (wt %)	Viscosity (mPa s)	Surface tension (mN/m)	Conductivity (μS/cm)	Breaking stress (MPa)	Breaking strain (%)	AFD (nm)
Neat (without drug)	10	66.0	35.3	126.4	—	—	—
	11	83.1	37.4	134.5	44.8	10.3	673
	12	—[c]	37.7	142.6	—	—	—
	13	—[c]	38.3	160.3	—	—	—
	14	—[c]	39.6	187.1	—	—	—
With drug	10[a]	67.5	35.0	197.2	—	—	246[b]
	11[a]	85.1	35.9	217.8	38.4	15.7	470[b]
	12[a]	—[c]	36.2	231.6	—	—	682[b]
	13[a]	—[c]	37.5	243.9	—	—	840[b]
	14[a]	—[c]	38.7	260.5	—	—	1011[b]

[a] For all the tape-like erythromycin-containing electrospun HPMCP fibers (HPMCP/erythromycin = 9/1), the HPMCP concentration were calculated from the HPMCP/(ethanol/acetone) solution, the weight of drug was excluded.
[b] AFD of the erythromycin-containing electrospun HPMCP fibers were measured from the width of the fibers.
[c] Insufficient materials were available for viscosity experiment.

respectively) was calculated as 44.8 and 38.4 MPa, respectively, while the breaking strain of the neat and the erythromycin-containing fiber mats was 10.3 and 15.7%, respectively. It indicated that the electrospun HPMCP fiber mats have enough strength to be used as a drug carrier.

Preparation of buffer solutions and determination of the drug calibration curves

The artificial gastric juice (pH 1.2) was made from diluted hydrochloric acid aqueous solution, containing 0.01 mg/mL pepsin. The artificial intestinal juice (pH 6.8) was made from the 0.034 mg/mL potassium dihydrogen phosphate aqueous solution and diluted sodium hydroxide aqueous solution, which contains 0.01 mg/mL trypsin. The calibration curves of erythromycin released in the buffer solutions were measured by a colorimetric method as the following procedure. An appropriate amount of erythromycin was placed in vials filled with 20 mL of the buffer solution each to prepare a series of solutions with various erythromycin concentrations. Each vial was incubated at 37°C in a thermostated shaker for half hour. Subsequently, 6 mL each erythromycin solution was transferred to a 60 mL separatory funnel, and 8 mL bromocresol purple and 10 mL of $CHCl_3$ were added into the funnel to extract the drug. Then, 6 mL extraction was measured by a UV–vis spectrophotometer (UV-2550, Shimadzu, Japan). A linear Beer–Lambert plot of absorbance at 408 nm versus concentration was measured and used as the calibration curve. According to this method, the calibration curves of erythromycin released in the two buffer solutions, the artificial gastric juice and the artificial intestinal juice, were determined respectively, [as shown in Fig. 1(a,b)].

Erythromycin Release profile and HPMCP dissolving profile evaluations

The erythromycin-containing electrospun HPMCP fiber mats were cut into 1 cm diameter round pieces (about 100 mg weight), and each sample was placed in a vial filled with 20 mL of the buffer solutions, the artificial gastric juice and the artificial intestinal juice, respectively. The vial was incubated at 37°C in a thermostated shaker. At appropriate intervals, the mat was transferred to another 20 mL of fresh buffer solution and the released erythromycin in the original buffer solution was measured by the colorimetric method and was monitored by the UV–vis spectrophotometer at the wavelength of 408 nm. The UV absorbance of erythromycin detected was converted to its concentration according to the predetermined calibration curve of erythromycin in the same buffer. Then the accumulative weight and relative percentage of the released erythromycin were calculated as a function of incubation time.

Since HPMCP could dissolve in the environment of pH greater than or equal to 5.5, the electrospun HPMCP fiber mats were found to be gradually dissolved in the artificial intestinal juice. To evaluate the effect of the dissolution of the electrospun HPMCP fiber mats on the release of erythromycin in the artificial intestinal juice, a linear Beer–Lambert plot of absorbance at 281 nm (characteristic wavelength of HPMCP) versus concentration was

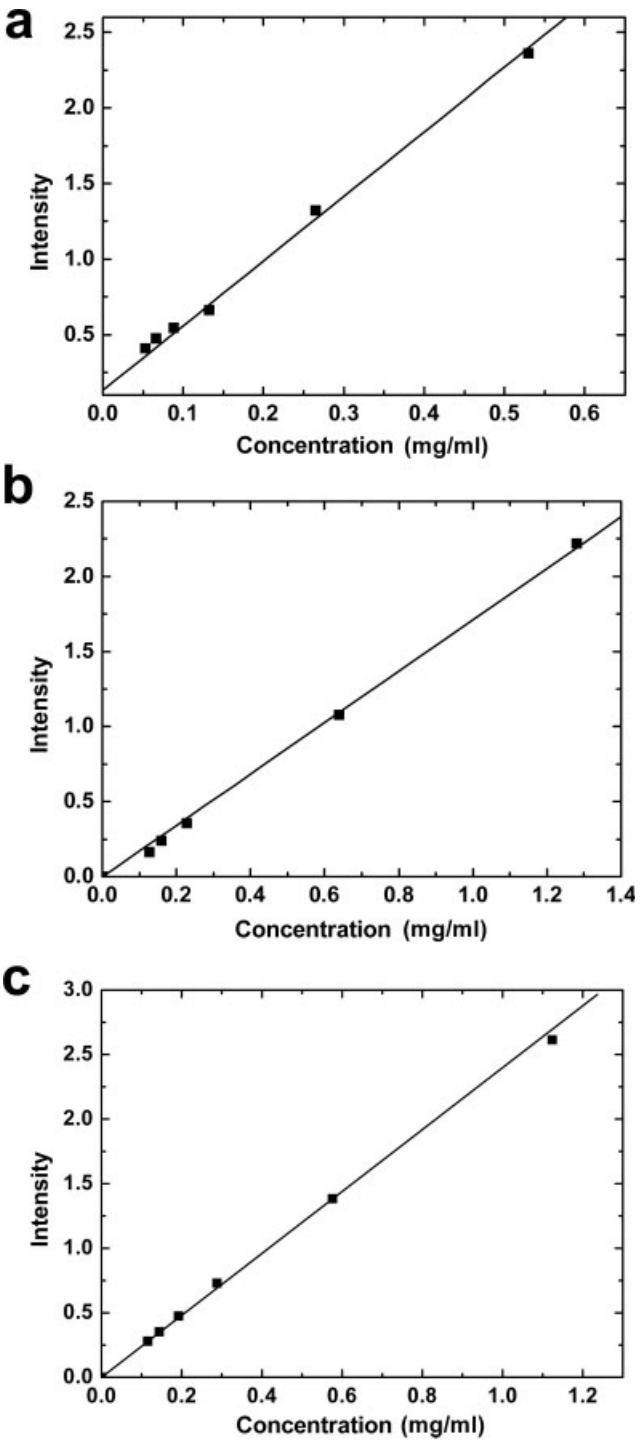

Figure 1 The calibration curves of erythromycin released in the artificial gastric juice (a) and in the artificial intestinal juice (b), and the calibration curve of HPMCP dissolved in the artificial intestinal juice (c).

measured and used as the calibration curve of HPMCP dissolved in the artificial intestinal juice, as shown in Figure 1(c). Therefore, similar to the evaluation of erythromycin release, the UV absorbance (281 nm) of HPMCP detected was converted to its concentration according to the predetermined calibration curve of HPMCP dissolved in the artificial intestinal juice. Then the accumulative weight and relative percentage of the dissolved HPMCP were calculated as a function of incubation time. All the measurements of the release of erythromycin and the dissolution of HPMCP were repeated three times.

It needs to mention that the actual drug-to-matrix ratio (HPMCP/erythromycin) in the fibers was measured, and it was the same as the initial drug-to-matrix ratio in the electrospinning solutions.

RESULTS AND DISCUSSION

Morphology of electrospun drug-load HPMCP fiber mats

Electrospinning is a very simple technique but involves a quite complicated process. The diameter and the morphology of the electrospun fibers are mainly influenced by two factors: the process parameters[24–26] such as applied voltage, flow rate, distance between needle and collector, etc., and the solution properties[25–28] such as viscosity, surface tension, etc. that can also dominate the diameter and the morphology of the fiber. It was found that, due to the fact that the solution viscosity is proportional to the polymer concentration, the higher the polymer concentration, the larger the resulting nanofiber diameter.[25,27]

In the present contribution, since the process parameters were fixed, the diameter and the morphology of the electrospun fibers were dominated by the solution concentration. The results showed that for the HPMCP solution, the concentration range suitable for electrospinning was between 7 and 16 wt %. When the HPMCP concentration was lower than 8 wt %, beads were formed. Then, with the increase of the HPMCP concentration, beads were not seen and the column fibers (HPMCP concentration from 10 to 14 wt %) were produced, as shown in Figure 2(a).

When erythromycin was added in the HPMCP solution, although the concentration range that is suitable for electrospinning has not noticeably changed, the morphological appearance of the fibers changed dramatically, from column to a tape-like or ribbon shape; while the surface of the fibers still looked smoothly, as shown in Figure 2(b) and Figure 3(a). It revealed that no presence of drug aggregates on the surface of the erythromycin-containing electrospun HPMCP fibers and erythromycin was embodied or encapsulated inside the fibers. Similar observation has also been reported in other literature.[10,13] Furthermore, the diameter range of the fibers was sharply decreased and the diameter distribution was also significantly narrowed. For the 11 wt % HPMCP solution, for instance, the diameter range of the

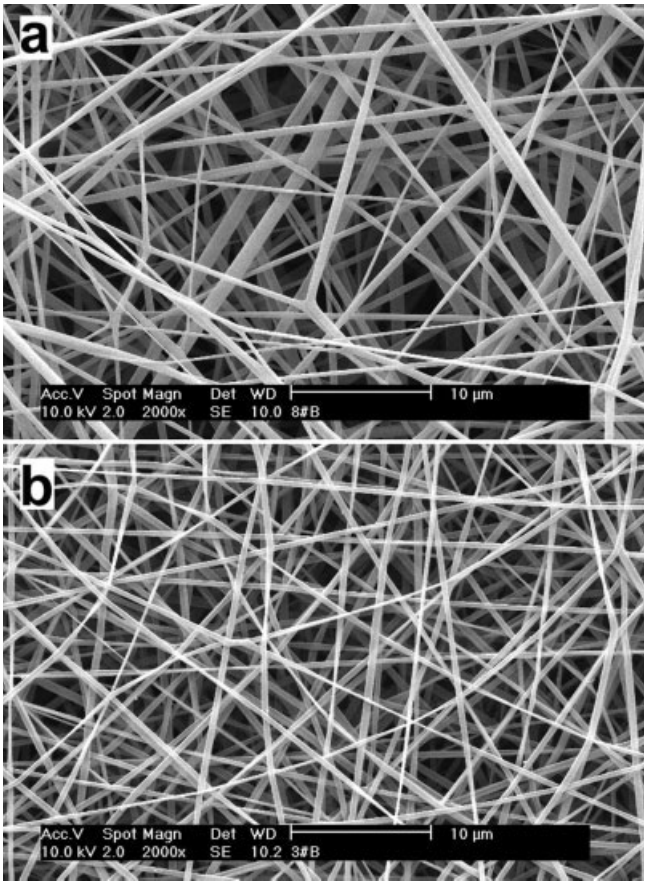

Figure 2 SEM micrographs of the fiber mats electrospun from the neat (a) and the erythromycin-containing (b) 11 wt % HPMCP solutions.

electrospun fibers from 0.3–1.3 μm [Fig. 2(a)] decreased to 0.3–0.6 μm [Fig. 2(b)]. The AFD (width) of the tape-like erythromycin-containing electrospun HPMCP fibers also increased with the HPMCP concentration (10–14 wt %), as summarized in Table I. It is believed that the addition of the drug varied the components of the solution and changed the solution properties.[10,11,13] Based on the data summarized in Table I, it obviously showed that for the erythromycin-containing HPMCP solution, when the viscosity and the surface tension increased slightly, the conductivity increased significantly. The considerable increase in the conductivity resulted in a marked increase of the *Coulombic repulsion*,[13] which caused the charged jet stretched or extended more, and formed thinner fiber.

Drug release process

As described in the Experimental section, the erythromycin-containing electrospun HPMCP fiber mats were put into the artificial gastric juice and into the artificial intestinal juice to study the drug release process. As observed under SEM, the morphology of the fiber mats displays no obvious change and still showed the smooth surface after their placement in artificial gastric juice for 12 h [see Fig. 3(b)], which is due to the fact that HPMCP cannot dissolve in the solution at low pH value of gastric juice. Since the dissolution of HPMCP, the drug was released by diffusion[13,29] from the surface of the erythromycin-containing electrospun HPMCP fibers to the artificial

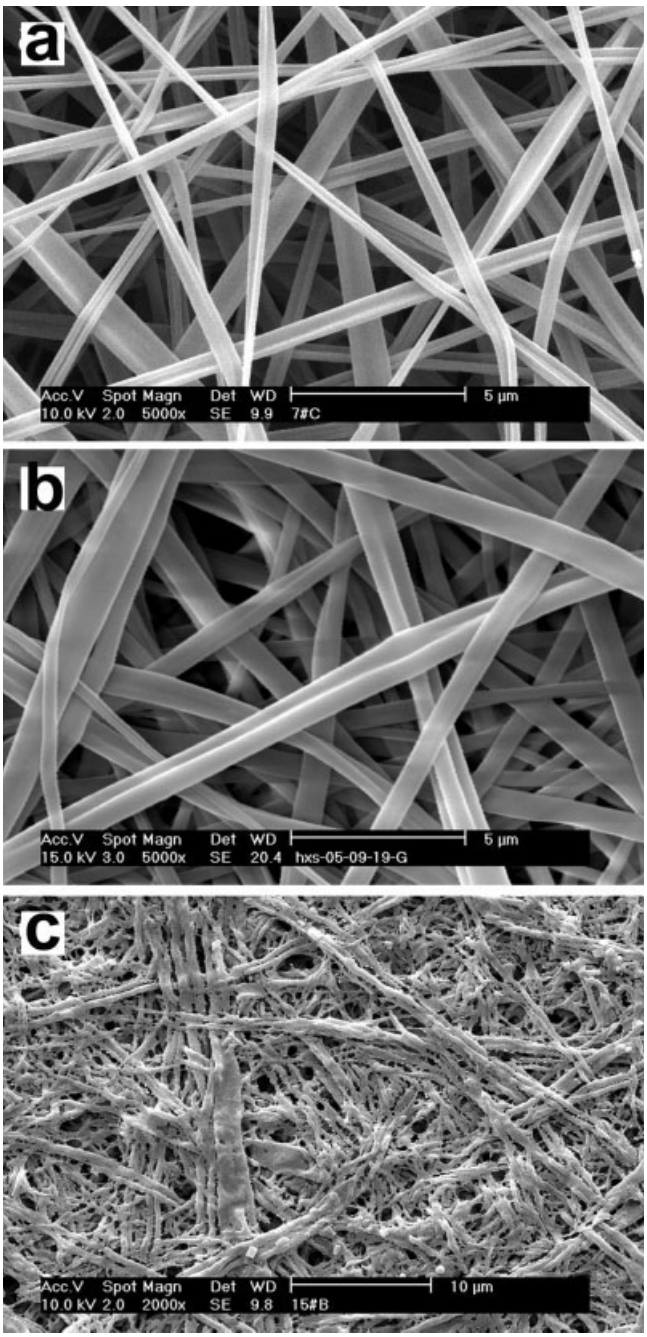

Figure 3 SEM micrographs of the erythromycin-containing electrospun HPMCP fiber mats from 13 wt % HPMCP solution (a), after put in the artificial gastric juice for 12 h (b), and after put in the artificial intestinal juice for about 4 h (c).

gastric juice. Based on the release profile of erythromycin [Fig. 4(a)], the total amount of erythromycin released from the electrospun fiber mats in the artificial gastric juice (at 12 h) was about 42.8, 33.9, and 25.0% for the AFD was about 246, 682, and 1011 nm, respectively, while the rate of erythromycin released in the artificial gastric juice (at 4 h) was about 4.5, 3.6, and 2.7%/h for the AFD was about 246, 682, and 1011 nm, respectively, (Fig. 5). It is obvious that the total amount and the rate of erythromycin released from the mats both vary inversely with the AFD. Since the area-to-volume ratio of fiber is dependent on the fiber diameter,[7] the thinner the diameter fiber, the larger the total surface area for the fiber mats with the same mass. Thus, naturally, more amount drugs were diffused from the fiber mats with small AFD (the same drug-to-matrix ratio, HPMCP/erythromycin = 9/1) in the same release time and that also resulted in a fast release rate [Fig. 4(a)]. Furthermore, the burst release was not seen in the release profile of erythromycin-containing electrospun HPMCP fiber mats in the artificial gastric juice, which should be attributed to the poor water-soluble property of erythromycin and the pH-sensitive property of HPMCP. It is a common phenomenon that has been found in other researches of electrospun water-insoluble polymer/hydrophobic drug fibers.[10,11,13]

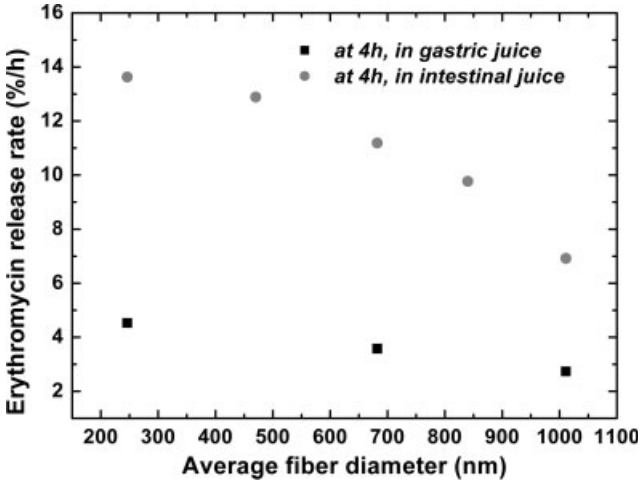

Figure 5 Rates of erythromycin released from the erythromycin-containing electrospun HPMCP fiber mats with the AFD in the artificial gastric juice and in the artificial intestinal juice for 4 h.

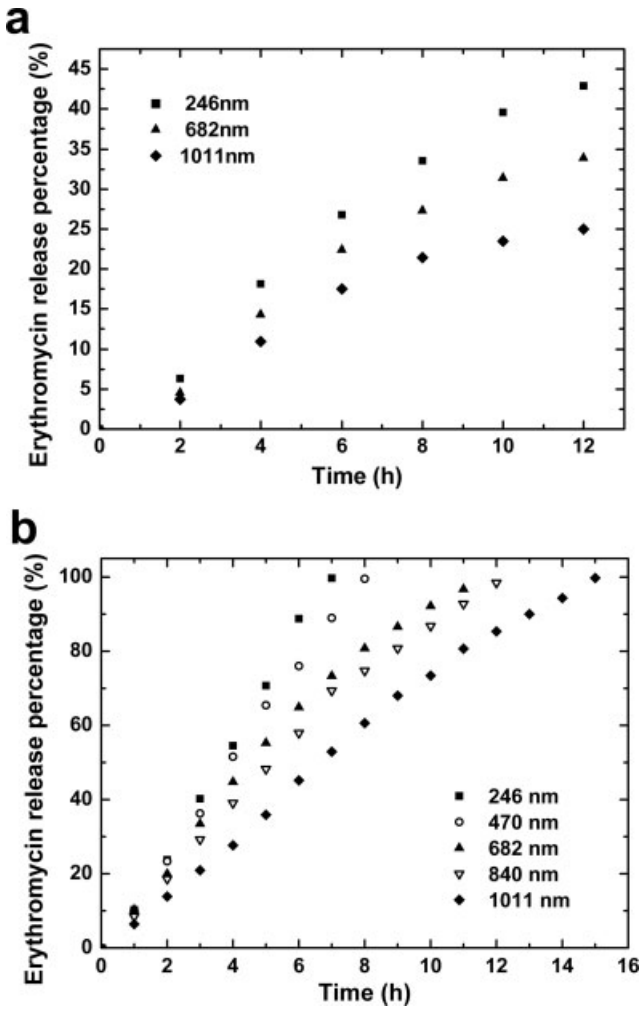

Figure 4 Profiles of erythromycin released from the erythromycin-containing electrospun HPMCP fiber mats with different average fiber diameter (AFD) (246 nm (■), 470 nm (○), 682 nm (▲), 840 nm (▽), and 1011 nm (◆) in the artificial gastric juice (a) and in the artificial intestinal juice (b), respectively. Those fiber mats were prepared from the erythromycin-containing HPMCP solutions within the HPMCP concentration range from 10 wt % to 14 wt % (shown in Table I).

However, when the erythromycin-containing electrospun HPMCP fiber mats were put into the artificial intestinal juice (pH = 6.8, and above the critical pH value 5.5 that could dissolve HPMCP), it could be observed obviously by the naked eyes that the fiber mats were gradually dissolved in the artificial intestinal juice over a long enough time. Under the observation of SEM [as shown in Fig. 3(c)], the erythromycin-containing electrospun HPMCP fibers were broken up and cracked after they were placed in the artificial intestinal juice for a couple of hours. According to the results shown in Figure 4(b), in the artificial intestinal juice, erythromycin was released proportional with the incubation time (nearly first-order kinetic process) from the erythromycin-containing electrospun HPMCP fiber mats, no burst release was observed. Within 12 h, erythromycin was released totally from all the electrospun fiber mats samples expect for the one with the largest AFD (1011 nm). For the thinnest erythromycin-containing electrospun HPMCP fiber mats (246 nm), it just took the shortest time, about 7 h, for all of erythromycin from the mats to be released.

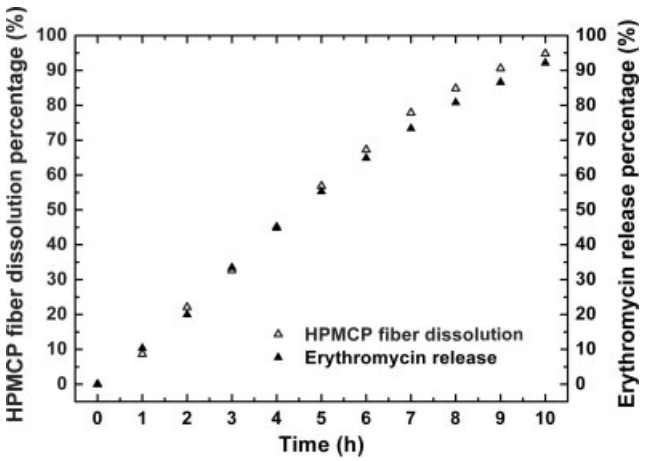

Figure 6 Profiles of erythromycin released (a) and HMPCP dissolved (b) from the erythromycin-containing electrospun HPMCP fiber mats (12 wt %) in the artificial intestinal juice.

Moreover, the rate of erythromycin released in the artificial intestinal juice (in 4 h) was found to be more rapid (more than 2.5 times) than that measured in the artificial gastric juice; it was about 13.6, 12.9, 11.2, 9.8, and 6.9%/h for the AFD about 246, 470, 682, 840, and 1011 nm, respectively, (Fig. 5). Similar to the release process of erythromycin in the artificial gastric juice, both the total amount and the rate of erythromycin released in the artificial intestinal juice were found to vary inversely with the AFD.

From Figure 6, it can be observed that the release rate of erythromycin and the dissolution rate of the electrospun HPMCP fiber mat in artificial intestinal juice were matched very well. This indicated that the release of erythromycin in the artificial intestinal juice is dominated by the dissolution of HPMCP. It also confirmed that erythromycin was embodied and dispersed very homogeneously in the electrospun fibers, and erythromycin released in nearly first-order kinetics in the artificial intestinal juice due to the first-order kinetics dissolution of the HPMCP fibers in the artificial intestinal juice.

Generally, in a regular process of gastric digestion, the drug dose would stay in stomach for about 2–4 h.[30] If the erythromycin-containing electrospun HPMCP fiber mats was used as a drug dose and taken by mouth, for example the fiber with AFD about 682 nm, based on the erythromycin release profile (see Fig. 4), the total amount of erythromycin released from the fiber mats in the artificial gastric juice would be just about 4.6% in the first 2 h, i.e. about 95% drug would be reserved in the fiber mats and would be delivered to intestine. If the electrospun fiber mats stayed in stomach for 4 h, then about 84% drug would be delivered to intestine. Since both the total amount and the rate of erythromycin released in the two buffer solutions were found to vary inversely with the AFD (see Fig. 4), the drug-loaded electrospun HPMCP fiber mats with the appropriate AFD should be prepared according to the practical need for being used as a target-released drug dose in intestine (enteric-coated dose).

CONCLUSIONS

Erythromycin-containing electrospun HPMCP fiber mats with various diameters were successfully prepared by the electrospinning technique. With the addition of the drug, the morphological appearance of the fibers changed dramatically from column to a tape-like shape. No presence of drug was found aggregated on the surface of the erythromycin-containing electrospun fiber mats, and that indicated the drug was embodied or encapsulated inside the fibers. Because of the pH-sensitive property of HPMCP, erythromycin was released from the erythromycin-containing electrospun HPMCP fiber mats by a slowly diffusion process in the artificial gastric juice, while it was released in nearly first-order kinetics in the artificial intestinal juice because of the first-order kinetics dissolution of the HPMCP fibers in the artificial intestinal juice. The diameter of the fibers plays an important role on the rate and the total amount of the drug released both in stomach and in intestine, the rate and the total amount of the drug released decreasing with increasing AFD. However, the rate of erythromycin released in the artificial intestinal juice was found about more than 2.5 times faster than that in the artificial gastric juice. Based on the results, mainly amount of the drug could be reserved in the electrospun fiber mats after they in stomach for a couple of hours and could be delivered to intestine. It indicated that such drug-loaded electrospun HPMCP fiber mats are expected to have biomedical applications.

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