Preparation and Properties of Poly(propylene carbonate maleate) Microcapsules for Controlled Release of Pazufloxacin Mesilate

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ABSTRACT: A novel biodegradable aliphatic polycarbonate, poly(propylene carbonate maleate) (PPCMA) was synthesized by terpolymerization of carbon dioxide, propylene oxide, and maleic anhydride (MA), using a polymer supported bimetallic complex as catalyst. The utility of PPCMA to encapsulate and control the release of drug pazufloxacin mesilate (PZFX), via microcapsules, was investigated. PPCMA microcapsules containing PZFX were elaborated by solvent evaporation method based on the formation of double W/O/W emulsion. The manufacturing parameters such as the volume ratio of V(PPCMA) : V(PZFX), the concentration of stabilizer gelatin in outer aqueous phase played major roles on microcapsule characters, and were altered to optimize the process parameters. The PPCMA-PZFX microcapsules were obtained with smooth and spherical surface under optimum condition, the mean diameter of microcapsules was $\sim$ 2 μm, and the drug loading and drug encapsulation efficiency of the microcapsules were 22.9 $\pm$ 1.05% and 82.1 $\pm$ 2.03%, respectively. PZFX released from PPCMA microcapsules was found to reach 89.8 $\pm$ 2.89% after 36d in a pH 7.4 phosphate-buffered solution, and the release profile obeyed the Higuchi equation. The results suggest that the new polymer PPCMA provides an alternative to degradable matrix polymers for long-term sustained releasing drug delivery systems. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 3248–3254, 2011

Key words: drug delivery systems; functionalization of polymers; microencapsulation; polycarbonates

INTRODUCTION

Controlled drug delivery systems offer numerous advantages compared to conventional dosage forms including improved efficacy, reduced toxicity, and improved patient compliance.[1] Biodegradable polymers have found increasing applications in the pharmaceutical industry as matrices for drug delivery systems.[2–6] Synthetic degradable polymers such as polyanhydrides,[7] homopolymers or copolymers of glycolide, lactide, and ε-caprolactone[8–15] have become increasingly important in the development of implantable biomaterials and drug delivery devices in the last few years. However, the applications of polylactide, polylactide-*co*-gly-colide, and poly (ε-caprolactone) may be limited for their hydrophobic character. The difference in physical–chemical properties between hydrophilic drug and hydrophobic polymer matrix leads to a lower drug encapsulation efficiency within microcapsules, and a higher burst effect of drug release from microcapsules.[16] Aliphatic polycarbonate represents one family of biodegradable materials used for biomedical applications such as drug carriers and implant materials because of their good biocompatibility, low toxicity, and biodegradability.[17–19] Importantly, they are also susceptible to enzymatic biodegradation.[20,21] Aliphatic polycarbonates can be modified by functional groups, such as ester,[22–24] carboxyl[25] to improve their thermal properties and degradability. In recent years, aliphatic polycarbonates have been explored in the search and design of new polyester-related structures for medical applications.[26] In recent years, functionalized polycarbonates has been reported as nonviral biodegradable vector for gene delivery system.[27,28] A degradable polymer, poly(propylene carbonate maleate) (PPCMA) was synthesized by introduction a third monomer, maleic anhydride (MA) into the backbone of poly(propylene carbonate) (PPC) in our laboratory, which resulted in an enhancement of the hydrophilicity and biodegradability of the

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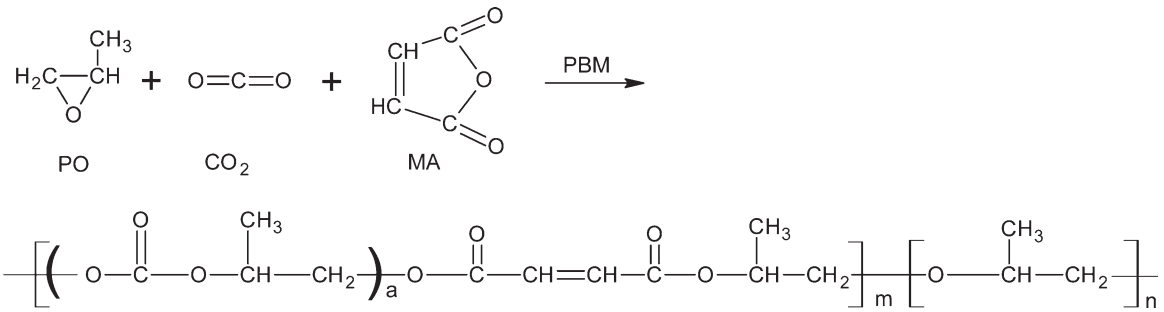


Scheme 1 Synthesis and structure of PPCMA.

polycarbonates,[29] synthesis and structure of PPCMA is shown in Scheme 1. PPCMA was used as matrix polymer for a hydrophobic drug finasteride delivery system; the finasteride-loaded PPCMA microcapsules were elaborated by a simple O/W emulsion-solvent evaporation method, and had a long release period of about 5 weeks.[30] Pazufloxacian mesilate (PZFX) is a novel injectable quinolone antibacterial agent, which has excellent therapeutic effects against a broad spectrum of bacterial infection.[31] The structure of PZFX is shown in Scheme 2. The use of extended release products offers potential advantages like sustained blood levels, attenuation of adverse effects and improved patient compliance. Hence, to develop its formulations in controlled release form is important in pharmaceutical study.

In this work, PPCMA was developed as drug carriers for controlled release system of a hydrophilic drug. PPCMA was used as a drug carrier material, the PZFX was used as a low-molecular weight and water-soluble drug model, and the drug-loaded microcapsules were prepared by the double W/O/W emulsion method. *In vitro* drug release profile was performed in pH 7.4 phosphate-buffered solution and pH 1.2 HCl media.

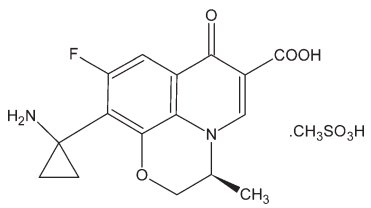
EXPERIMENTAL

Materials

Propylene oxide, PO, (Shanghai Chemical Reagents, A.R. grade) and toluene (Hengyang Organic Chemical Reagents Plant, A.R. grade) were dehydrated by 0.4 nm molecular sieves prior to use. MA (A.R. grade) was purchased from Shantou Xilong Chemical Factory, Guangdong. CO_2 and N_2 (purity more than 99.5%) were purchased from Hunan Special Gas Factory (China). The copolymer PPCMA ([η] = 0.772, M_w = 67,400, M_n = 32,100, M_w/M_n = 2.10) was prepared in our laboratory (Central South University, China), and its synthesis and characterization were detailed in our earlier work.[29] Briefly, 1.0 g PBM catalyst, 70 mL toluene, 0.5 mol propylene oxide, and 0.3 mol maleic anhydride were added into an FYX-0.3 300 mL stainless steel autoclave equipped with an electromagnetic stirrer in the absence of oxygen. The autoclave was then pressurized to ± 4 MPa with a CO_2 cylinder. The reaction mixture was stirred magnetically at 60°C for 24 h. When the reaction was finished, the resulting viscous mixture was removed, washed, and dried. Finally, it was purified. PPCMA was random copolymer with terminal hydroxyl and carboxyl groups. The composition of PPCMA was calculated by integrating areas of ^{1}H-NMR spectra, and the molar fractions of CO_2, PO, and MA were 19.39, 52.79, and 27.82, respectively. Gelatin was produced by the Foshan Chemical Factory and was medical-grade. Pauzufloxacin mesilate of purity 99.8% was purchased from Shangdong Zhongke Taidou Chemical. All other reagents and solvents were of analytical grade and used without further purification.

Preparation of PPCMA-PZFX microcapsules

PPCMA was used as drug carriers. The W/O/W emulsion method was applied to the fabrication of PPCMA-PZFX microcapsules. The amount of 10 wt % aqueous pazufloxacin Mesilate (PZFX) solution was dispersed in 20 mL of 1 wt % polymer dichloromethane (DCM) solution. Span-80 (0.6 g) used as an emulsifier, was also put into the solution. Then the mixture was emulsified with a JHS-1/90 mechanical stirrer (Hangzhou Instrument Electrical Factory, China) at 500 rpm to form stable water-in-oil (W/O) initial emulsion. The initial emulsion was added to 200 mL of external water phase containing stabilizers gelatin in drops with mechanical stirring at 400 rpm. The W/O/W ultimate emulsion was obtained, and the solvent was simultaneously evaporated at



Scheme 2 Structure of pauzufloxacin mesilate.

TABLE I
Properties of PPCMA-PZFX Microcapsules Prepared by Various Volume Ratio of V(PPCMA) : V(PZFX) (±S.D., $n = 3$)

Formulation code	V(PPCMA) : V(PZFX)	Drug loading (%)	Encapsulation efficiency (%)	Particle size D (μm)
F1	20 : 1	13.5 (±0.46)	85.6 (±2.19)	1.85 (±0.07)
F2	10 : 1	19.6 (±0.57)	83.7 (±1.96)	1.93 (±0.09)
F3	8 : 1	22.9 (±1.05)	82.1 (±2.03)	2.09 (±0.11)
F4	5 : 1	25.6 (±1.13)	46.9 (±1.28)	2.27 (±0.10)

room temperature. The emulsion was mechanically stirred continuously for 5 h until DCM was removed completely. The microcapsules were collected by centrifugation, washed in distilled water, frozen, and lyophilized with a FD-1CE freeze drier (Beijing Dtianyou Technology Development, China).

Characterization of PPCMA-PZFX microcapsules

The morphology of microcapsule was observed with a XSP-2C light microscope (China) and a JSM-6360 scanning electron microscope, the structure of microcapsule was observed with a Tecnai G2 20 ST transmission electron microscope. The particle size and distribution of the microcapsules were measured with a laser diffraction particle size analyzer (Malven, Mastersizer 2000, British). To determine the drug-loading content and the encapsulation efficiency, a weighted quantity of microcapsules were dissolved in DCM and PZFX was extracted into aqueous PBS(0.1*M*, pH 7.4). PZFX concentrations were determined at 249 nm using UV spectroscopy (UV-3802, Shanghai Younike Instrument, China), compared with a standard curve of data obtained by assaying known concentrations of PZFX solutions. The drug loading and encapsulation efficiency were determined for all batches using eqs. (1) and (2), respectively.

$$\text{Drug loading} = (\text{weight of PZFX in microcapsules} / \text{microcapsules sample weight}) \times 100\% \quad (1)$$

$$\text{Encapsulation efficiency} = (\text{actual weight of PZFX in sample} / \text{theoretical weight of PZFX}) \times 100\% \quad (2)$$

Wide-angle X-ray diffraction analysis

To clarify the structure of the microcapsules, the wide-angle X-ray diffraction (WXRD) measures of PZFX and drug-loaded microcapsules were performed at room temperature using a Rigaku D/max 2550 VB^+ 18 Kw X-ray diffractometer.

In vitro release study

In vitro release study was conducted in the way reported by Xiong et al.,[32] briefly: dialysis bags (dialysis tubing, molecular weight cut off 12,000–14,000 Da) containing suspension of a weighted quantity of microcapsules in 5 mL of HCl (pH 1.2) or PBS (pH 7.4) were placed in 195 mL of the same release media. The whole solution was then placed in a shaking water bath at 37°C for the drug release study, intervals released PZFX solution (5 mL) outside the dialysis bags was withdraw and measured at wavelength of 249 nm by UV spectroscopy to determine the concentration of PZFX. Fresh solution (5 mL) was added to replenish the sample that was removed to maintain a constant volume. Each experiment was repeated at least three times.

RESULTS AND DISCUSSION

Influence of V(PPCMA) : V(PZFX) ratio on the properties of microcapsules

The PZFX solution was the inner aqueous phase, the polymer solution of DCM was the oily phase, and gelatin solution was the outer aqueous phase. Gelatin kept the droplets stable and prevented aggregation during solidification. The drug loading, encapsulation efficiency, and mean diameter of drug-loaded PPCMA microcapsules elaborated by emulsion-solvent evaporation methods with different volume ratio of V(PPCMA) : V(PZFX) were summarized in Table I. The results indicated that as the ratio of V(PPCMA) : V(PZFX) decreased, the drug loading increased, the particle size increased slightly, and entrapment efficiency decreased. When the V(PPCMA) : V(PZFX) ratios was 8 : 1, the microcapsule F3 showed optimum drug-loading content, entrapment efficiency among the four formulations.

Influence of concentration of gelatin on the properties of microcapsules

The effect of concentration of stabilizer gelatin on the properties of microcapsules was studied. Properties of PPCMA-PZFX microcapsules prepared by various concentration of gelatin in external aqueous phase were shown in Table II. It revealed that the particle size increased with increasing concentration of gelatin, encapsulation efficiency increased as ω(gelatin) changed from 0.05 to 0.2%, and it had maximum value at 0.2%, then the encapsulation efficiency dropped when ω(gelatin) increased from 0.5

TABLE II
Properties of PPCMA-PZFX Microcapsules Prepared by Various Concentration of Gelatin in External Aqueous Phase (±S.D., $n = 3$)

Formulation code	ω (gelatin) (%)	Drug loading (%)	Encapsulation efficiency (%)	Particle sizeD (μm)
F5	0.05	23.1 (±1.04)	75.4 (±1.86)	1.93 (±0.08)
F6	0.1	24.2 (±1.15)	80.6 (±2.14)	2.01 (±0.10)
F3	0.2	22.9 (±1.05)	82.1 (±2.03)	2.09 (±0.11)
F7	0.5	20.6 (±0.94)	79.2 (±1.98)	2.13 (±0.07)
F8	0.8	15.1 (±0.63)	72.1 (±1.83)	2.21 (±0.11)

to 0.8%. Microcapsule F3 prepared by 0.2% ω(gelatin) achieved the highest entrapment efficiency (82.1 ± 2.03%), appropriate drug-loading (22.9 ± 1.05%), and particle size (2.09 ± 0.11 μm) among the five formulations, the manufacturing parameters of microcapsule F3 was the optimum preparative condition.

Size and morphological characterization of microcapsules

The morphology of microcapsules was observed with light microscopy and scanning electron microscopy. The inner structure of microcapsules was observed with transmission electron microscope. Figure 1(a) shows light micrograph of PPCMA-PZFX microcapsule F3 (magnification = 1000 times), The optical microscopy (OM) and transmission electron microscope (TEM) photo [Fig. 1(b)] revealed that PPCMA-PZFX microcapsule was spherical in shape, the outer shell of microcapsules was polymer PPCMA, and in the center of the microcapsules was the encapsulated PZFX. Further studies using scanning electron microscope provided a better understanding of the morphological characteristics of the microcapsules. SEM image of PZFX-loaded PPCMA microcapsule F2 and F3 were shown in Figure 1(c) and (d), respectively. The particles were smooth and spherical. Smooth surface reveals complete removal of dichloromethane from microcapsules. The microcapsule diameter was about 2 μm. The morphology of the studied samples microcapsules was similar to each other. The particle size and distribution of the microcapsules were measured with a laser diffraction particle size analyzer, as shown in Figure 2, the mean diameter of microcapsule F3 was 2.09 μm, the microcapsules particle size range was 0.6–25 μm, and diameter span was 2.94.

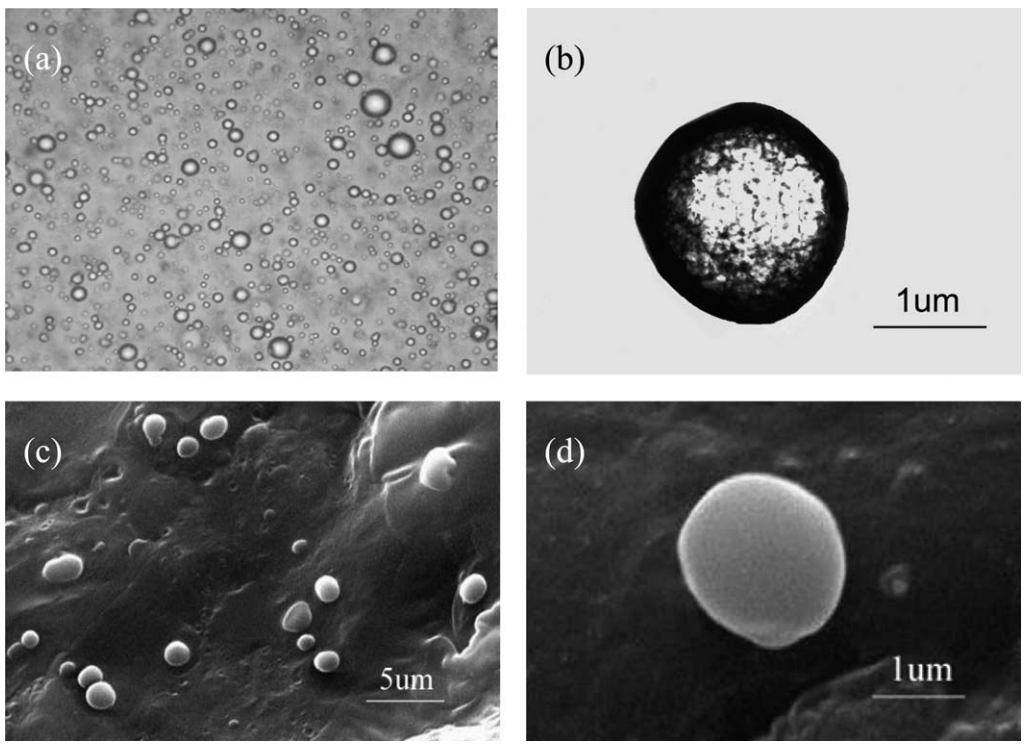


Figure 1 (a) Light micrograph (magnification = 1000 times), (b) TEM image of PPCMA-PZFX microcapsule F3, (c) SEM image of PPCMA-PZFX microcapsule F2, and (d) SEM image of PPCMA-PZFX microcapsule F3.

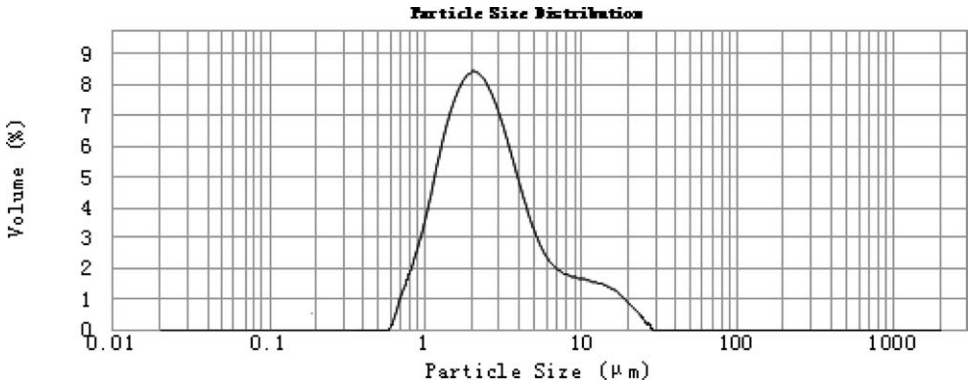


Figure 2 Particle size distribution of PPCMA-PZFX microcapsule F3.

WXRD analysis

The WXRD spectra recorded for the pure PZFX, and PPCMA-PZFX microcapsule F3 were presented in Figure 3. WXRD analysis was useful to investigate crystallinity of the drug in polymer microcapsules. Figure 3(a) was the WXRD spectra of pure PZFX indicated that PZFX was crystalline drug. Figure 3(b) was the WXRD spectra of PPCMA-PZFX microcapsule F3, PZFX was amorphous in the microcapsules, which gave the observed lack of diffraction pattern in Figure 3(b). The WXRD spectra of other samples microcapsules were similar to those of microcapsule F3. In view of the X-ray results mentioned above, it was believed that PZFX had been encapsulated inside the microcapsules, this indicated that drug was dispersed at the molecular level in the polymer matrix and hence no crystals were found in the drug-loaded matrices.[33]

Release behavior of drug

The release of a drug is a rather complicated process, which is affected by many factors, such as the properties of polymer matrix, the size and structure of the delivery system, drug loading content, release media, and so on. Many manufacturing parameters determine the drug release behavior from microcapsules. The effect of V(PPCMA) : V(PZFX) ratio on release profiles of microcapsules was studied. The manufacturing parameters and properties of microcapsule F1, F2, F3, and F4 were shown in Table I. *In vitro* release studies of the four kinds of microcapsules were investigated. Figure 4 showed the release profiles of PZFX from the microcapsules prepared with various V(PPCMA) : V(PZFX) ratio in pH 7.4 PBS. After 36d, microcapsule F4 with the lowest ratio of V(PPCMA) : V(PZFX) (5 : 1) showed 95.6 ± 4.01% drug release which was higher than 89.8 ± 2.89% release of PZFX from microcapsule F3, 84.3 ± 3.64% release of PZFX from microcapsule F2, and 59.1 ± 3.05% release of PZFX from microcapsule F1. The release rate of PZFX increased with decreasing ratios of V(PPCMA) : V(PZFX). In view of the results mentioned above and the results of drug loading in Table I, interestingly, it has been found that as the amount of PZFX incorporated increased, drug released from microcapsules was also increased.

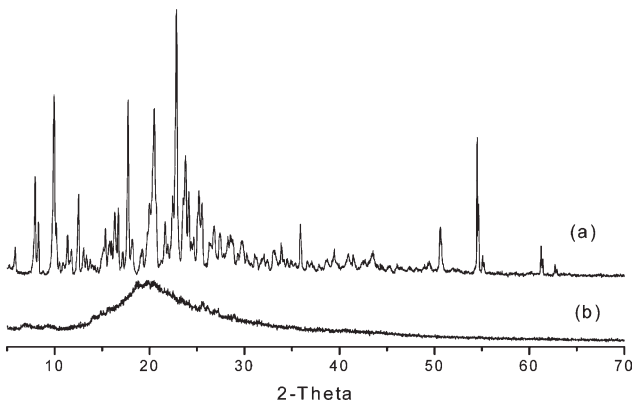


Figure 3 The wide-angle X-ray diffraction spectra of (a) pure PZFX and (b) PPCMA-PZFX microcapsule F3.

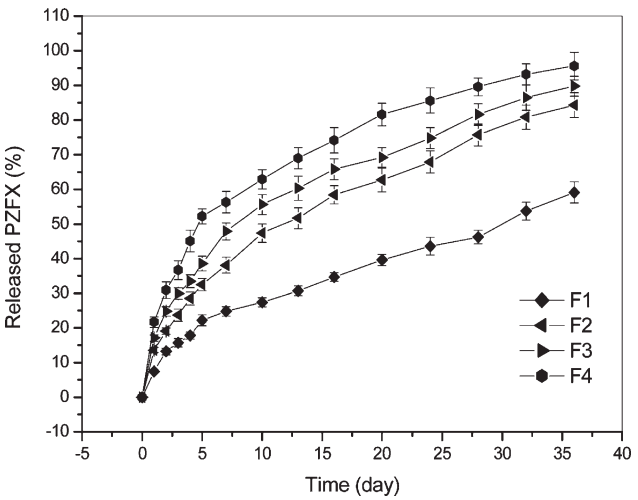


Figure 4 The release profiles of PZFX from microcapsules prepared with various V(PPCMA) : V(PZFX) ratios in pH 7.4 PBS (±S.D., $n = 3$).

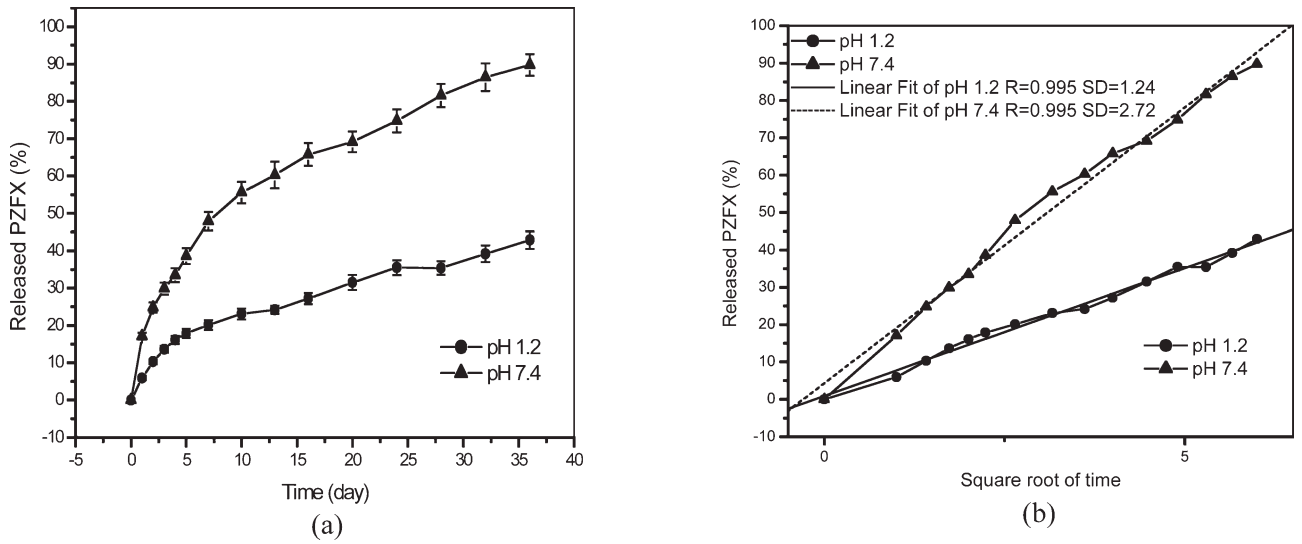


Figure 5 (a) The release profiles of PZFX from microcapsules F3 in pH 7.4 PBS and pH 1.2 HCl media (±S.D., $n = 3$) and (b) functional profiles of the square root of time and the percentage of released PZFX.

This suggests that the level of drug loading was a main factor that controlled the rate of drug release, the microcapsules with a higher loading released PZFX faster. These results agree with the observations by Spenlehauer et al.[34] and Sah et al.[35]

In vitro release behaviors of PZFX from PPCMA microcapsule F3 were studied in pH 7.4 PBS and pH 1.2 HCL media, respectively. Figure 5(a) showed the percent release of PZFX from microcapsules against incubation time in various media. The PZFX release profiles in pH 7.4 and pH 1.2 media were found to occur in a biphasic manner, with an initial fast release phase followed by a slower release phase. After release for 36d, the release of PZFX from microcapsules in pH 7.4 media showed 89.8 ± 2.89% drug release, which was higher than the 42.9 ± 2.36% release of PZFX in pH 1.2 media. For the mathematical evaluations, we characterized drug release kinetics by fitting Higuchi equation to the experimental data from Figure 5(a). Plotting the PZFX release as a function of the square root of time resulted in a linear correlation [Fig. 5(b)], PZFX release from PPCMA microcapsules followed the Higuchi matrix model. Table III showed the release equations of dissolution results according to the Higuchi models. This indicated that the release of PZFX from the copolymers was controlled by either swelling or diffusion.[36] The results suggested that PPCMA microcapsules might be optimized as carriers in drug delivery system for different purposes.

TABLE III
The Release Equations, R and SD of Dissolution Results According to the Higuchi Models

Formulation codes	Release media	Higuchi equations	R	SD
F3	pH 7.4 PBS	$Q_t = 4.53 + 14.76\ t^{1/2}$	0.995	2.72
F3	pH 1.2 media	$Q_t = 0.88 + 6.85\ t^{1/2}$	0.995	1.24

CONCLUSIONS

The copolymer PPCMA was developed as drug carriers for controlled release of PZFX in the present study. A series of PPCMA microcapsules containing PZFX were elaborated by solvent evaporation method based on the formation of double W/O/W emulsion. More than 80% encapsulation efficiency, appropriate drug loading, and particle size were obtained by varying the process parameters. The microcapsules had a spherical, smooth morphology, and a diameter of ~ 2 μm. *In vitro* release studies were performed in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4) medium, respectively. The microcapsules were shown to retard drug release under physiologically simulated pH conditions. The release profiles of PZFX from PPCMA microcapsules were biphasic and obeyed the Higuchi equation. This results suggested that PPCMA was a potential polymer matrix for drug controlled release delivery systems.

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