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A novel pH-dependent gradient-release delivery system for nitrendipine
II. Investigations of the factors affecting the release behaviors of the system

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Abstract

Nitrendipine, a dihydropyridine calcium antagonist, was used as a poorly water-soluble model drug. To improve its dissolution rate and extend the therapeutic period in vivo as well, a novel pH-dependent gradient-release drug delivery system for nitrendipine having a solid dispersed matrix structure was developed. Four factors, i.e. the amount of excipients, the pH of the dissolution medium, the rotating speed of the paddle of the dissolution apparatus and the particle size of the microspheres, all of which affect the drug-release behavior of the pH-dependent microspheres of the system were investigated in detail. The release profiles of the pH-dependent drug delivery system under simulated gastrointestinal tract pH conditions were also investigated. The results showed that the release rate of drug from the microspheres increased on increasing the amount of respective pH-dependent polymers formulated. Due to the fact that the active drug was incorporated in pH-dependent polymers and was present in a solid dispersion state in the microspheres, the release rate of the drug from the microspheres depended on the dissolution rate of the polymers, which was mainly influenced by the pH of dissolution medium, whereas the rotating speed of the paddle and the particle size of the microspheres had only a relatively minor effect. The release behavior of the system under simulated gastrointestinal tract conditions exhibited obvious gradient-release characteristics, showing that the release rate of the active drug could be controlled efficiently before the microspheres reached the appropriate region of the gut for absorption. These findings suggest that the pH-dependent drug delivery system could be fabricated by using present microspheres.

Keywords: Nitrendipine; pH-dependent gradient-release drug delivery system; Release behaviors; Solid dispersion; pH-dependent microspheres

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1. Introduction

In recent years, pH-dependent drug delivery systems have been the focus of much formulation research because these are considered as a suitable method for designing many dosage forms for specific purposes, such as sustained-release drug delivery systems, colon-targeted delivery systems and other site-specific drug delivery systems (Ashford et al., 1993; Risbud et al., 2000; Palmieri et al., 2000; Gupta et al., 2001). In general, the coating technique is the main approach used to manufacture pH-dependent drug delivery systems using pH-dependent polymers (Watts and Illum, 1999). Firstly, the active drug is prepared in an appropriate dosage form such as tablets, pellets or granules with some excipients. Then the resultant products are coated with appropriate pH-dependent polymers depending on the ultimate purpose of the pH-dependent drug delivery system. For example, Khan et al. prepared core tablets of mesalazine and then coated them with a spray dispersion of Eudragit L100-55 and Eudragit S-100 mixed at a suitable combination-ratio using a fluid bed coating apparatus to obtain a colon-targeted drug delivery system (Khan et al., 1999, 2000). In the reports of Song et al., the active drugs were prepared in fine spherical pellets using a centrifugal granulator and then coated with different pH-dependent polymers to obtain a pH-dependent delivery system for a traditional Chinese medicine compound recipe (Song et al., 2002). However, pH-dependent systems having a matrix structure have seldom been investigated although the matrix structure is much preferred over the coating structure due to its ease of manufacture and in-process control.

The coating method for pH-dependent drug delivery systems is suitable for water-soluble drugs. However, in designing a system for poorly water-soluble drugs by this method, the low absorption rate of the drug will be the main obstacle due to its poor dissolution rate, even though the active drug can be released promptly after the coating materials of the systems dissolve at the target site. Here the suitable method involves handling the poorly water-soluble drug using some pharmaceutical techniques, such as solid dispersion, using an inclusion compound to improve its dispersal, fabricating it in an appropriate dosage form, and finally, coating the resultant products with pH-dependent polymers. This results in a more complex manufacturing process and careful control of each step is required to produce reproducible and reliable preparations.

So, in our previous research (Yang et al., 2004), a novel pH-dependent gradient-release drug delivery system for poorly water-soluble drugs was developed using a combination of the preparation of pH-dependent microspheres and the solid dispersion in one step. The system involved three types of pH-dependent microspheres, which were fabricated with Acrylic resins Eudragit E-100 (EuE-100), hydroxypropylmethylcellulose phthalate (HP-55) and hydroxypropylmethylcellulose acetate succinate (AS-H), respectively. Due to the fact that the three types of pH-dependent polymers dissolve under acid conditions, pH values of $\geq$5.5 and $\geq$6.5, respectively, the active drug could be released in the stomach, duodenum and lower segment of the small intestine, respectively. Also, it was expected to extend the therapeutic period of the active drug in vivo while the system was transported down the gastrointestinal tract. The quasi-emulsion solvent diffusion method was employed in the manufacturing process of the microspheres, the resultant pH-dependent microspheres of the system had a matrix structure, in which the poorly water-soluble drug was present as a solid dispersion. Not only was the preparation process of the pH-dependent drug delivery system simplified, but also the dissolution rate of the poorly water-soluble drug was improved. The matrix structure of the microspheres could also overcome some problems, such as rupture of the coating film and emerging intact in the faeces without releasing any drug, an event that is common with coating preparations.

To evaluate the release behaviors of the pH-dependent gradient-release drug delivery system in vitro, in this paper, we investigated the following factors: the amount of excipients, the pH of the dissolution medium, the rotating speed of the paddle of the dissolution apparatus and the particle size of the microspheres, which affect the drug-release behavior of the respective pH-dependent microspheres of the system. The release profiles of the respective pH-dependent microspheres, i.e. EuE-100, HP-55 and AS-H microspheres, were evaluated under simulated gastrointestinal pH conditions. Finally, the release behavior of the pH-dependent gradient-release drug delivery system under the simulated gastrointestinal tract pH conditions was investigated, and the release mechanisms

of each pH-dependent microspheres were also discussed.

2. Materials and methods

2.1. Chemicals and reagents

Nitrendipine was obtained from Nanjing Pharmaceutical Factory (China); the acrylic resin Eudragit E-100 (EuE-100) was from RÖhm Pharma (Germany); hydroxypropylmethylcellulose phthalate (HP-55) and hydroxypropylmethylcellulose acetate succinate (AS-H) were from Shin-Etsu Chemical Ind. Co. Ltd., Japan; light anhydrous silicic acid (Aerosil, hydrophilic) was obtained from Guangzhou People Chemical Plant (China). All other chemicals, such as sodium dodecyl sulfate (SDS), Tween-80, dichloromethane, acetone, ethanol, and inorganic salt, were of analytical grade.

2.2. Preparation of pH-dependent nitrendipine microspheres

2.2.1. Preparation of EuE-100 microspheres

Nitrendipine (0.5 g) was dissolved with EuE-100 (1.0, 1.5, 2.0 g) in a mixed solution of acetone (good solvent, 5.0 mL) and dichloromethane (bridging liquid, 2.5 mL). Then, Aerosil (1.0, 1.5, 2.0 g) was suspended uniformly in the drug-polymer solution under vigorous agitation. The resultant drug-polymer-Aerosil suspension was poured into 150 mL distilled water containing 0.08% of SDS (poor solvent) under agitation (500 rpm) and the temperature was controlled at 20 °C. After agitating the system for 20 min, another 150 mL of poor solvent was added slowly and agitation was continued for another 40 min till the translucent quasi-emulsion droplets turned into opaque microspheres. The solidified microspheres were recovered by filtration and washed with water. The resultant products were dried in an oven at 50 °C for 24 h. In this study, Aerosil was mainly used as an antiadhesion agent introduced in the formulation of EuE-100 microspheres.

2.2.2. Preparation of HP-55 microspheres

Nitrendipine (0.5 g) was dissolved with HP-55 (0.67, 1.0, 1.5, 2.0 g) in a mixed solution of acetone (good solvent, 8.0 mL) and dichloromethane (bridging liquid, 7.5 mL). The manufacturing process was same with that used for the preparation of EuE-100 microspheres, except for without adding Aerosil.

2.2.3. Preparation of AS-H microspheres

Nitrendipine (0.5 g) was dissolved with AS-H (1.0, 1.5, 2.0, 2.5 g) in a mixed solution of ethanol (good solvent, 8.0 mL) and dichloromethane (bridging liquid, 7.5 mL). The manufacturing process was same with that used for the preparation of HP-55 microspheres.

The microspheres were fractionated by the sieving method using the standard sieve stipulated in the Chinese Pharmacopoeia 2000 ed. (Ch. P. 2000 ed.). To investigate the effects of dissolution condition and the amounts of excipients formulated on the drug-release behavior of the microspheres more precisely, the general formulations of the microspheres were fixed as follows, except when stated otherwise. The amount of nitrendipine was 0.5 g, and that of the excipients was as follows, EuE-100 (1.5 g) and Aerosil (1.5 g) for EuE-100 microspheres, HP-55 (1.5 g) for HP-55 microspheres and AS-H (2.0 g) for AS-H microspheres, respectively.

2.3. Release behavior of each pH-dependent microspheres

As specified in method II for enteric dissolving preparations in the Ch. P. 2000 ed., the release test was carried out at 37 ± 0.5 °C in the dissolution medium nominated for the type of microspheres used by the paddle method specified in the Ch. P. (ZRD6-B, Shanghai Huanghai Pharmatest Apparatus Factory, China). The microspheres were fractionated (280–900 μm) and weighed to be equivalent to 20 mg nitrendipine for the dissolution test. Five milliliters of the dissolution medium was sampled at appropriate intervals, and fresh dissolution medium was simultaneously replaced in the apparatus to maintain a constant volume. The withdrawn sample was passed through a membrane filter (0.8 μm), and the filtrate was assayed spectrophotometrically at 355 nm to determine the dissolved drug concentration using a spectrophotometer (Mode 752, Shanghai the Third Analytical Instrument Plant, China). Three trials of each release test were carried out, and the mean and standard deviations of each release rate were reported.

Table 1
Specific condition of release test of each pH-dependent microspheres

Sample	Volume of dissolution medium (mL)	pH of dissolution medium	Rotating speed (rpm)	Time (h)
EuE-100 microspheres	500	1.2	100	2
	500	3.0	100	2
	500	5.0	100	2
	500	1.2	50	2
	500	1.2	150	2
HP-55 microspheres	700	5.5	100	2
	700	6.0	100	2
	700	6.4	100	2
	700	6.4	50	2
	700	6.4	150	2
AS-H microspheres	900	6.5	100	3
	900	7.0	100	3
	900	7.5	100	3
	900	7.8	100	3
	900	7.8	50	3
	900	7.8	150	3

The specific conditions of release test of each pH-dependent microspheres were listed in Table 1.

In this work, hydrochloride acid was employed as the dissolution medium for EuE-100 microspheres to keep acid condition, and phosphate buffers of different pH were employed as the dissolution medium for HP-55 microspheres and AS-H microspheres. One percent (w/v) of Tween-80 was added in each a dissolution medium to maintain sink conditions for the drug. Except where mentioned otherwise, the rotating speed was fixed at 100 rpm, and the pH of the dissolution medium for EuE-100 microspheres, HP-55 microspheres and AS-H microsheres were fixed at 1.2, 6.4 and 7.8, respectively.

2.4. Release test of HP-55 microspheres under simulated gastric pH conditions

The drug-release tests on HP-55 microspheres were performed for 2 h in 500 mL hydrochloric acid solution containing 1.0 % (w/v) Tween-80. The release tests were then continued for another 2 h after adding 200 mL sodium phosphate solution (0.132 M) containing 1.0% (w/v) Tween-80 to adjust the pH and the volume of the dissolution medium to 6.4 and 700 mL, respectively.

2.5. Release test of AS-H microspheres under simulated gastric and upper intestinal pH conditions

Firstly, the drug-release tests on AS-H microspheres were performed for 2 h in 500 mL hydrochloric acid solution (pH 1.2) containing 1.0% (w/v) Tween-80. The release tests were then continued for another 2 h after adding 200 mL sodium phosphate solution (0.132 M) containing 1.0% (w/v) Tween-80 to adjust the pH and the volume of the dissolution medium to 6.4 and 700 mL, respectively. Finally, another 200 mL sodium phosphate solution (0.066 M) containing 1.0% (w/v) Tween-80 was added to adjust the pH of the dissolution medium to 7.8 and the release test was continued for 3 h, where the final volume of the dissolution medium was 900 mL.

2.6. Release test of the pH-dependent gradient-release delivery system for nitrendipine under simulated gastrointestinal pH conditions

Three types of microspheres (i.e. EuE-100 microspheres, HP-55 microspheres and AS-H microspheres) which formulations had been stated in the Section 2.2.3, were weighed accurately and mixed in the capsules at a ratio of 1:1:1 of nitrendipine, ensuring each capsule

contained 20 mg nitrendipine. The release behavior of nitrendipine from system was tested at pH 1.2 for 2 h, pH 6.4 for 2 h, and then pH 7.8 for 3 h at the rotating speed of 100 rpm. The adjusting of the pH of the dissolution medium was same as above process (2.5.). These pH values are corresponding to the values in the stomach, duodenum and small intestine, respectively.

3. Results

3.1. Factors affecting the release behavior of EuE-100 microspheres

The effect of the amount of excipients formulated on the release rate of EuE-100 microspheres is illustrated in Fig. 1.

When the ratio of the amount of drug to Aerosil was fixed at 1:3, increasing the amount of EuE-100 resulted in a marked increase in drug release. When the ratio of EuE-100 to drug exceeded 3:1, no significant changes could be observed from the release profiles. In addition, the data of Fig. 1 demonstrated that the release rate of the drug was not affected by the amount of Aerosil when the ratio of drug to EuE-100 was fixed at 1:3. In addition, the original powder of nitrendipine exhibited a very low dissolution rate under the same dissolution conditions.

The effects of the pH of the dissolution medium, the rotating speed of the paddle and the particle size of the microspheres on the release behavior of EuE-100 microspheres are shown in Fig. 2. The results showed that the release rate of the microspheres having particle diameter of 280–900 μm at 100 rpm reduced with an increase in the pH of the dissolution medium, whereas the other two factors have no

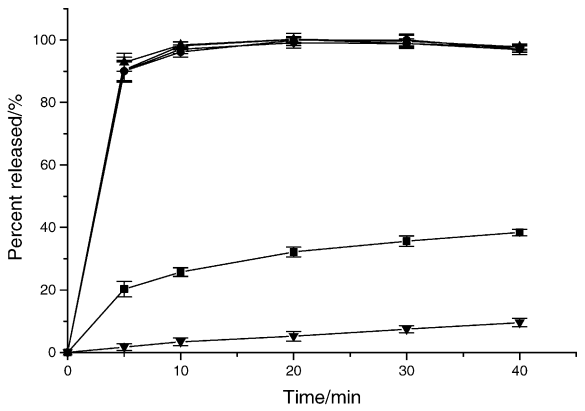

Fig. 1. Effect of the amount of EuE-100 and Aerosil on the release profiles (pH = 1.2, rotating speed: 100 rpm, particle size: 280–900 μm). Nitrendipine:EuE-100:Aerosil = 1:0:0 (▼); 1:2:3 (■); 1:3:2 (♦); 1:3:3 (●); 1:3:4 (▲); 1:4:3 (+).

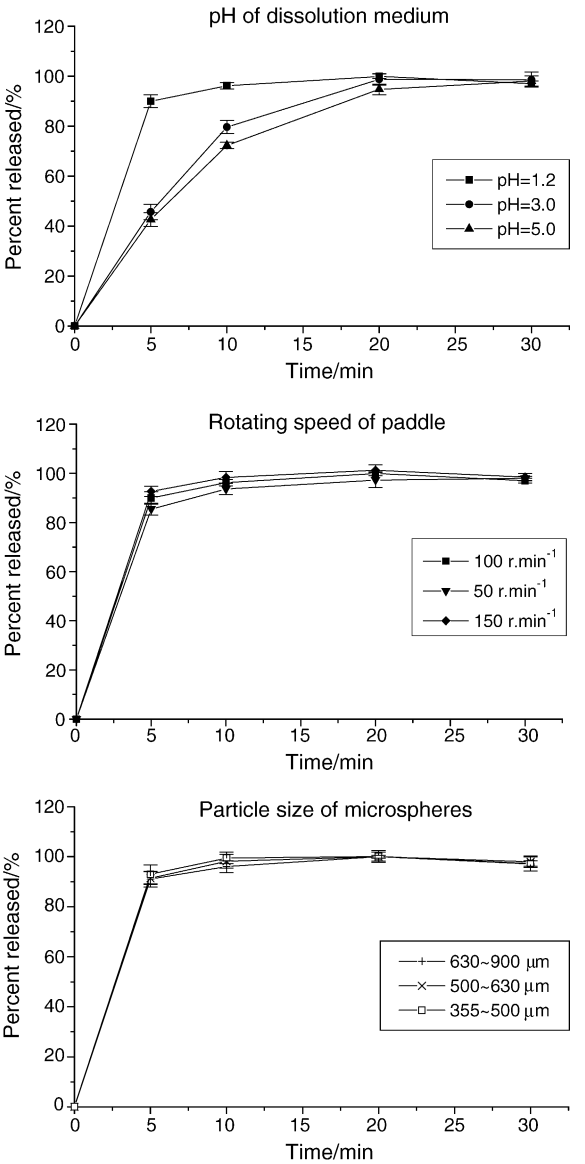

Fig. 2. Effect of pH of dissolution medium, rotating speed of paddle and particle size of microspheres on the release rate of nitrendipine from EuE-100 microspheres.

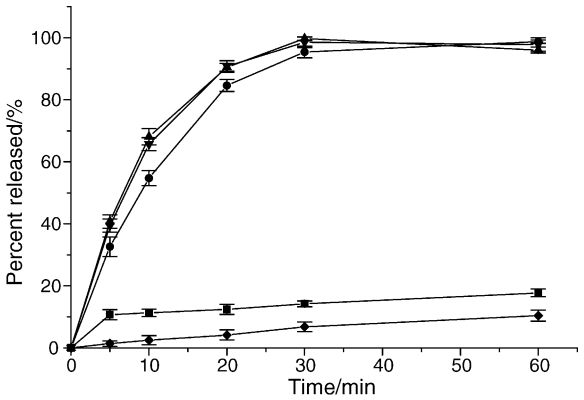

Fig. 3. Effect of the amount of HP-55 on the release profiles (pH = 6.4, rotating speed: 100 rpm, particle size: 280–900 μm). Nitrendipine:HP-55=1:0 (♦); 1:1.33 (■); 1:2 (●); 1:3 (▲); 1:4 (▼).

evident effect on the release behavior of EuE-100 microspheres.

In Figs. 1 and 2, the percent released of the drug tended to be decreased with time once it reached nearly 100%. The same phenomenon was also observed in the results of later release tests. It was believed due to the amount of drugs sampled from the release media was not considered in calculation.

3.2. Factors affecting the release behavior of HP-55 microspheres

The data presented in Fig. 3 show that the release rate of the drug from HP-55 microspheres increased markedly on increasing the amount of HP-55 formulated. It was also found that the release rate was not affected significantly by the amount of HP-55 when the ratio of HP-55 to drug exceeded 2:1.

The effects of the pH of the dissolution medium, the rotating speed of the paddle and the particle size of the microspheres on the release behavior of HP-55 microspheres are shown in Fig. 4. The results showed that the release rate of the drug from HP-55 microsphers having particle diameter of 280–900 μm at 100 rpm increased in increasing the pH value of the dissolution medium and the rotating speed of the paddle, whereas no evident difference could be seen under paddle conditions of the 100 and 150 rpm. In addition, the release rate of the drug only decreased slightly in increasing the particle size of the microspheres.

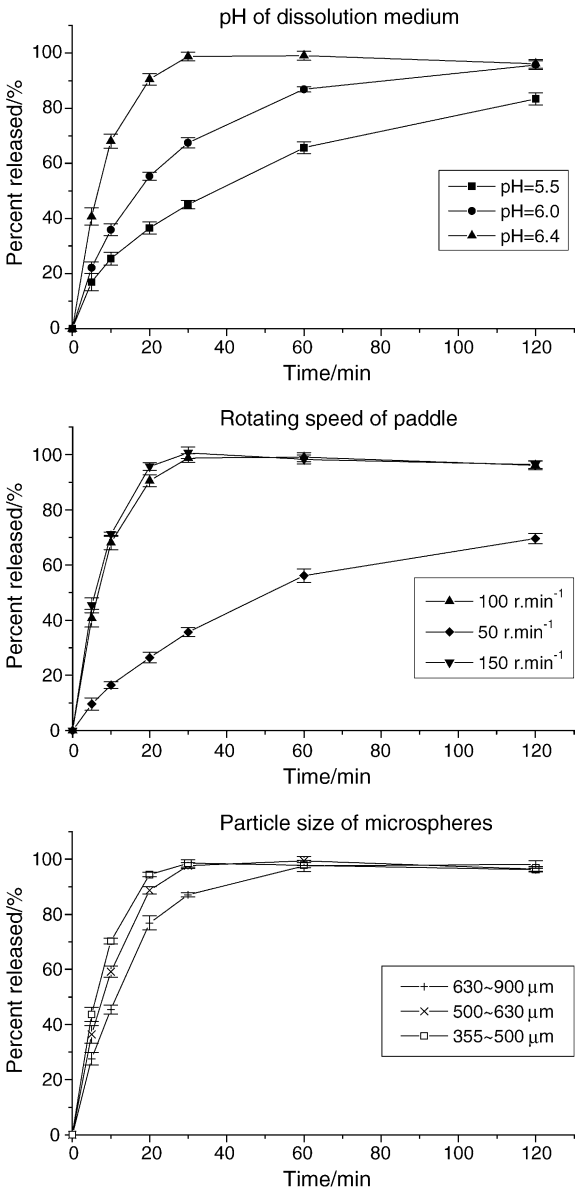

Fig. 4. Effect of pH of dissolution medium, rotating speed of paddle and particle size of microspheres on the release rate of nitrendipine from HP-55 microspheres.

3.3. Factors affecting the release behavior of AS-H microspheres

The effects of the amount of AS-H and the other three factors on the release behavior of AS-H microspheres are shown in Figs. 5 and 6, respectively. The release rate of the microspheres increased in increasing

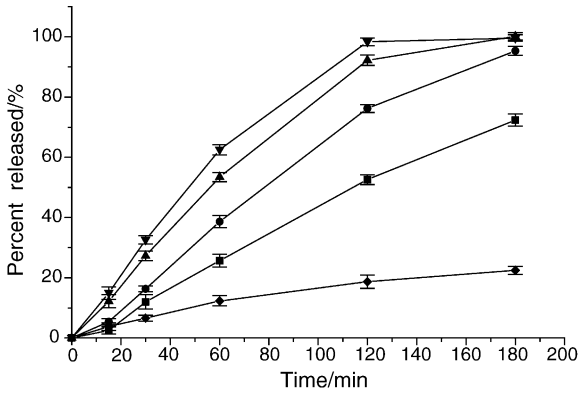

Fig. 5. Effect of the amount of AS-H on the release profiles (pH = 7.8, rotating speed: 100 rpm, particle size: 280–900 μm). Nitrendipine:AS-H = 1:0 (♦); 1:2 (■); 1:3 (●); 1:4 (▲); 1:5 (▼).

the amount of AS-H formulated. In increasing the pH of the dissolution medium with the particle size of 280–900 μm at 100 rpm and the rotating speed of the paddle with the particle size of 280–900 μm in pH = 7.8 dissolution medium, the release rate of the AS-H microspheres was increased. The data in Fig. 6 also showed that the release rate of the drug decreased in increasing the particle size of the microspheres in pH = 7.8 dissolution medium at 100 rpm.

3.4. Effect of simulated gastric and upper intestinal pH conditions on the release behavior of HP-55 and AS-H microspheres

The effect of the simulated gastric and upper intestinal pH conditions on the release behavior of HP-55 and AS-H microspheres is shown in Figs. 7 and 8, respectively.

The data shown in Fig. 7 showed that less than 1% of the drug in HP-55 microspheres was released under the simulated gastric conditions (pH = 1.2). However, the release rate of HP-55 microspheres increased rapidly after the pH value of dissolution medium was adjusted to the pH = 6.4 of the simulated upper segment of the intestine. The data presented in Fig. 8 showed that the cumulative released percentages of AS-H microspheres under the simulated gastric pH conditions (pH = 1.2) and the pH = 6.4 of the simulated upper segment of the intestine were around 2% and 10%, respectively. In addition, the release rate increased immediately after the pH of the dissolution medium was adjusted to the pH = 7.8 of the simulated lower segment of the intestine.

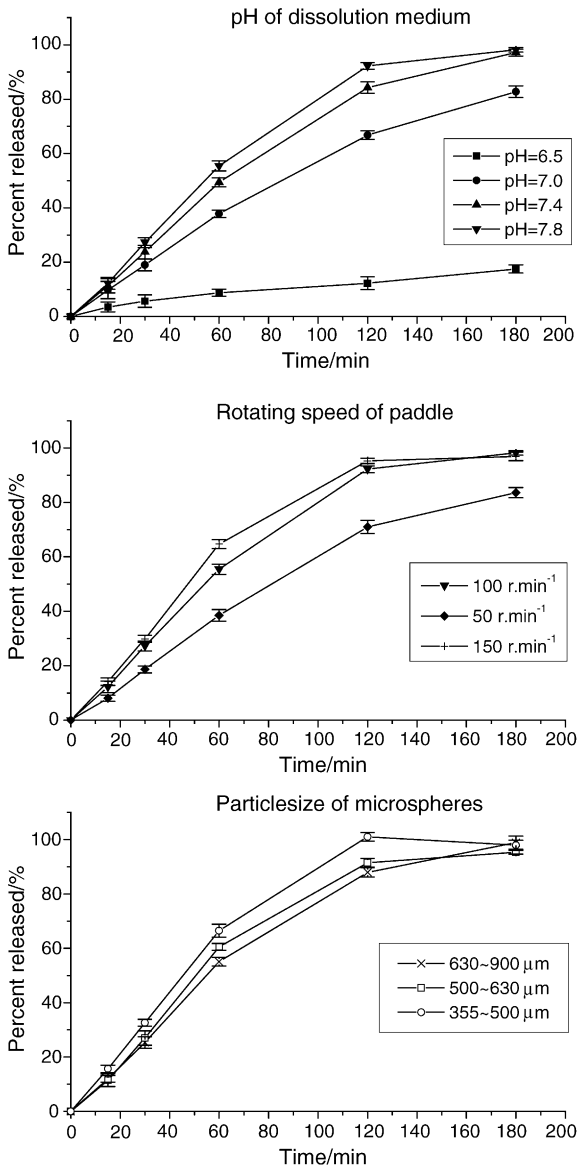

Fig. 6. Effect of pH of dissolution medium, rotating speed of paddle and particle size of microspheres on the release rate of nitrendipine from AS-H microspheres.

3.5. Drug-release behavior of the pH-dependent gradient-release delivery system for nitrendipine under the simulated gastrointestinal pH conditions

Fig. 9 shows the release behavior of pH-dependent gradient-release delivery system for nitrendipine, which was obtained by mixing the three types of pH-

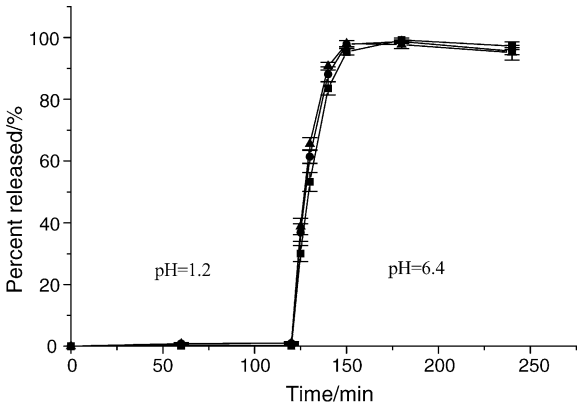

Fig. 7. Effect of simulated gastric pH conditions on release profiles of HP-55 microspheres. Nitrendipine:HP-55 = 1:2 (■); 1:3 (▼); 1:4 (▲).

dependent microspheres at a ratio of 1:1:1 of nitrendipine. It can be seen that EuE-100 and HP-55 microspheres have a fast release rate, which could release drug completely within 30 min, followed by a plateau. However, the release behavior of AS-H microspheres displayed slight sustained-release characteristics.

3.6. Release mechanism

In order to investigate the release mechanism of present drug delivery system, the release data of three types of pH-dependent microspheres were fitted to classic drug-release kinetics models. The release rates were analyzed by least-squares linear regression method.

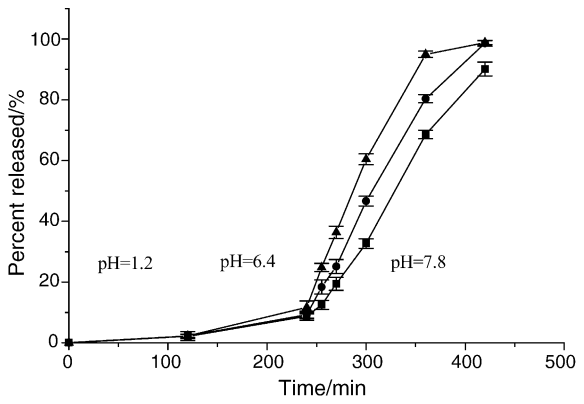

Fig. 8. Effect of simulated gastrointestinal pH conditions on release profiles of AS-H microspheres. Nitrendipine:AS-H = 1:2 (■); 1:3 (▼); 1:4 (▲).

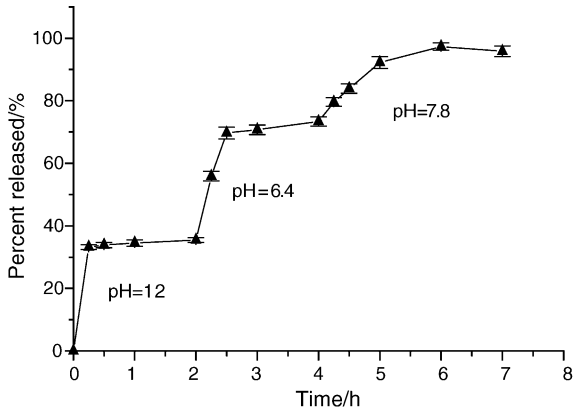

Fig. 9. Release profiles of pH-dependent gradient-release delivery system for nitrendipine under simulated gastrointestinal pH conditions (n = 6).

Except the main models, i.e. zero-order model, first-order model and Higuchi model, which have been suggested to describe drug-release kinetics from microspheres, other models such as Hixson-Crowell's corrosion model (Dredan et al., 1996), Baker-Lonsdale's spherical diffusion model (Akbuga, 1991) and Ritger-Peppas empirical model (Peppas, 1985) were also discussed in this paper. The analysis results are summarized in Table 2.

The formulation of each pH-dependent microspheres in Table 2 is same with that of the microspheres used in the release test of the pH-dependent gradient-release delivery system under the simulated gastrointestinal pH conditions. A survey of Table 2 shows that the coefficient of determination (r^2) of equation for AS-H microspheres was about 0.9 in each case, however, that of EuE-100 and HP-55 microspheres was always less than 0.9.

4. Discussion

The results of the dissolution test demonstrated that the original crystals of nitrendipine had a very low dissolution rate in vitro. However, the drug-release rate from the pH-dependent microspheres was increased clearly in increasing the amount of excipients formulated, because the pH-dependent polymers were used as solid dispersion carriers to improve the dispersing state of the poorly water-soluble drug used in this study.

Table 2
Kinetic data of three types of pH-dependent microspheres

Kinetics		EuE-100 microspheres	HP-55 microspheres	AS-H microspheres
Zero-order	K	1.387	0.325	0.343
	r^2	0.419	0.372	0.923
First-order	K	−8.941	−1.615	−1.391
	r^2	0.398	0.379	0.985
Higuchi	K	1.332	0.657	0.642
	r^2	0.716	0.669	0.952
Hixson-Crowell	K	−5.739	−1.317	−1.212
	r^2	0.517	0.422	0.987
Bake-Lonsdale	K	0.283	0.046	0.162
	r^2	0.351	0.637	0.976
Ritger-Peppas	n	0.047	0.255	0.861
	r^2	0.708	0.708	0.967

k, Release rate constant; r^2, coefficient of determination; n, release exponent.

Especially, when the ratio of the pH-dependent polymer to drug in the microspheres was 3:1 for EuE-100 microspheres, and 2:1 for HP-55 microspheres, the drug-release rate was dramatically increased compared with that of the original crystals, indicating that solid dispersions of drug were formed at these ratios. The release profiles of AS-H microspheres exhibited minor sustained-release characteristics compared with the other two microspheres, so the increase in the release rate of AS-H microspheres was not so evident as that of the other two types of microspheres in increasing the amount of AS-H formulated. However, the release rate of AS-H microspheres at a ratio of polymer to drug of 2:1 was still improved compared with that of the original drug. In addition, the results of X-ray diffractograms also suggested that nitrendipine was molecularly dispersed as amorphous state in microspheres at a ratio of AS-H to drug of 2:1, which are reported elsewhere (Yang et al., 2004). The release rate of drug from EuE-100 microspheres exhibited no change on increasing the amount of Aerosil, although it is also a good solid dispersion carrier (Liao and Jarowski, 1984; Yang et al., 2003). This was believed to be due to the fact that the drug was highly dispersed in EuE-100 microspheres when the ratio of EuE-100 to drug was 3:1, and the EuE-100 dissolved so rapidly that the effect of the Aerosil was masked.

The data in Fig. 2 demonstrated that the release rate of EuE-100 microspheres was not affected by the rotating speed of the paddle, because EuE-100 dissolved very rapidly in dissolution medium of pH 1.2, the effect of the rotating speed being masked. However, the release rate of HP-55 and AS-H microspheres was much lower at a rotating speed of 50 rpm than at 100 and 150 rpm. This was due to the fact that most of these two types of microspheres were settled at the bottom of the dissolution vessel at a low rotating speed and agglomerated after the polymers had swelled and dissolved. This resulted in a retardation of the release rate of the drug from the microspheres. The release profiles, however, exhibited no significant difference between 100 and 150 rpm after the microspheres had been thoroughly dispersed at a high paddle rotating speed. It was found that the rotating speed of the paddle was not the main factor affecting the release rate of the microspheres after it exceeded a critical value. These findings suggest that the release rate of drug from these two microspheres would be reduced under the conditions of low motility in the intestine.

It seems that the particle size of the microspheres has no clear effect on the release behavior of EuE-100 and HP-55 microspheres. The reason for this was that these two microspheres dissolved rapidly in the respective dissolution medium, and the effect of the particle size on the release rate could not be observed clearly under such dissolution testing conditions. However, the release rate of the drug from AS-H microspheres increased in reducing the particle size of the microspheres due to the increased surface area available for dissolution. And it has been experimentally confirmed that no particle size dependence of the crystalinity of drug in the microspheres was shown in the present study.

In this study, the active drug was incorporated with polymers and was present as a solid dispersion in the

microspheres. The release rate of the drug from the microspheres depended on the dissolution rate of the polymers. All the factors affecting the dissolution rate of the polymers would determine the release rate of the drug from the microspheres. Due to the fact that all three types of microspheres consist of pH-dependent polymers, the pH of the dissolution medium would have a clear effect on the release behavior of the microspheres. As anticipated, with increasing the pH of the dissolution medium, the dissolution rate of EuE-100 was reduced, resulting in a reduced release rate of the drug from EuE-100 microspheres. However, the dissolution rate of HP-55 and AS-H improved on increasing the pH of the dissolution medium, which led to an increase in the release rate of drug from these two types of pH-dependent microspheres. These findings suggest that the pH of the dissolution medium is the most important factor affecting the release behavior of microspheres, compared with the other three factors. This is in agreement with our purpose in designing the pH-dependent gradient-release drug delivery system, i.e. that the active drug was released from the system at different regions of the gastrointestinal tract as the pH-dependent microspheres dissolved under corresponding pH conditions in the gut. This will result in extending the therapeutic period of the active drug as the system is transported down the gastrointestinal tract. In addition, the sustained-release characteristics of AS-H microspheres would be expected to prolong the pharmacological action of the active drug in vivo as the microspheres are retained at the lower intestinal tract.

The results of the dissolution testing of HP-55 microspheres under the simulated gastric pH conditions suggest that these microspheres are stable under acid conditions and HP-55 could efficiently prevent the release of drug before HP-55 microspheres reach the upper segment of the intestine. Moreover, the microspheres could release the drug completely within a short time (30 min), followed by a plateau in the release profiles after the pH of the dissolution medium was adjusted to 6.4. Also, the results of the release behavior of AS-H microspheres under the simulated gastric pH conditions indicated that they are stable under acid conditions for 2 h. Although a less release of drug was observed at a pH of 6.4, about 90% of the accumulated released percentages of drug from AS-H microspheres were retained after the pH value of the dissolution medium was adjusted to the pH conditions of the simulated lower segment of the intestine. This finding indicated that AS-H would effectively reduce the release of drug from AS-H microspheres before arriving at the target region of the gut. These results suggest that the microspheres fabricated with HP-55 and AS-H have a dense matrix structure and the release rate of the active drug can be controlled efficiently before the system reaches the target region. This could be supported from the SEM of these two kinds of microspheres, which were reported in another article (Yang et al., 2004). This is a key point for the reliable design of gradient-release preparations. It shows that these two types of polymers are suitable for designing pH-dependent gradient-release drug delivery systems with a matrix structure.

At last, the drug-release behavior of the pH-dependent gradient-release delivery system for nitrendipine was investigated under the simulated gastrointestinal pH conditions. The results showed that the release profiles of the system exhibited obvious gradient-release characteristics, which suggested that the drug delivery system with evident pH-dependent gradient-release behaviors could be obtained using present method, i.e. preparing respectively three types of pH-dependent microspheres firstly, then mixing and filling them into a capsule at a certain ratio.

Due to the fact that the release behavior of the present drug delivery system under the simulated gastrointestinal pH conditions was a complex profile of three types of pH-dependent microspheres, it is difficult to study its release kinetics by simulating the whole curve with release kinetic models. The release mechanisms of the present drug delivery system were investigated by fitting the release data of each pH-dependent microspheres with classic drug-release kinetic models. The results showed that these models were not suitable for estimating the release kinetics of EuE-100 microspheres and HP-55 microspheres. It was believed to be due to the immediate release of these two types of microspheres caused by rapid dissolution of related polymers. The coefficient of determination for AS-H microspheres was about 0.92–0.99, indicating that the data represent diffusion control. This result was attributable to the slight sustained-release of the drug from AS-H microspheres. In addition, this finding suggested that the release of nitrendipine from AS-H microspheres contained diffusion-controlled

process due to the relatively slow dissolution rate of AS-H, although the drug were mainly released with the dissolution of AS-H in corresponding pH condition.

In conclusion, the release rate of the drug from the microspheres improved on increasing the amount of respective pH-dependent polymers formulated, and it was controlled by the dissolution rate of the polymers, since the active drug is present as a solid dispersion in the microspheres. Besides the amount of excipients formulated, the pH of dissolution medium was the most important factor affecting the release rate of the pH-dependent microspheres compared with the other two factors, while the particle size and the rotating speed of the paddle had only a minor effect on the release behavior of the microsphers. The release behavior of the present drug delivery system exhibited obviously pH-dependent and gradient-release characteristics under simulated gastrointestinal pH conditions, even though the system involves a matrix structure. It suggested that the present method was suitable for improving the availability of poorly water-soluble drug, i.e. improving its dissolution rate and retarding the therapeutic period in vivo.

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References

Akbuga, J., 1991. Furosemide-loaded ethyl cellulose microspheres prepared by spherical crystallization technique: morphology and release characteristics. Int. J. Pharm. 76, 193–198.

Ashford, M., Fell, J.T., Attwood, D., Woodhead, P.J., 1993. An in vitro investigation into the suitability of pH-dependent polymers for colon targeting. Int. J. Pharm. 91, 241–245.

Dredan, J., Antal, I., Racz, I., 1996. Evaluation of mathematical models describing drug release from lipophilic matrices. Int. J. Pharm. 145, 61–64.

Gupta, V.K., Beckert, T.E., Price, J.C., 2001. A novel pH- and time-based multi-unit potential colonic drug delivery system. I. Development. Int. J. Pharm. 213, 83–91.

Khan, M.Z.I., Prebeg, Z., Kurjakovic, N., 1999. A pH-dependent colon targeted oral drug delivery system using methacrylic acid copolymers. I. Manipulation of drug release using Eudragit L100-55 and Eudragit S 100 combinations. J. Control. Release 58, 215–222.

Khan, M.Z.I., Stedul, H.P., Kurjakovic, N., 2000. A pH-dependent colon-targeted oral drug delivery system using methacrylic acid copolymers. II. Manipulation of drug release using Eudragit L100 and Eudragit S100 combinations. Drug Dev. Ind. Pharm. 26, 549–554.

Liao, C.H., Jarowski, C.I., 1984. Dissolution rates of corticoid solutions dispersed on silicas. J. Pharm. Sci. 72, 401–403.

Palmieri, G.F., Michelini, S., Martino, P., Martelli, S., 2000. Polymers with pH-dependent solubility: possibility of use in the formulation of gastroresistant and controlled-release matrix tablets. Drug Dev. Ind. Pharm. 26, 837–845.

Peppas, N.A., 1985. Analysis of fickian and non-fickian drug release from polymers. Pharm. Acta Helv. 60, 110–111.

Risbud, M.V., Hardikar, A.A., Bhat, S.V., Bhonde, R.R., 2000. pH-sensitive freeze-dried chitosan-polyvinyl pyrrolidone hydrogels as controlled release system for antibiotic delivery. J. Control. Release 68, 23–30.

Song, H., Guo, T., Zhang, R., Zheng, C., Ma, Y., Li, X., Bi, K., Tang, X., 2002. Preparation of the traditional Chinese medicine compound recipe heart-protecting musk pH-dependent gradient-release pellets. Drug Dev. Ind. Pharm. 28, 1261–1273.

Watts, P.J., Illum, L., 1999. Colonic drug delivery. Drug Dev. Ind. Pharm. 23, 893–913.

Yang, M.S., Cui, F.D., You, B.G., Fan, Y.L., Wang, L., Yue, P., Yang, H., 2003. Preparation of sustained-release nitrendipine microspheres with Eudragit RS and Aerosil using quasi-emulsion solvent diffusion method. Int. J. Pharm. 259, 103–113.

Yang, M.S., Cui, F.D., You, B.G., You, J., Wang, L., Zhang, L.Q., Kawashima, Y., 2004. A novel pH-dependent gradient-release delivery system for nitrendipine. I. Manufacturing, evaluation in vitro and bioavailability in healthy dogs. J. Control. Release 98, 219–229.