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Preparation of Novel Cationic Copolymer Microspheres and Evaluation of Their Function by In Vitro and In Vivo tests as Ph-Sensitive Drug Carrier Systems

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ABSTRACT Novel pH-sensitive copolymer microspheres containing methylacrylic acid and styrene cross-linking with divinylbenzene were synthesized by free radical polymerization. The microspheres that were formed were then characterized by Fourier-Transform infrared (FT-IR) spectroscopy, differential scanning calorimetry (DSC), size analysis, and X-ray analysis. The copolymer microspheres showed pulsatile swelling behavior whenthe pH of the media changed. The pH-sensitive microspheres were loaded with diltiazem hydrochloride (DH). The release characteristics of the free drug and the drug-loaded microspheres were studied under both simulated gastric conditions and intestinal pH conditions. The in vivo evaluation of the pulsatile preparation was subsequently carried out using beagle dogs as experimental subjects. The results demonstrated that the drug release exhibited a pulsatile character both in vitro and in vivo.

KEYWORDS pH-sensitive, Poly (methylacrylic acid/styrene), Diltiazem hydrochloride, Lag time, Pulsatile release

INTRODUCTION

During the last couple of decades, much research has been focused on realizing sustained drug delivery to reach a constant drug blood level over an extended period of time. However, recent investigations based on clinical cases and on chronopharmacology theory have revealed that sustained drug delivery is not suitable for all therapeutic agents (Qiu & Zhu, 2001; Forse & Mass, 1992; Giuseppe, 1991). On the other hand, pulsatile drug delivery systems have the advantage of avoiding drug tolerance problems and also of matching the chronotherapeutic needs of the patient. The oral pulsatile release system is used mainly for the treatment of various diseases, such as hypertension, ischemic heart disease, asthma and rheumatoid arthritis, the symptoms of which exhibit circadian rhythms (Fan et al., 2001; Vyas et al., 1997; Greasy & Jaffe, 1991; Langer, 1990). In pulsatile release systems, the required amount of the drug is administered within a short period of time, preceded and followed by a specified

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lag time in which little or no drug is released to the patient. For this purpose, two kinds of pulsatile release systems have been proposed. The first one is a preprogrammed delivery system which delivers the drug depending on the device structure of the release system; the other is a delivery system that releases the drug in response to certain environmental or physiologic variables in vivo (Jiang & Zhu, 2000).

In general, delayed or pulsatile systems that are based on the dispersion of the drug through enteric coated systems have serious shortcomings, such as low loadings of water-soluble drugs, large bursts, complex operations, and significant leaching of the active drugs during the delay period (He & Cao, 2004; Lowman et al., 1999; Horak et al., 2001; Morishita et al., 2002). In order to overcome these limitations, stimuli-responsive copolymers have recently been developed to trigger the release of an active drug from the polymeric systems. Polymeric systems that modify their structures and properties in response to changes in the physical and chemical characteristics of the physiological medium are very promising candidates for achieving optimum control of the drug release process (Lorenzo & Concheiro, 2002). These systems are able to respond to external stimuli such as pH, ionic strength, temperature and electric current. The pH-sensitive properties of polymers are due to the ionization of weakly acidic and/or basic functional groups on their backbones. (Soppimath & Kulkarni, 2001; Bettini & Colombo, 1995) Studies involving the use of polyelectrolyte polymers have been reported (Varshosaz & Falamarzian, 2001; Qiu & Kinam, 2001; Kost & Langer, 2001).

Sodium polystyrene sulfonate has been used as a type of ion exchange resin in the investigation of drug delivery systems. It has a number of advantages over other polymers, such as better stability, better taste, fewer side effects, as well as more uniform absorption and sustained release (Sriwongjanya & Bodmeier, 1998; Farag, 1988; Amesel et al., 1984). However, few investigations have been published, to the best of our knowledge, on the possibility of modifying its structure in order to obtain additional advantages, such as a pulsatile character. In this article, we will describe the process of combining ionic functional groups onto the cross-linked polymer in order to produce a novel pH-sensitive polyelectrolyte polymer.

Diltiazem hydrochloride (DH) is a calcium-channel blocker which is widely used for the treatment of angina pectoris, arrhythmia and hypertension. There have been a number of studies on the drug carrier system for diltiazem hydrochloride (Levis & Deasy, 2003; Kakish et al., 2002; Corrigan & Heelan, 2001; Chuo et al., 1996). According to the human circadian rhythm, the diseases listed above often occur during the night or in the early morning hours, and thus can prove to be very disturbing to the afflicted patient. Therefore, if DH could be released after a 2–3 h lag time, this would greatly benefit the patients to whom it is administered. In this article, we have used DH as a model drug for achieving pulsatile drug release according to the pH-sensitivity of the novel polyelectrolyte polymer that was used in the study.

The purpose of this study was to incorporate methylacrylic acid into the styrene backbone in order to obtain a novel pH-sensitive polyelectrolyte polymer. The copolymers prepared were characterized by Fourier-Transform infrared (FT-IR) spectroscopy, differential scanning calorimetry (DSC), size analysis and X-ray analysis. Then, the pH-sensitive copolymer was loaded with DH. Swelling and drug release in vitro were allowed to occur at gastric and intestinal pH conditions in order to investigate the pH-sensitivity of the copolymer that had been developed. The in vivo evaluation of the pulsatile preparation was subsequently carried out in beagle dogs.

MATERIALS AND METHODS

Materials

The two monomers, namely methylacrylic acid (MAA) and styrene (St), were obtained from the Tianjin Chemical Reagent Company (Tianjin, China). The monomers were vacuum distilled prior to use. Divinylbenzene (DVB) was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Benzoyl peroxide (BPO), dichloroethane, methanol and PVA with a molecular weight of 125,000 Daltons were obtained from the Shenyang chemical reagent factory (Shenyang, China). Diltiazem hydrochloride was received as a gift sample from the Huai Hai Pharmaceuticals Company (Shanghai, China). All reagents used in this study were of analytical grade.

Preparation of pH-sensitive Copolymer Microspheres

Free radical polymerization was used to prepare the MAA/St microspheres cross-linked with DVB. At first,

10 ml of 10% (w/w) PVA solution was dispersed in 150 ml of water under constant stirring in a 250 ml round-bottomed flask. Then, a mixture of MAA and styrene (molar ratios: 20/80, 50/50, 80/20) was added to this solution, followed by the addition of 0.7 wt% DVB (as a cross-linking agent) and 0.5 wt% BPO (as an initiator). Nitrogen was bubbled through the mixture for 30 min to remove any dissolved oxygen that would act as an inhibitor during the reaction. The polymerization was carried out at 80°C under stirring for 10 h. Then, a sufficient amount of acetone was added in order to bring the reaction to an end. The polymers that had been formed were subsequently filtered and washed thoroughly with methanol and hot water in order to extract any untreated components. Finally, the copolymer microspheres were air dried at room temperature overnight, followed by a vacuum-drying cycle at 60°C for 12 h.

The prepared copolymer was immerged in dichloroethane for 20 min. Then, 95%-concentrated sulfuric acid was added to the mixture and the sulfonation reaction was carried out at 82°C with constant stirring for 12 h. Afterward, the mixture was slowly diluted with distilled water until it became neutral. 1 mol/l NaOH was then used to adjust the pH of the mixture to 10–12. Finally, the cationic copolymer microspheres that had been formed were washed repeatedly with an excess amount of distilled water to remove the leftover alkali and they were dried under vacuum overnight at a temperature of 60°C. The cross-linked microspheres were designated, respectively, as MAA (20/80), MAA (50/50) and MAA (80/20).

Fourier-Transform Infrared (FT-IR) Spectra

The microspheres were crushed under a hydraulic pressure of 500 kg/cm^2 to make the KBr pellets. The FT-IR (BRUKER IFS55; Fällanden, Switzerland) spectra were recorded in the wavelength region from 400 to 4000 cm^{-1}.

Differential Scanning Calorimetric (DSC) Studies

DSC analysis was performed using a DSC Analyzer (Perkin Elmer, Norwalk, CT, USA) to investigate the thermal stability of the pure drug and of the drug-loaded microspheres. The DSC profiles were recorded with a heating rate of 10°C/min. in nitrogen from 25°C to 350°C.

X-ray Analysis

X-ray analysis was performed using a D/MAX-2A transfer target radial diffraction instrument (Rigaku, Tokyo, Japan) to investigate the pure drug and the drug-loaded microspheres. The analysis method used was as follows: graphite-curved crystal homochromy implement was used to make the diffraction bind into a single crystal with high pressure at 40 KV, pipe current at 50 mA, and a scan rate of 5 degrees/min.

Particle-Size Analysis

The mean particle size of the microspheres that were prepared was measured using a laser light scattering particle-size analyzer (LS230; Beckman Coulter, Fullerton, CA, USA). A sample of about 100 mg of the microspheres was suspended in 100 ml of distilled water. This suspension was stirred under sonication in order to avoid agglomeration of the particles during measurement.

The Analysis of the Content of the Carboxylic Groups and Sulfonic Acid Group

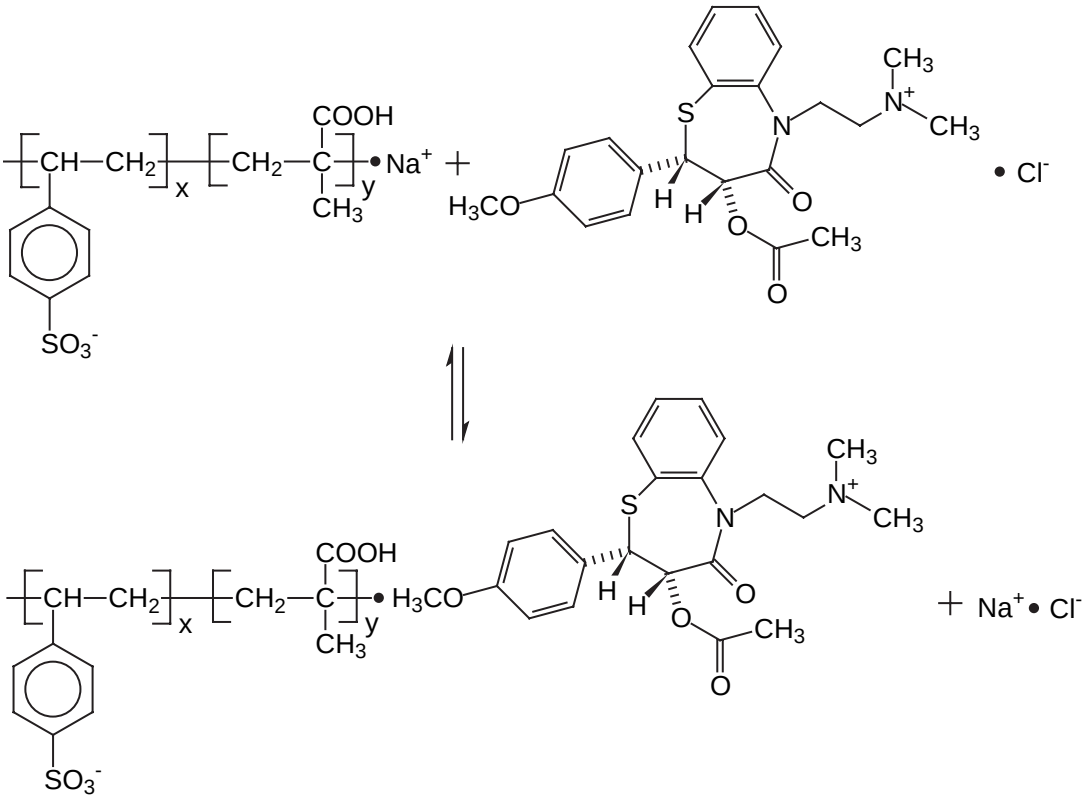
Elemental analyses of the microspheres formed were made using an EA1110 analyzer (Thermoquest; CE Instruments, Milan, Italy) and the percent estimations of sulphur, carbon, and hydrogen were calculated.

The amount of –COOH functional groups was estimated by using the acid-base titration method (Tripathy & Sing, 2000). In brief, a known quantity of microspheres was equilibrated in water for 12 h. The hydrogen ion concentration was estimated with a standard alkali solution. Thereafter, the equivalent mass of carboxylic acid functional group was calculated.

Preparation of the Drug-loaded Microspheres

The drug-loaded microspheres were prepared by using the batch method. For this purpose, 200 mg of DH was first dissolved in 40 ml of water. Then, 200 mg of the prepared copolymer microspheres were added to the DH solution with agitation until the amount of DH in the water reached an equilibrium. The amount of DH in the water was measured at 240 nm by UV spectrophotometry, and the drug-loaded microspheres were formed by the ion exchange reaction.

Then, the drug-loaded microspheres were washed with deionized water in order to remove the remaining exchange salt and any free drugs. Finally, the drug-loaded microspheres were dried in an oven at 50°C for 8 h. The reaction equation of the drug microsphere was as follows:



The Determination of Drug Content of the Drug-loaded Microspheres

The DH content of the drug-loaded microspheres was determined by the following method: Precisely 30 mg of dry drug-loaded microspheres was placed in a conical flask. 100 ml of 1 mol/l NaCl solution was then added to the flask, and the mixture was surged for 10 hours at 65°C. The solution was then filtrated, and the amount of DH in the filtrated solution was determined using UV spectroscopy at 240 nm.

Swelling Study

The pH-dependent equilibrium swelling of the cross-linking copolymer microspheres was studied under conditions in which the pH was increased from 1.2 to 6.8. The microspheres were allowed to swell completely for about 24 h in order for them to attain equilibrium at 25°C. Excess surface liquid was removed by blotting the swelling with tissue papers, and the swollen microspheres were weighed on an electronic balance (Model 20; Mettler, Greifensee, Switzerland). The microspheres were then dried in an oven at 60°C for 5 h until no change could be observed in the dry mass of the samples. The equilibrium degree of the swelling (Q) was calculated by applying the following equation:

$$Q = (W_\infty - W_0)/W_0$$

where W_∞ is the mass of the microspheres after absorbing water, and W_0 is the mass of the microspheres after drying.

In Vitro Drug Release

The studies of in vitro drug release were performed using the USP paddle (apparatus II) method and a ZRS-8G Intelligent Dissolution Tester (Tian Jin

University Radio Factory, Tian Jin, China) at a rotation speed of 50 rpm. For this purpose, drug release from the microspheres was studied both in simulated gastric fluid (pH 1.2 HCl solutions) and in intestinal fluid (pH 6.8 phosphate buffer) at 37 ± 0.1°C. The drug-loaded microspheres were accurately weighed in order to attain an equivalent of 30 mg DH. The 5 ml dissolution medium was sampled at predetermined time intervals. These samples were passed through a 0.45 μm membrane filter, and the amount of the drug released was measured at 240 nm by UV spectrophotometry.

In Vivo Study

The in vivo evaluation was performed by a crossover treatment in six male beagle dogs (each weighing 10–12 kg) with a washout period of 7 days. The beagle dogs were deprived of food overnight for at least 12 h, although they were allowed to have free access to drinking water. During the course of the experiment, water was not provided to the subjects until 6 h after the administration of the two preparations. The two preparations were: **(1)** the drug-loaded microspheres with DH pulsatile released in vitro (PM) and **(2)** the conventional DH tablet (CT). Both treatments contained 30 mg of DH. All studies were conducted in accordance with the Principles of Laboratory Animal Care (U.S. Dept. Health and Human Services, NIH Publication No. 85-23, 1985), and were approved by the Department of Laboratory Animal Research at Shenyang Pharmaceutical University. Blood samples were taken immediately prior to administering the drug and at the following times, respectively, for each treatment: **(1)** 0, 2, 3, 4, 5, 5.5, 6, 6.5, 7, 8, 9, 10, 12, 16 and 24 h; **(2)** 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24 h. Plasma samples were stored at –20°C until assayed.

Assay of DH in Plasma Samples and Validation of the Analysis Methods

The DH in the plasma was assayed as follows: plasma aliquots (700 μl) were mixed with a 2 μg/ml verapamil (internal standard) solution. Acetonitrile (900 μl) was then added to each of the samples. The samples were subsequently centrifuged and kept frozen overnight at –20°C to facilitate the separation of the aqueous and the organic phases. 600 μl of the organic phase from the frozen samples were evaporated to dryness, and they were then reconstituted in the mobile phase, vortexed and centrifuged. Aliquots were transferred to an autosampler vial. The HPLC analysis was conducted using the following parameters: a column (Lichrosorb RP-18, 5 um, 250 mm; Alltech Associates, Deerfield, IL, USA), an injection loop of 40 μl, mobile phase (acetonitrile-0.01 M phosphate solution (Na_2HPO_4)–triethylamine, 15:85:1), a flow rate of 0.7 ml/min, a UV detector wavelength of 237 nm, a column temperature of 20°C, a temperature of 10°C in the autosampler, and a run time of 13 min. All reagents were of analytical grade.

Analysis methods were validated according to established international guidelines and requirements (Validation of Analytical Methods: Definitions and Terminology, ICH Topic Q2A, 1994; Validation of Analytical Procedure: Methodology, ICH Topic Q2B, 1995). No interfering peaks were detected at the retention time of DH (6.2 min) and the retention time of verapamil (9.1 min). A linear correlation ($r >$ 0.999) was obtained between the ratio of peak area and DH concentration in the range of 10–150 ng/ml. The coefficient of variation of the slope was 7.2%. The limit of quantification (the background noise multiplied by 10) was 10 ng/ml. The precision and the accuracy of the method were evaluated at concentrations of 150, 75, and 10 ng/ml. The precision of the method was assessed on the basis of the coefficient of variation in quality-control samples and accuracy was measured as the bias percentage of these samples. The coefficients of variation of the intra- and inter-day precision were, respectively, 2.2–5.3% and 2.7–6.2% at all concentration levels. The bias percentages of the intra- and inter-day accuracy were 0.8–2.3% and 0.6–0.9%. No decrease was observed in the contents of the quality control samples in the freezer or the autosampler.

Pharmacokinetics Study

Model-independent parameters, including the maximum plasma concentration (C_{max}) and the time to the maximum plasma concentration (T_{max}) were determined through the observation of the plasma concentration–time curve. The areas under the serum concentration-time curve (AUC_{0-24h}) were calculated by using the trapezoidal method. The lag time values (t_{lag}) were also observed from the plasma

concentration-time curve. They were calculated from the time when the drug was administered to the time when the drug was detected. The time difference between t_{max} and t_{lag} was defined as t_{psi} in order to compare the in vivo drug release rates of the two preparations. Results from the two preparations were analyzed by using the SPSS statistical package, and an analysis of variance was performed to assess any significant ($P < 0.05$) differences between the two preparations.

RESULTS AND DISCUSSION

FT-IR Spectroscopic Study

Fig. 1 shows the FT-IR spectra of the cationic microspheres. The results demonstrate that an apparent peak of the carbonyl groups appears at 1680 cm^{-1}, which proves the presence of carboxylic acid in MAA on the backbone of the microspheres. The absence of the peak of the alkenyl groups illustrates that the copolymer was formed as a result of the reaction between MAA and styrene. The absorption band at 2921 cm^{-1} is caused by the C-H stretching of the CH_2 groups, which were formed as a result of the cross-linking reaction with DVB. The peak at 3438cm^{-1} was formed by the remnant water and the OH group of the methacrylic acid monomeric unit.

Differential Scanning Calorimetric (DSC) Studies

The DSC graphs of diltiazem hydrochloride and diltiazem hydrochloride-loaded microspheres are presented in Figs. 2 and 3. The DSC curve of the pure drug shows a sharp peak at about 212.6°C, which is due to the melting of the pure drug. The drug-loaded microspheres did not show any peak at 212.6°C, as was observed in the curve of the pure drug. This indicates that the drug is connected to the cross-linking structure of the microspheres by chemical bonds, without any physical mixtures. Therefore, no separate peak is observed.

X-ray Analysis

The X-raygraphs of diltiazem hydrochloride and diltiazem hydrochloride-loaded microspheres are presented in Figs. 4 and 5. The X-raycurve of the pure drug shows an obvious crystal peak. The drug-loaded microspheres did not show any peak as was observed in the curve of the pure drug. This indicates that the drug is connected to the cross-linking structure of the microspheres by chemical bonds, without any physical mixtures. Therefore, no separate peak is observed. X-ray analysis shows that the drug is entrapped in an amorphous state.

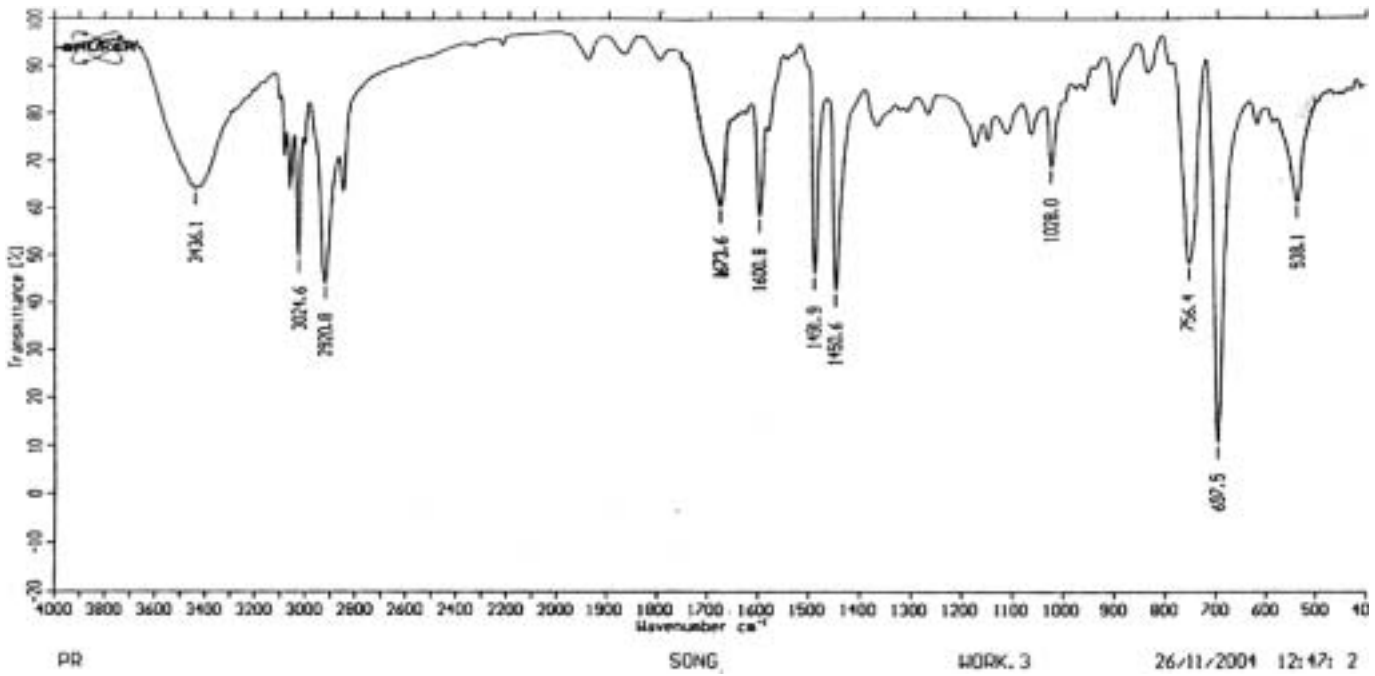


FIGURE 1 FT-IR Spectrum of Cationic Microspheres of MAA/Styrene.

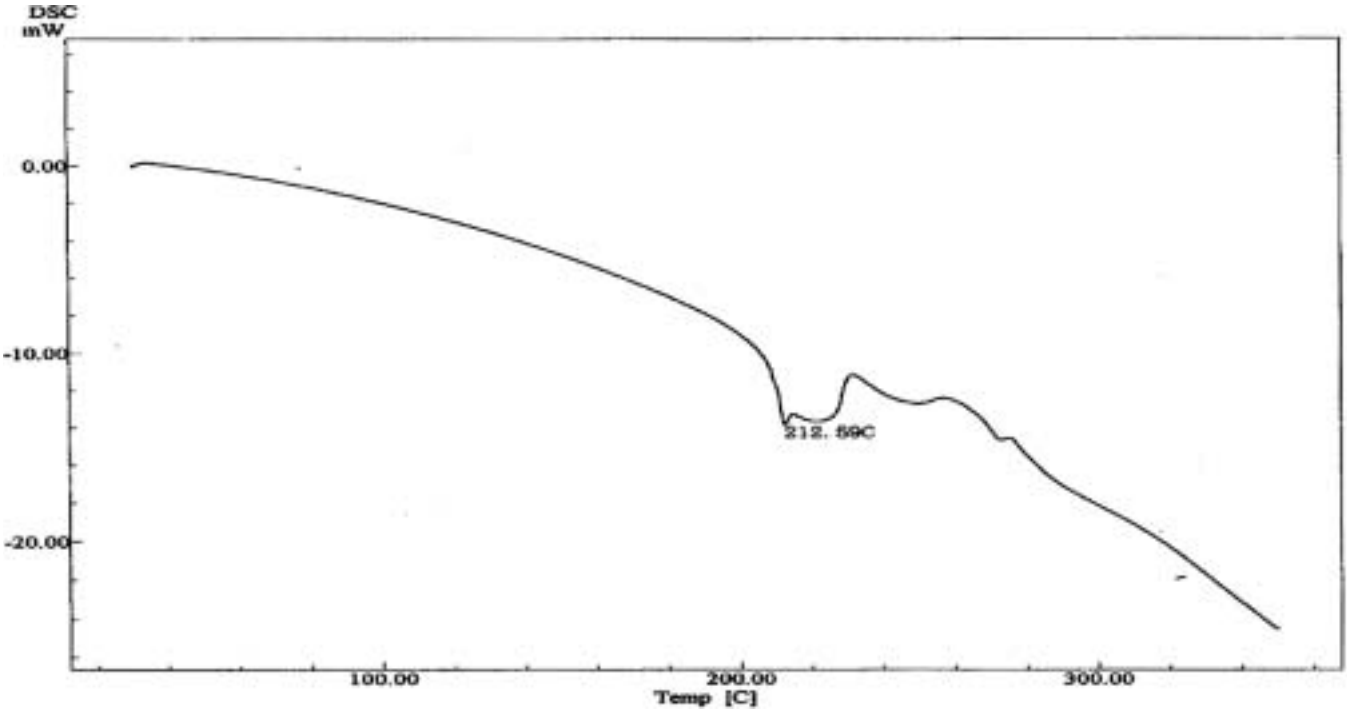


FIGURE 2 DSC Curves of Diltiazem Hydrochloride.

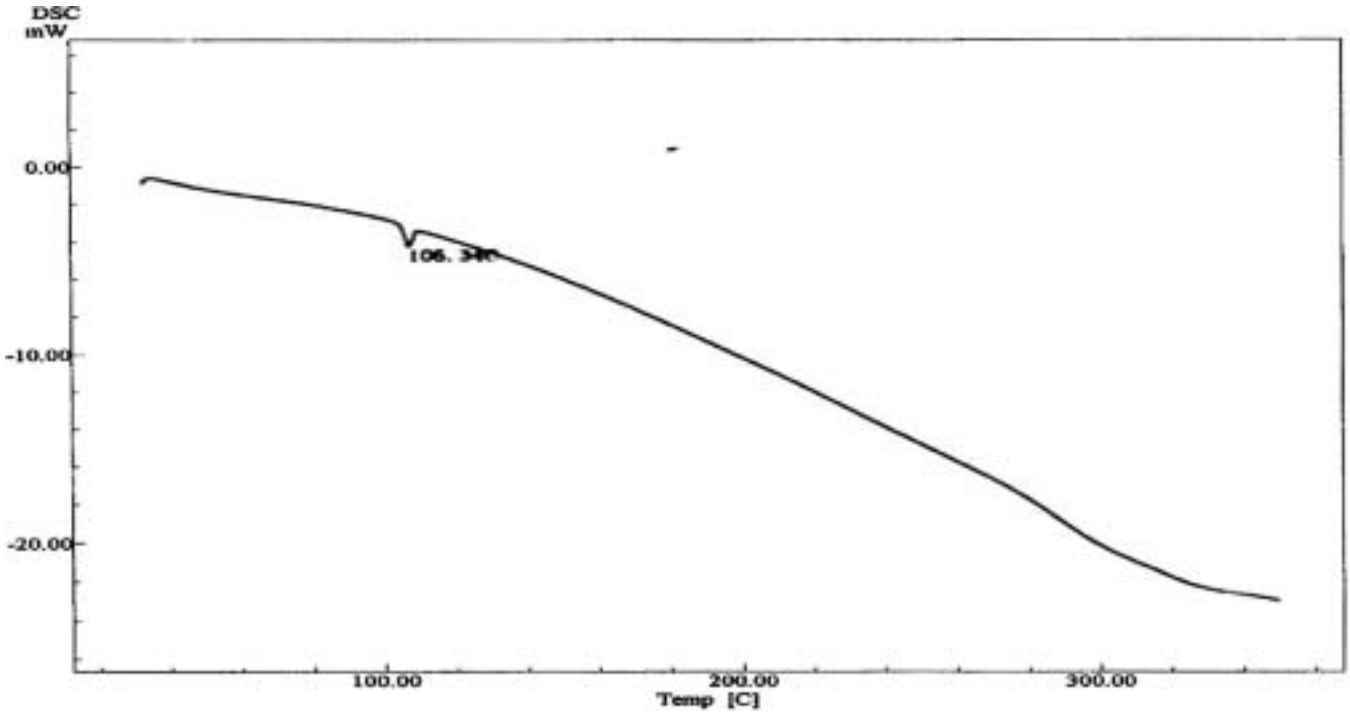


FIGURE 3 DSC Curves of Diltiazem Hydrochloride-loaded Microspheres.

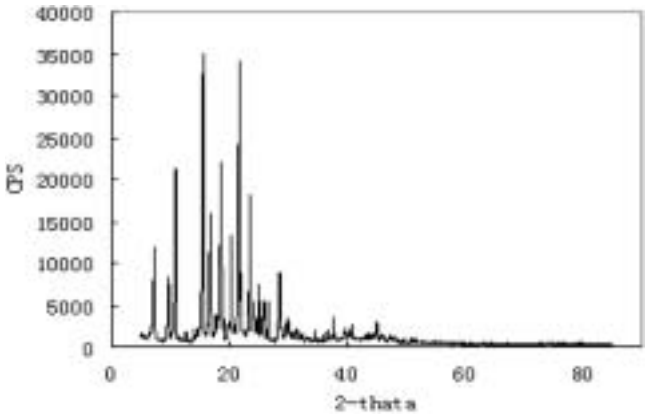


FIGURE 4 X-ray Curves of Diltiazem Hydrochloride.

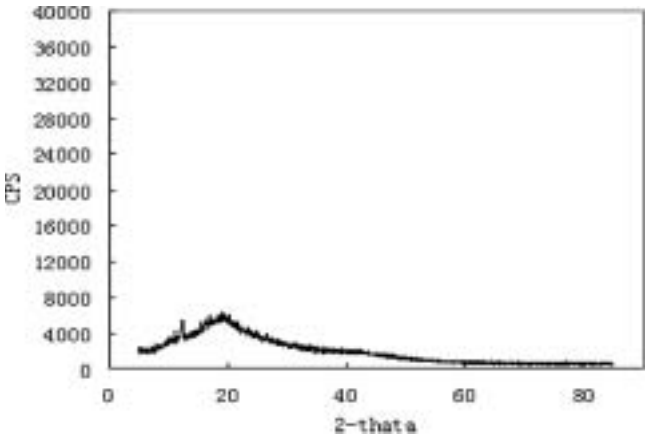


FIGURE 5 X-ray Curves of Diltiazem Hydrochloride-loaded Microspheres.

Particle-Size Analysis

The particle sizes of the microspheres are presented in Fig. 6. A nearly standard normal distribution of particle sizes was observed in this study. The mean particle size of these microspheres was about 180 μm.

The Analysis of the Content of Carboxylic Groups and of the Sulfonic Acid Group

The elemental analyses data and titration data are presented in Table 1. In the case of ionic microspheres, the sulphur percentage decreased as the content of MAA in the microspheres was increased. The concentration of –COOH in the microspheres as estimated by the titration method is in the range 4.45–4.88 mequiv/g of microspheres.

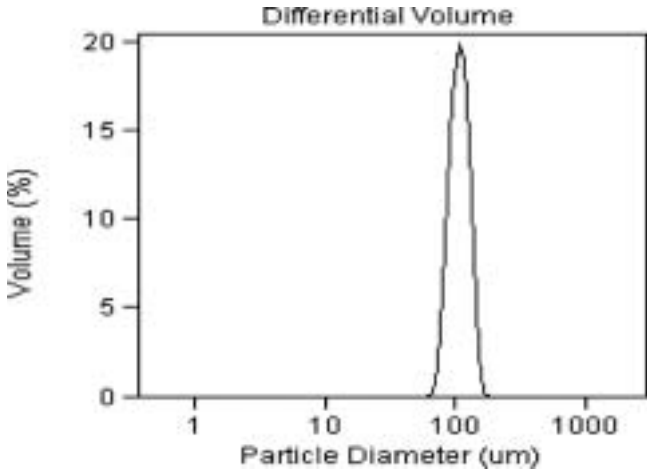


FIGURE 6 Histogram of the Size Distribution of the Microspheres.

TABLE 1 Elemental Analyses and Titration Data

Sample	% Sulphur	% Carbon	% Hydrogen	-COOH (mequiv/g)
MAA-20	13.14	36.42	6.11	4.45 ± 0.13
MAA-50	9.65	41.13	5.84	4.66 ± 0.08
MAA-80	5.84	44.56	5.61	4.88 ± 0.11

The Determination of Drug Content in the Drug-Microsphere Complexes

The results of the drug content study are listed in Table 2. The results illustrate that when the MAA content is increased, the exchange group content in the microspheres decreases, and the drug content decreases as well.

Swelling Study

Fig. 7 shows the equilibrium swelling data for the copolymer microspheres at 25°C in a buffer solution ranging from pH 1.2 to pH 6.8. The results indicate that a significant increase in water uptake occurs for all the microspheres when the pH level is increased from

TABLE 2 The Drug Content in the Three Kinds of pH-sensitive Microspheres

MAA (%)	(20/80)	(50/50)	(80/20)
Drug content($g \cdot g^{-1}$)	0.42	0.35	0.22

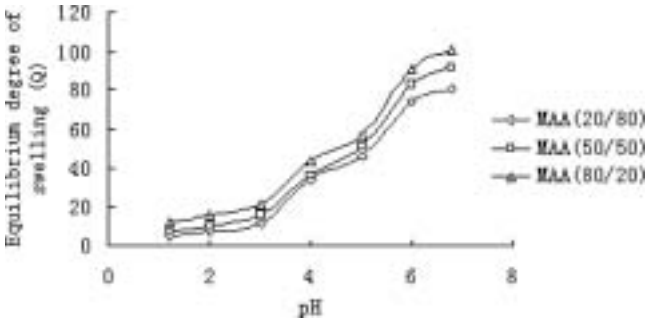


FIGURE 7 **Effect of pH on the Swelling Degree of Copolymer Microspheres Buffer in Buffer Solution from pH 1.2 to pH 6.8 at 25°C (*n* = 3).**

1.2 to 6.8. At low pH values, the carboxyl groups of MAA would be protonated, the polymer chains would take the form of aggregates, and the microspheres would undergo shrinkage. At high pH values, the microspheres swell rapidly and they can achieve a much higher equilibrium swelling ratio. This is caused by the partial or complete ionization of the carboxyl groups of MAA, which results in the dissociation of the hydrogen bond between the carboxylic acid groups of MAA. The dissociation of the hydrogen bonds, combined with the electrostatic repulsion force, causes the network of microspheres to swell rapidly.

In Vitro Drug Release

Figs. 8 and 9 show the cumulative release data of microspheres at pH 1.2 and at pH 6.8. Fig. 10 illustrates the in vitro dissolution profile of free diltiazem at pH 1.2 and at pH 6.8. The free diltiazem release was rapid in both pH 1.2 and pH 6.8 dissolutions. The dissolution figures for the microspheres demonstrate

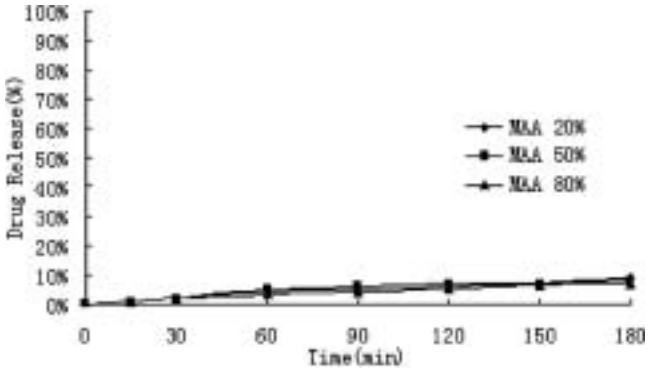


FIGURE 8 **Drug Released from the Copolymer Microspheres in the Gastric Dissolution Medium Mean (S.D) Value (*n* = 3).**

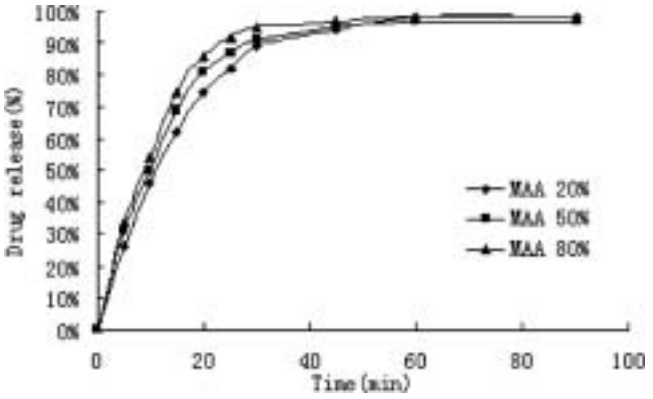


FIGURE 9 **Drug Released from the Copolymer Microspheres in the Intestinal Dissolution Medium Mean (S.D) Value (*n* = 3).**

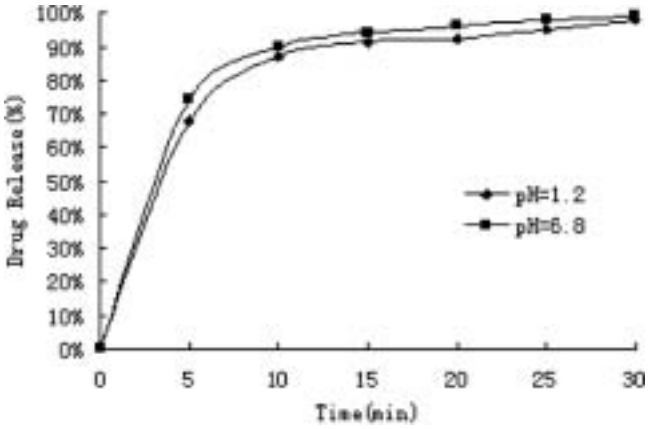


FIGURE 10 **Pure Drug Released in the Gastric Dissolution Medium and Intestinal Dissolution Medium Mean (S.D) Value (*n* = 3).**

that, at pH 6.8, about 90% of the DH is rapidly released in 10 minutes and that the DH is completely released in less than 20 minutes. On the other hand, the fraction of the drug released at pH 1.2 is almost negligible. The disparity between the dissolution profiles of the drug-loaded microspheres and the free drug can be explained by the swelling of the microspheres.

At pH 1.2, a small amount of the drug is released from the microspheres, possibly due to the exchange between the drug and the ions occurring on the surface of the microspheres during the dissolution process, although the microspheres were not swollen at this pH level. In contrast to the results obtained at pH 1.2, a pronounced change was observed in the release data for pH 6.8. This difference was attributable to the presence of the carboxyl groups, which are responsible for the greater swelling in media at higher pH levels.

As the pH level of the media increased, the ionization of the carboxyl groups resulted in the swelling of the microspheres, which then caused the enlargement of the size of the pores. The ions in the media subsequently entered the microspheres through the microchannels of the pores and exchanged with the drug which was combined with the sulfonic groups. The results of the swelling and the in vitro release show that the preparation demonstrates a heightened level of pH-sensitivity when the MAA content of the microsphere is increased. Therefore, we finally used microspheres with 80% MAA to prepare the pH-sensitive ion exchange resins in order to carry out the in vivo evaluation.

In Vivo Study

Fig. 11 shows a comparison of the plasma concentration-time profiles of DH after oral administration of DH (30 mg) of each formulation to six beagle dogs. The CT did not exhibit any lag time prior to drug release. On the other hand, the PM exhibited a rapid increase in the DH plasma concentration following a lag time of about 2.6 h, which is approximately equivalent to the transit time of the preparation through the gastrointestinal system. Table 3 summarizes the pharmacokinetic parameters. A comparison of the parameters with the two preparations reveals no significant differences for C_{max} and AUC_{0-24h}, but shows significant variations for t_{max} and t_{lag}. These results suggest that the pulsatile release microspheres prolonged the lag time of drug release, but they did not lower the drug release rate.

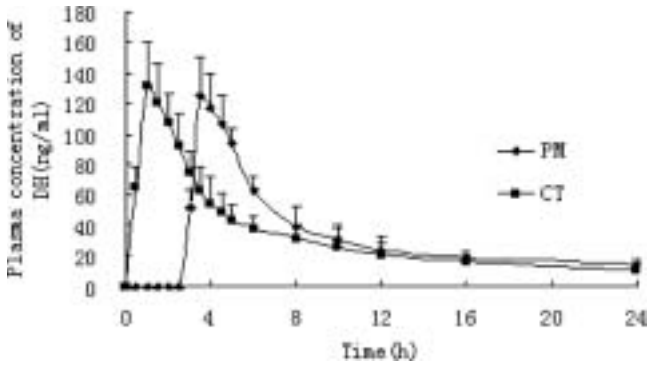


FIGURE 11 **Plasma Concentration-time Curve of DH Following Oral Administration of PM and CT. Each Point Represents the Mean ± S.D. (n = 6).**

TABLE 3 **Pharmacokinetic Parameters of Diltiazem Hydrochloride Pulsatile Release Preparation (PM) and Conventional Tablet (CT)**

	PM		CT		
	Mean	±S.D	Mean	±S.D	ANOVA
T_{max} (h)	3.7	0.7	1.3	0.5	P<0.05
T_{lag} (h)	2.6	0.5	0.3	0.4	P<0.05
T_{psi} (h)	1.1	0.3	1.0	0.4	P>0.05
C_{max} (ng/ml)	125	26.3	132	28.1	P<0.05
AUC_{0-24h} (ngh/ml)	751.5	79.2	789.0	68.8	P<0.05

Bioequivalence of the two preparations was ensured with AUC_{0-24h} and C_{max} by conducting the two one-side test. The relative bioavailability of pulsatile release microspheres to conventional tablets was 1.05 (105%). This result suggests that the absorption of DH was not influenced by the in vivo behavior of the pulsatile release preparation.

CONCLUSIONS

A novel pH-sensitive polyelectrolyte copolymer was prepared by incorporating MAA into a styrene backbone. The results of Fourier-Transform infrared spectroscopy (FT-IR), differential scanning calorimetry (DSC), size analysis, and X-ray analysis showed that a pH-sensitive polyelectrolyte polymer had been obtained. The swelling and drug dissolution results indicated that the cationic copolymer microspheres exhibit strongly pH-dependent swelling behavior, which is caused by the presence of ionizable carboxylic functional groups. The pulsatile release of DH was achieved through the swelling of the copolymer microspheres in response to the changes in the pH level. Finally, the in vivo study also displayed an apparent lag time of approximately 2.6 h.

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