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Preparation and *in vitro/in vivo* evaluation of a pantoprazole sodium drug-resin liquid delayed release suspension

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Abstract: A drug-resin liquid delayed-release suspension of pantoprazole sodium (PAZ-Na) was prepared to improve the effectiveness, convenience and safety of peptic ulcer treatment in children, the elderly, and patients with dysphagia. Pantoprazole sodium drug-resin complexes (PAZ-Na-DRC) were prepared using the bath method. The fluidized bed coating method is used to coat it and then add excipients to make a dry suspension prepared before use. The parameters of the in vitro release experimental conditions were optimized and the drug release curve showed delayed release. Rats were given commercial PAZ-Na enteric-coated pellet capsules and the PAZ-Na delayed release suspension via intragastric administration. The results showed that the T_{max} of the PAZ-Na delayed release suspension was increased from 2h to 4h compared with the PAZ-Na enteric-coated pellet capsules. Similarly, the C_{max} was reduced from 6.162μg/mL to 3.244μg/mL with the concentration-time curve is very gentle compared with the commercial drug capsules. After oral administration, the relative bioavailability of PAZ-Na delayed release suspension (AUC_{0-24} of 19.578 $\mu g \cdot h \cdot mL^{-1}$) compared with the commercial drug (AUC_{0-24} of 17.388 $\mu g \cdot h \cdot mL^{-1}$) was 112.67%. The findings showed that the PAZ-Na delayed release suspension for oral administration was successfully formulated with highly improved pharmacokinetic indices.

Keywords: Anion exchange resin, pantoprazole sodium, delayed release suspension.

INTRODUCTION

Peptic ulcer disease (PUD) is a sources of significant morbidity and mortality worldwide. The prevalence of peptic ulcer disease in China is estimated to be 10% and its mortality is 7% (Kavitt *et al.*, 2019). PUD refers to ulcerative disease that occurs in the upper part of the gastrointestinal tract from top to bottom in the lower esophagus, lower stomach, and upper duodenum (Dunlap and Patterson, 2019). Its pathogenesis is due to excessive secretion of acidic gastric juice in the body, which results in pH imbalance in the stomach, and eventually causes reactions such as mucosal damage and gastrointestinal pain (Ebell, 1992). In recent years, the incidence of peptic ulcer disease in China has continued to increase year by year, and the onset age of this disease is younger (Minli, 2016). Peptic ulcer disease has also been found to be associated with increasing age. A significant decrease in the diagnosis of peptic ulcer disease and its associated complications has been observed with age, both in China and elsewhere in the world. The disease seriously affects the quality of people's lives and work, and therefore, anti-ulcer drugs will have a great market demand.

Proton pump inhibitors (PPIs) are currently the most effective anti-ulcer drugs, with fast action, long duration, and good acid suppression (Hu, 2017). PAZ-Na, a third-generation proton pump inhibitor, can cause hydrogen, potassium, and adenosine triphosphatase in the cell membrane of the gastric parietal to lose the function of acidity and reduce the degree of acidification of the gastric mucosa. The PAZ-Na also has a weak inhibitory effect on cytochrome P450 enzymes, which are relatively stable under neutral and weak acidic conditions, but rapidly activated under strong acidic conditions. The pH-dependent activation properties of PAZ-Na make it more selective for the action of H^+/K^+ ATP pumps (Zou *et al.*, 1999; Liu and Wang, 2001; Shi *et al.*, 2005). Studies have shown that the use of pantoprazole sodium in the treatment of patients with peptic ulcer or bleeding has a shorter treatment period, a lower recurrence rate and better treatment response, better recovery of gastric pH, a higher level of safety, and safe for both adults and children (Dipasquale *et al.*, 2022). PPI is a common pediatric drug, and now the issue of children's medication has received great attention from the state. In China, the Drug Review Center has successively issued four technical guidelines, including "the Technical Guidelines for Clinical Trials of Drugs in Pediatric Populations". Gradually establish and improve a scientific technical review system for children's medication (Chen *et al.*, 2021). Currently, PPIs suitable for children are available

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on the foreign market, including pantoprazole. However, the corresponding products have not been approved in China (Arenas-López *et al.*, 2004).

Ion exchange resin (IER) is an insoluble ionic material consisting of a structural moiety (usually consisting of a polymer matrix cross-linked with styrene and divinylbenzene) and a functional moiety (ionic active groups). Resins can be divided into cationic and anion exchange resins as they contain positively or negatively charged groups (Guo *et al.*, 2009). IER has important functions in the development of drug delivery systems to optimize drug treatment and improve patient compliance (Zhang *et al.*, 2022). Because of its excellent properties such as high exchange capacity, good stability, good adsorption, and almost insoluble in any solvent, IER has become an ideal carrier choice for taste masking and control drug release (Shah *et al.*, 2009). Benzimidazole the basic chemical structure of PAZ-Na, is unstable to humidity and heat, especially in acidic environments, but relatively stable under alkaline conditions. Therefore, loading PAZ-Na into the resin can keep the drug properties stable. The ion exchange effect of the resin gives the drug release a sustained-release effect (Bhise *et al.*, 2008).

In this paper, PAZ-Na was selected as the model drug, ~~and~~ the self-pharmaceutical grade anion exchange resin was used as the carrier, and ion exchange technology and extended release microencapsulation technology were used to improve the stability and bioavailability of the drug. Compared with conventional dosage forms, PAZ-Na was made into enteric-coated extended-release suspension, which had the advantages of easy swallowing, flexible adjustment of drug dose, high patient compliance, and a wider range of suitable groups (children, elderly, and patients with dysphagia). And it has a sustained-release effect, which can prolong the time of administration.

MATERIALS AND METHODS

Materials

PAZ-Na was purchased from Zhuhai Berry Pharmaceutical Technology Co., Ltd. (Zhuhai, China). Polyethylene glycol 4000 was purchased from Sinopharm Chemical Reagent Company (Shanghai, China). Ethanol was purchased from Xilong Science Co., Ltd. (Shantou, China). CYP compound enteric-coated film coating premix was purchased from Shanghai Puli Film Preparation Excipients Co., Ltd. (Shanghai, China). Oppa was purchased from Colorcon. Pantoprazole sodium enteric-coated pellets were purchased from Hubei Weisen Pharmaceutical Co., Ltd. (Ezhou, China). Methanol, sodium chloride, sodium hydroxide, sodium acetate, toluene, dichloromethane, potassium dihydrogen phosphate, silicon dioxide, glucose, HPMC, ether, ethyl acetate, and sodium heparin purchased were from Shanghai Test Agent Co., Ltd. (Shanghai, China). Anhydrous citric acid, poloxamer, and tinidazole were purchased from Aladdin. Xanyuan gum was purchased from Hubei Gedian Renfu Pharmaceutical Co., Ltd. (Wuhan, China). The sweet orange flavor was purchased from Shenzhen trading house Piaoxiang Garden (Shenzhen, China). Propylparaben was purchased from Zhejiang Shengxiao Chemical Co., Ltd. (Quzhou, China). CMC-Na was purchased from J. RETTENMAIER & SÖHNE。

Animals

Twelve SD rats (6 male SPF rats and 6 female SPF rats, weighing 200-220g) were purchased from the Experimental Animal Center of Jiangsu University. The qualification certificate number was N0.202101115. All animal study conditions were carried out in strict accordance with the standard protocol approved.

Preparation of the drug-resin complexes

Pantoprazole sodium drug-resin complexes (PAZ-Na-DRC) was prepared using the bath method. PAZ-Na solution was prepared by dissolving a certain amount of PAZ-Na in 100mL of NaOH solution (pH=9). After that, resins were dispersed into PAZ-Na solution with magnetic stirring at constant temperature for 2 h. Samples were timed and diluted after filtration with a 0.45 μm water film to determine the absorbance at 289 nm. The drug resin was filtered and washed with pure water 3 times when the concentration was stable (the ion exchange process reached dynamic equilibrium). The drug-resin complex was obtained by drying at 45°C.

Optimization of parameters of resin loading process

The parameters of resin loading process were investigated as follows: drug loading temperature (25°C, 37°C, 45°C, 60°C), PAZ-Na drug concentration (1mg/mL, 2mg/mL, 3 mg/mL, 4mg/mL, 4.5mg/mL), dosage ratio of drug to resin (4:1, 2:1, 1:1, 2:3). The drug loading process parameters were optimized by the drug load capacity (Qt), drug utilization rate (E) and drug loading degree (F) of the resin (Yang *at al.*, 2021). Its calculation formula is as follows.

$$Q_t = \frac{(C_0 - C_t)V}{W_R} \quad (1)$$

$$E = \frac{(C_0 - C_t)}{C_0} \quad (2)$$

$$F = \frac{Q}{Q_\infty} \quad (3)$$

In the above formula, V(mL) is the volume of pH = 9 NaOH solution; W_R (mg) is the amount of resin added; C_0 (mg/mL) is the initial concentration of PAZ-Na solution; C_t (mg/mL) is the concentration of PAZ-Na solution in t time; Q_∞ (mg/mg) is the amount of resin loaded after drug loading.

Characterization of drug-resin complexes

Scanning electron microscope (SEM)

The S-4800 Field Emission Scanning Electron Microscope (Hitachi Limited, Tokyo, Japan) was used. The sample is washed and vacuum-dried and then sprayed with gold. Test voltage: 15KV; Magnification: 500. Observe the morphology of blank resin and PAZ-Na-DRC.

X-ray diffraction (XRD)

The D8 ADVANCE X-ray diffractometer (BRUKER, NASDAQ, USA) was used. PAZ-Na, resin, PAZ-Na resin physical mixture (1:1), and PAZ-Na-DRC were sent for testing. The test conditions are as follows: room temperature; Cu target; Detector: semiconductor array detector; Angle test range (2θ): 10~80°; Scanning speed 7°/min.

Tests and evaluation of in vitro release

In vitro release tests were carried out using the USP paddle (apparatus II) method with an RC806D Intelligent Drug Dissolution Meter (Tianjin Tianda Tianfa Technology Co., Ltd, Tianjin, China) at 100 rpm with 900mL of 0.15 M NaCl at 37.0± 0.5ºC. Regularly collect samples (5 mL each) and rehydrate. The samples were also passed through a 0.45μm membrane. The absorbance was determined with a UV-1800PC type UV spectrophotometer (Shanghai Meipuda Instruments Co., Ltd., Shanghai, China) at a wavelength of 289 nm. The effects of release temperature, rotational speed, medium volume, and other factors on *in vitro* release were investigated to determine the final method.

The similarity factor method was used to study the influencing factors of drug release. The formula for the method is as follows:

$$f_2 = 50\lg\{[1+(1/n)\sum W_t(R_t-T_t)^2]^{-0.5}\times 100\} \quad (4)$$

Where Rt and Tt are the accumulated dissolution data at time t in different experimental conditions; n is the total number of samples. The larger the f_2, the higher the fitting degree. When f_2 >=50, the release behavior is similar.

Investigation of in vitro release test

The parameters of *in vitro* release test were investigated as follows: medium volume (200mL, 600mL, 900mL), medium ion composition (0.15mol/L CH_3COONa, 0.15mol/L NaCl), medium temperature (25.0±0.5℃, 37.0±0.5℃, 45.0±0.5℃), medium ion concentration (0.15mol/L NaCl, 0.6mol/L NaCl, 1.0mol/L NaCl), paddle speed (30, 50, 100r/min). The final choice of parameters is determined based on *in vitro* release behavior.

Impregnation of the drug-resin complexes

Measure a certain volume of deionized water into a beaker, and add an appropriate amount of PEG 4000 to it to make the solution concentration 20% (w/v). Stir to 45°C by magnetic heating. When PEG was completely dissolved, PAZ-Na drug resin was added, stirred for 45 min, and then filtered, dried, and sifted.

Coating process of PAZ-Na-DRC

PAZ-Na microencapsulated drug-resin complexes (PAZ-Na-MC) was prepared based on the fluidized bed coating method. The WBF-1G multifunctional fluidized bed (Chongqing Yingge Granulation Coating Technology Co., Ltd., Chongqing, China) was selected. The enteric coating material selected for this project is CYP compound enteric-coated film coating premix, and a solvent (dichloromethane: ethanol=3:1) is added according to the instructions to prepare enteric coating solution with a certain solid content. According to the previous research of the research group, the PAZ-Na-DRC isolation layer coating was carried out using Opadry. The fluidized bed coating parameters of PAZ-Na-DRC were as follows: inlet air quantity 40 m^3/h, inlet air temperature 35 °C, and liquid injection rate 2 mL/min. The coating prescription was determined to have a solid content of 15% in the coating solution and a 30% weight gain from continuous coating to the microencapsulated membrane.

Characterization of PAZ-Na-MC

Scanning electron microscope

The S-4800 Field Emission Scanning Electron Microscope (Hitachi Limited, Tokyo, Japan) was used. The sample is washed and vacuum dried and then sprayed with gold; Test voltage: 15KV. The apparent morphology of PAZ-Na drug resin coating before and after was observed.

Particle size

Using a dynamic light scattering meter (Malvern Instrument Co., Ltd., Shanghai, China), take an appropriate amount of PAZ-Na-MC and evenly disperse it in a suitable medium, and start recording data when the measured response value is reached. Samples are measured three times in parallel and averaged.

Content

PAZ-Na-MC solution was prepared by dissolving a certain amount of PAZ-Na-MC in 200 mL media solution (pH=6.8). After that, the solution magnetic stirred at a constant temperature for 4 h. The samples were also passed through a 0.45μm membrane. The absorbance was determined with a UV-1800PC type UV spectrophotometer (Shanghai Meipuda Instruments Co., Ltd., Shanghai, China) at a wavelength of 289 nm.

Prescription screening of liquid delayed release suspension

Precisely weigh the prescribed amounts of PAZ-Na-MC (containing PAZ-Na 40 mg), anhydrous citric acid, glucose, poloxamer, sweet orange flavor, propylparaben,

and different suspensions to be investigated. After vortexing, add 100 mL of deionized water and mix well. Taking the sedimentation volume ratio and redispersibility as the evaluation index, the suspension aids of different types of suspension agents (xanthan gum, HPMC, CMC-Na) were investigated.

Quality investigation of PAZ-Na delayed release suspension

Sedimentation volume ratio (F)

Place the sample (50 mL) in a graduated cylinder with a stopper, keep the container tightly closed and shake vigorously for 1 min, at which point the initial height is recorded as H_0. After standing for 2 h, the final height was recorded as H, and the settlement volume ratio (F) was calculated according to the following formula. The F value should be greater than 0.9, and the larger the F value, the more stable the suspension (Miao *et al.*, 2021).

$$F = \frac{H}{H_0} \quad (5)$$

Redispersibility (RI)

The suspension placed in the clogged tube is centrifuged at 500 r/min for 5 min and then allowed to stand for 24 h. Rotate and invert the test sample by 180°. Record the number of rotations required to disperse into a homogeneous suspension after shaking. The fewer rotations required, the better the dispersion (Zhou *et al.*, 2021).

Content

Precision weighing of PAZ-Na delayed release suspension 10 mL (containing PAZ-Na 4 mg) in 200 mL media solution (pH=6.8). After that, the solution magnetic stirred at a constant temperature for 4 h. The samples were also passed through a 0.45μm membrane. The absorbance was determined with a UV-1800PC type UV spectrophotometer (Shanghai Meipuda Instruments Co., Ltd., Shanghai, China) at a wavelength of 289 nm.

In vitro release

Weigh PAZ-Na-MC and PAZ-Na delayed release suspension with consistent drug content. *In vitro* release tests were carried out using the USP paddle (apparatus II) method with an RC806D Intelligent Drug Dissolution Meter (Tianjin Tianda Tianfa Technology Co., Ltd, Tianjin, China) at 100 rpm with 900mL of 0.1 M HCl at 37.0±0.5°C. After 2h, discard the solution in the dissolution cup, and add 900 mL of phosphate buffer (pH 6.8) at 37.0±0.5°C. Keep the speed constant, and take samples at the specified sampling time. The sample solution at each time point was filtered through a 0.45μm membrane, and the absorbance was determined with a UV-1800PC type UV spectrophotometer (Shanghai Meipuda Instruments Co., Ltd., Shanghai, China) at a wavelength of 289 nm. Finally, the f_2 factor method was used to evaluate the release behavior of the two.

Amount of drug leakage

Store PAZ-Na delayed release suspension for a certain period at room temperature, and centrifuge for 10 min at 4000 r/min speed. Determine the drug content as described above. The amount of drug leakage (L) is calculated according to equation 6 (Tong *et al.*, 2010).

$$L = \frac{C_t}{C_0} \quad (6)$$

Stability studies

The PAZ-Na delayed release suspension's settlement volume ratio (F), redispersibility (RI), content (C), and amount of drug leakage (L) were tested initially and after 1, 2, 3 and 6 months to confirm their stability.

In vivo study

The *in vivo* evaluation was performed using six male SD rats and six female SD rats (weighing 200-220g). The rats were randomly and equally assigned to groups A and B (half male and half female). They were fasted for at least 12 hours (free access to water was allowed). A dose of 20mg/kg body weight was given by gavage. Group A was fed on commercially available PAZ-Na enteric-coated microcapsules after grinding and dissolving, while group B was fed on PAZ-Na delayed release suspension. The blood collection was set at different time points (0.25h, 1h, 1.5h, 2h, 3.5h, 4h, 5h, 6h, 8h, 12h, 24h) after the drug administration.

Assay of PAZ-Na in plasma samples

Precise aspiration of 500 μL of rat plasma sample is placed in a centrifuge tube. Add 50μL of methanol-water (methanol: water = 50:50), 50μL of internal standard solution (tinidazole) and 3 mL of extraction solvent ether-ethyl acetate (volume ratio 3:2). Vortex for 5 min to mix well and extract well, and centrifuge at 10 °C and 12000r/min for 10 min (Wang *et al.*, 2016; Yang *et al.*, 2010). The upper organic phase was placed in another test tube, heated in a water bath at 40 °C and dried with nitrogen. Add 100μL of mobile phase reconstituted sample, centrifuge at 10000r/min for 6min, and the supernatant injected into the High Performance Liquid Chromatography (HPLC).

The HPLC was equipped with a column (Unitary Silica 4.6mm×250mm, 5μm, 100A) and connected with a 289nm ultraviolet detector. The flow rate of the mobile phase (methanol: water = 40: 60) was monitored at 1.0mL/min with an injection volume of 20μL. The experiment was performed at a column temperature of 25°C and a run time of 15 min.

Standard curves were established and the precision, accuracy, specificity, and recovery of the HPLC system were verified and it met the analysis requirements. In the range of 2.5~50 μg/mL, the ratio of peak area to PAZ-Na concentration (R2 = 0.9907) was linearly correlated with

PAZ-Na. A solution of PAZ-Na plasma samples at a concentration of 2.5 μg/mL under the standard curve *in vivo* was serially diluted and injected into a liquid chromatograph. The final test results showed that the limit of quantification and detection of PAZ-Na plasma samples in the *in vivo* analysis method was 450 ng and 125 ng.

Pharmacokinetics study

In vivo data were analysed using a non-compartmental model. Using the data in the plasma concentration-time curve, the relevant pharmacokinetic parameters were calculated with DAS 2.0 pharmacokinetic software.

Relative bioavailability

In this study, rats with gavage the PAZ-Na delayed release suspension were used as the experimental group, and rats with gastric gavage of commercially available PAZ-Na enteric-coated pellet capsules were used as the control group, and the relative bioavailability (F_r) study was carried out using formula (7), and the results were as follows:

$$F_r = \frac{AUC_{0-24}(test)}{AUC_{0-24}(Reference)} \quad (7)$$

In the formula, AUC_{0-24} (test) is the area under the drug-time curve of the self-made PAZ-Na suspension, and AUC_{0-24} (reference) is the area under the drug-time curve of commercially available PAZ-Na enteric-coated pellet capsules.

In vitro and in vivo correlation studies

The cumulative release percentage in vitro was taken as the independent variable, and the absorption fraction *in vivo* was used as the strain variable, and the least squares linear loop was performed Return. The correlation equation and correlation coefficient were obtained to determine the correlation between in vitro release and *in vivo* absorption.

STATISTICAL ANALYSIS

Data were obtained at least in triplicate and expressed as mean + standard deviation (SD). Statistical differences were determined by student's two-tailed t-test. Differences are considered statistically significant at $p < 0.05$.

RESULTS

Optimizing the parameters of the resin loading process

Loading temperature

As the loading temperature gradually increases, the sooner the resin reaches ion exchange equilibrium. When the temperature rises from 25°C to 37°C, the drug load and drug utilization rate show an upward trend. However, when the temperature continued to increase (45ºC to 60ºC), the drug load and drug utilization rate decreased and stabilized (fig. 1). Therefore, 37 ºC was chosen as the loading temperature for the static method in this experiment.

PAZ-Na concentration

With the change of drug concentration, the drug load and drug utilization rate of resin change significantly. When the concentration of PAZ-Na solution increased from 1 mg/mL to 4 mg/mL, the drug load increased significantly, and the drug utilization rate gradually decreased. As the concentration continued to increase to 4.5 mg/mL, the drug load increased slowly (fig. 2). It shows that the exchangeable ion group of the resin is basically exchanged by the drug ion, and the excess drug continues to increase the drug load, but the drug utilization rate is low. Considering the drug load and drug utilization rate, the initial concentration of 4 mg/ml of PAZ-Na solution was determined for drug loading.

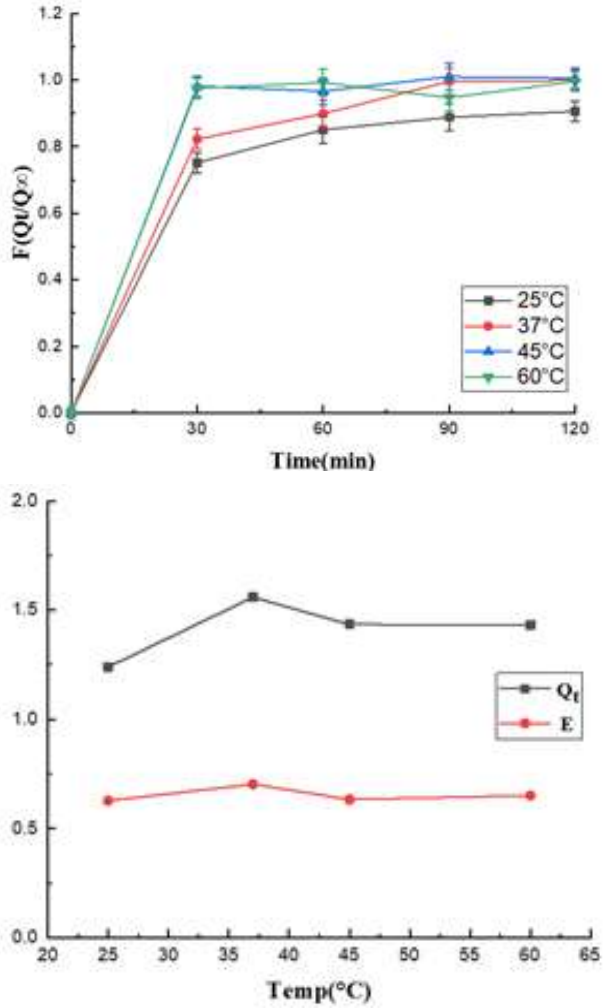


Fig. 1: The influence of temperature on drug loading. The influence of temperature on drug loading and drug utilization.

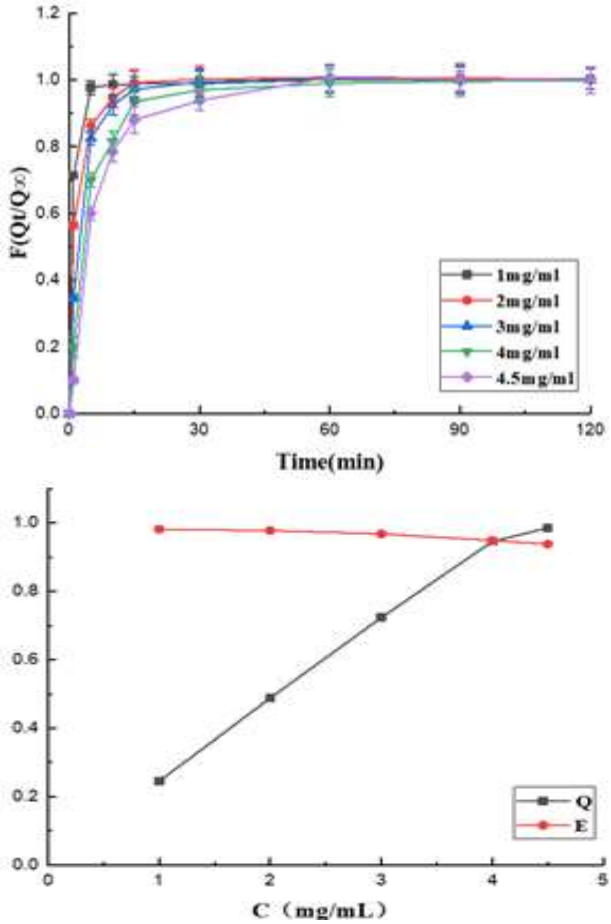


Fig. 2: The influence of PAZ-Na concentration on drug loading, Ion exchange isotherm (25℃±0.5℃)

Drug-resin ratio

When the drug concentration is constant, with the increase of resin addition, the drug load gradually decreases, and the drug utilization rate gradually increases and tends to be stable (fig. 3). Under the premise of high drug load and drug utilization, the ratio of drug to resin was determined to be 1:1.

Through the above single-factor investigation of the bath method, the optimal process for drug loading was determined as follows: precision weighing 400mg PAZ-Na was added to 100mL pH=9 NaOH solution and dissolved with magnetic stirring at 37°C. Add 400mg of resin and stir at constant temperature for 2h. The drug load of PAZ-Na-DRC was measured to be 0.93mg/mg, and the drug utilization rate was 93.57%.

Characterization of drug-resin complexes

The blank resin after crushing and the PAZ-Na-DRC were investigated by SEM and the result was shown in fig. 4. PAZ-Na, resin, PAZ-Na resin physical mixture (1:1), and PAZ-Na-DRC were sent for testing by XRD and the result was shown in fig. 5.

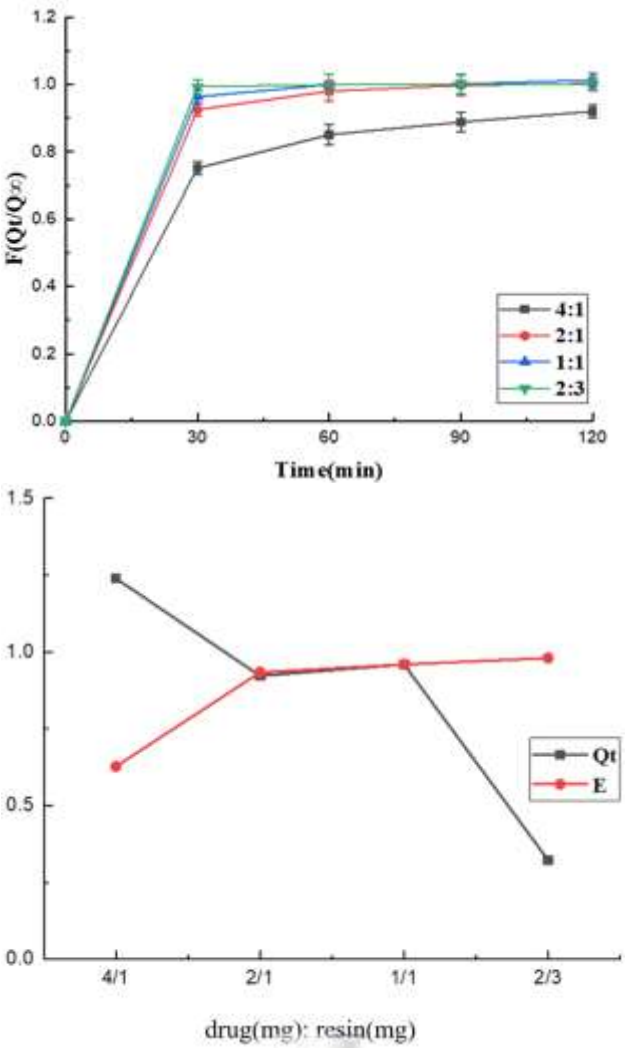
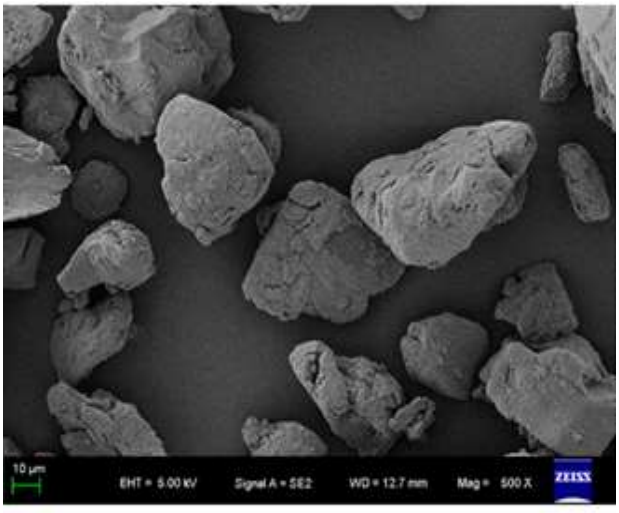
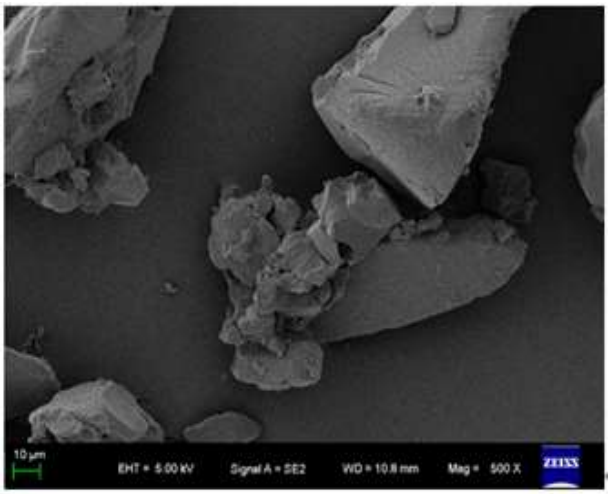


Fig. 3: The influence of resin ratio on drug loading. The influence of resin ratio on drug loading and drug utilization.



A (before drug loading)



A (after drug loading)

Fig. 4: SEM before and after loading with resin

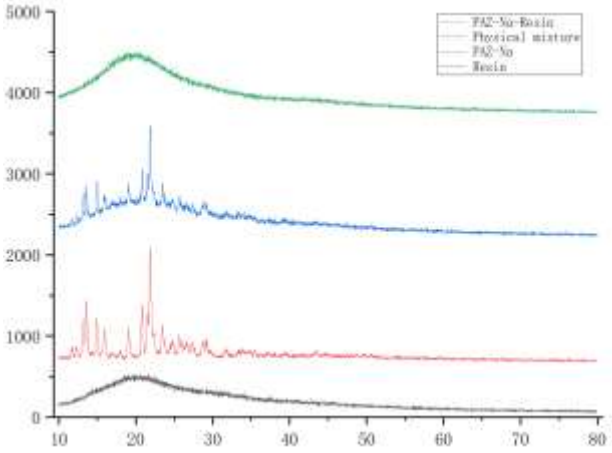


Fig. 5: XRD of blank resin, PAZ-Na, physical mixture and PAZ-Na-resin

Investigation of in vitro release test

Medium volume

When the volume of the medium is small, the drug release is incomplete, and the drug release tends to be complete as the volume of the medium increases. From the f_2 value in fig. 6, the release behavior is similar when the media volume is 600mL and 900mL. To provide enough exchange ions for complete drug release, a medium volume of 900mL was determined.

Medium ion composition

It can be seen from fig. 7 that the degree of drug release is obviously different under different ionic media conditions. When sodium acetate is used as the medium, the drug release is low. Considering the difference in ionic strength between Cl^- and $CH3COO^-$, the ability to replace the drug from the drug-loaded resin is different, and the f_2 value calculated by comparing the two curves is less than 50. In order to make the drug release more thorough, NaCl was selected as the dissolution medium.

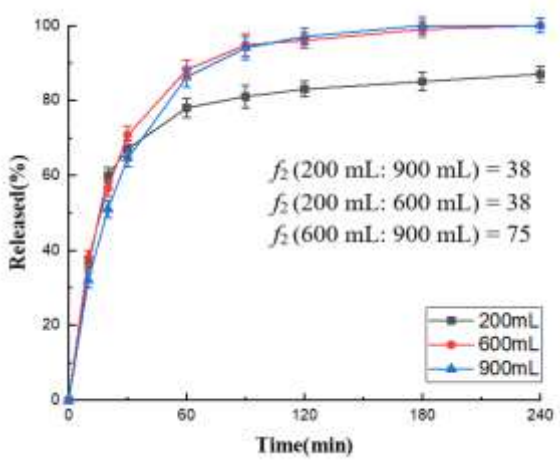


Fig. 6: The influence of dissolution medium volume on PAZ-Na-resin release *in vitro*

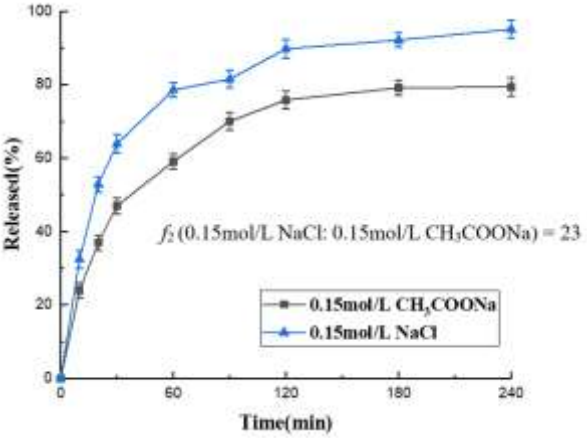


Fig. 7: The influence of medium ion composition on PAZ-Na-resin release *in vitro*

Medium temperature

It can be seen from fig. 8 that the release of drugs increases with the increase of temperature, and the release rate is relatively fast when the temperature is higher. When comparing the two curves with media temperatures of 25°C and 45°C, the f_2 value is 45 and the release behavior of the drug resin is different at different temperatures. Although the f_2 value of the drug-resin complex is greater than 50 in the comparison cases of 25°C and 37°C, 37°C and 45°C, considering that 37°C is the normal human body temperature and the cumulative release of the drug is also high, 37°C is selected as the medium temperature.

Medium ion concentration

It can be seen from fig. 9 that when the ion concentration increases from 0.15mol/L to 0.6mol/L, the drug release rate increases and the release is gradually complete. However, when the concentration continues to increase to 1.0mol/L, the drug release decreases, and the release becomes relatively slow. Although there was no significant difference in the release curve between

0.15mol/L and 0.6mol/L, the concentration of medium was 0.15mol/L NaCl in combination with the human *in vivo* environment.

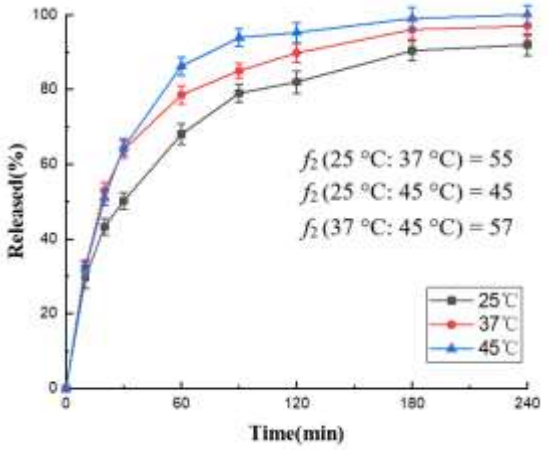


Fig. 8: The influence of dissolution medium temperature on PAZ-Na-resin release *in vitro*

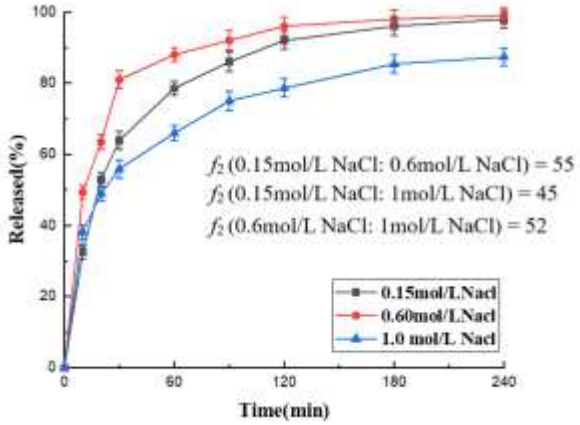


Fig. 9: The influence of medium ion concentration on PAZ-Na-resin release *in vitro*

Paddle speed

It can be seen from fig. 10 that the higher the speed, the faster the drug release speed and the release amount gradually increases. Although the release behavior of 50r/min was similar to that of 100r/min (f_2 value greater than 50), a speed of 100r/min was determined for complete drug release.

Through the determination of the above factors, the method of *in vitro* release determination in this experiment was as follows: at 37.0°C±0.5°C, the drug-resin complex was added to 900mL of 0.15mol/L NaCl solution, and stirred at 100r/min speed.

Characterization of PAZ-Na-MC

The color of PAZ-Na drug resin is light yellow after coating. It can be seen from fig. 11 that the resin surface is smoother after coating, there is obvious wrapping phenomenon, and the coating film is complete and dense. The average particle size of PAZ-Na-MC in table 1 is 157.3μm and the coating content of PAZ-Na-MC in table 1 was greater than 30%, which can be prepared into a suspension preparation.

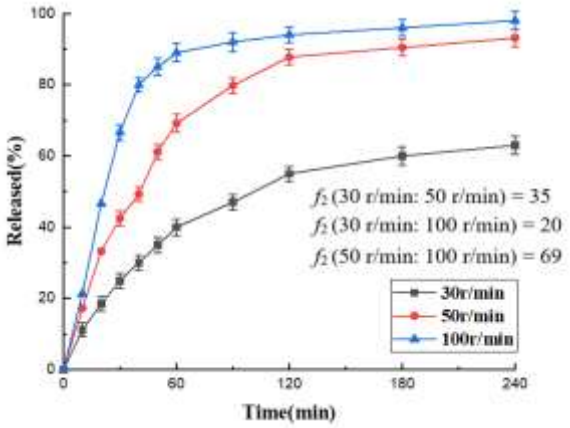
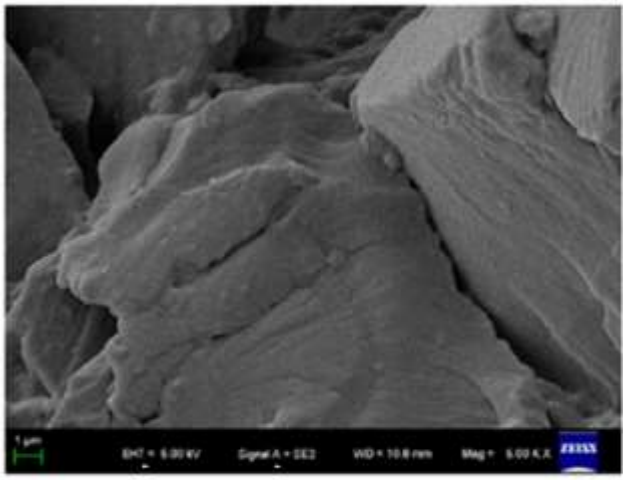
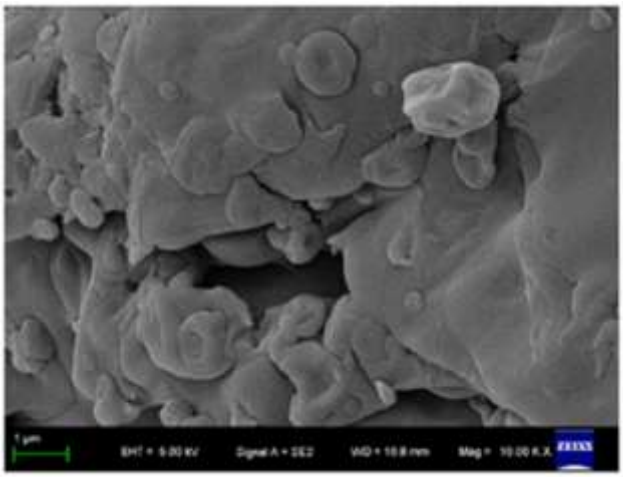


Fig. 10: The influence of paddle speed on PAZ-Na-resin release *in vitro*



a (before coating PAZ-Na-resin



b (after coating PAZ-NA-Re=resin

Fig. 11: SEM before and after coating with PAZ-Na-resin

Reproducibility of in vitro release of PAZ-Na-MC

It can be seen from fig. 12 that the f_2 factor of the three batches of PAZ-Na-MC is greater than 75, indicating that the *in vitro* release behavior of the three batches of samples is similar and the reproducibility is good.

Formulation of PAZ-Na delayed release suspension

After research, the combination of xanthan gum and CMC-Na was finally selected as a suspension aid, and the relevant prescriptions are shown in table 2.

Quality investigation of PAZ-Na delayed release suspension

The drug content, *in vitro* release, and drug leakage of the PAZ-Na delayed release suspension were investigated. The content of three batches of PAZ-Na delayed release suspension was in the range of 98%~105%, and the RSD content of different batches was less than 2% (table 3). It indicates that the uniformity of suspension content is good. The f_2 factor between PAZ-Na-MC and PAZ-Na delayed release suspension is 78 (fig. 13). It was shown that the addition of medium in the suspension had no significant effect on the release of the drug in the PAZ-Na-MC. After the delayed release suspension was left at room temperature for 15 days, the drug leakage was determined to be 0.18%, indicating that the PAZ-Na delayed release suspension had good stability. Therefore, PAZ-Na-MC can be made into a suspension.

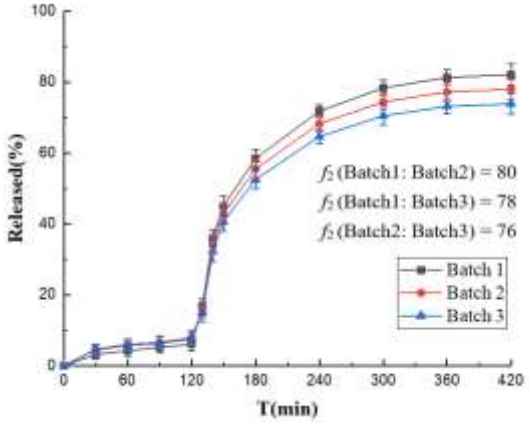


Fig. 12: The *in vitro* release curves of three batches of PAZ-Na-resin

Stability studies

Under the set experimental conditions, the leaked amount of the sample was 0.50% after standing for 6 months, which accounted for a small proportion (table 4).

In vivo and pharmacokinetic studies

PAZ-Na enteric coated capsules and delayed release suspension were administered by gavage in rats, and the drug time curves were shown in fig. 14, and the pharmacokinetic parameters were shown in table 5.

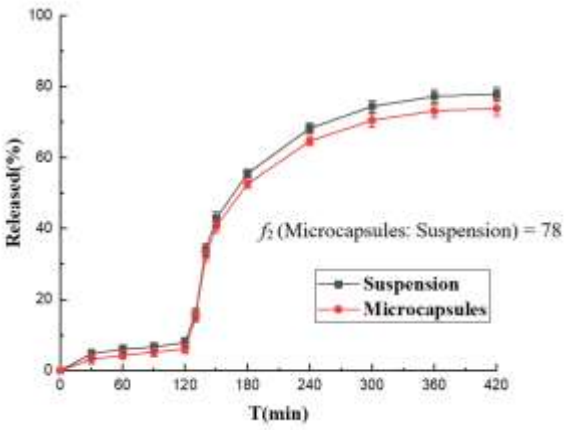


Fig. 13: *In vitro* release curves of PAZ-Na-resin and PAZ-Na delayed release suspension

In vitro and in vivo correlation studies

The data of *in vitro* and *in vitro* drug release are shown in table 6. The regression equation was Fa = 0.8572 F + 13.102 and R^2 = 0.9527, indicating that the correlation *in vitro* and *in vivo* was good.

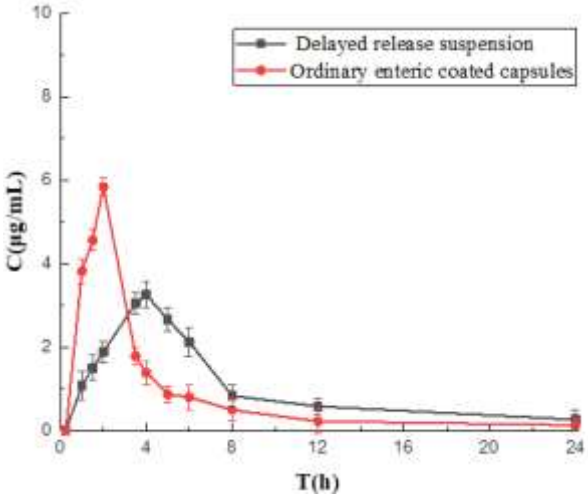


Fig. 14: Concentration-time curves of PAZ-Na enteric coated capsules and delayed release suspension

DISCUSSION

Preparation of the drug-resin complexes

The drug-resin complexes were commonly made by bath method and column method. The counterions exchanged in the column method are continuously eluted, so that the resin has a high equilibrium exchange amount and a long operation process (Liu, *et al.*, 2021). The bath method is simple to operate, time-consuming, can be carried out in batches, and has a wide range of applications (Xie *et al.*, 2008). So the bath method is selected. Referring to the relevant literature (Wang *et al.*, 2015; Malladi *et al.*, 2010), ion exchange reactions usually occur first on the resin surface.

Table 1: Average grain diameter and content of PAZ-Na-resin

No.	Average grain diameter (μm)	Content (%)
1	157	33.18
2	159	32.79
3	156	31.91
Mean	157.3	32.63
RSD (%)	0.971	1.99

Table 2: Formulation process of PAZ-Na delayed release suspension

Component	Reagent	Dosage
PAZ-Na	/	equivalent to 40 mg PAZ-Na
Corrigent	Sweet orange essence	0.04g
Corrigent	Anhydrous citric acid	0.4g
Filler	Glucose	1g
Wetting agent	Poloxamer	0.002g
Suspending agent	CMC-Na	0.2g
Suspending agent	Xanthan gum	0.2g
Antiseptic substance	Propylparaben	0.02g
Anticaking agent	Superfine silica powder	0.18g

Table 3: The content of PAZ-Na delayed release suspension

Suspension	Actual content (%)			Average (%)	RSD (%)
	1	2	3		
1	102.3	103.6	101.8	102.56	0.905
2	101.5	104.2	103.8	103.16	1.412
3	99.7	101.6	102.1	101.13	1.252

Table 4: The stability of PAZ-Na delayed release suspension (n=3)

T (month)	C (%)	L (%)	RI	F	Character
0	99.1	0.23	good	0.99	Homogeneous yellow liquid
1	98.6	0.27	good	0.98	Homogeneous yellow liquid
2	98.3	0.33	good	0.98	Homogeneous yellow liquid
3	97.8	0.38	good	0.97	Homogeneous yellow liquid
6	97.4	0.50	good	0.96	Homogeneous yellow liquid

Table 5: Pharmacokinetics relative data of PAZ-Na enteric coated capsules and delayed release suspension

Parameter	PAZ-Na enteric coated capsules	PAZ-Na delayed release suspension
$t_{1/2}$ *(h)*	0.466	1.091
T_{max} *(h)*	2	4
C_{max} *(μg/mL)*	6.162	3.244
MRT_{0-24}*(h)*	4.819	7.408
AUC_{0-24} *(μg·h·mL^{-1})*	17.388	19.578
AUC_{∞} *(μg·h·mL^{-1})*	17.487	19.704

Table 6: Drug release of PAZ-Na delayed release suspension *in vitro* and *in vivo*

T (h)	1	2	3	4	5	6	7
F (%)	8	10	56	70	78	81	82

Particle size affects the rate of ion exchange reactions. When the particle size of the drug resin increases, its drug load and release rate decrease. When the particle size of the resin decreases, the specific surface area increases, and more reaction sites encounter the drug ions in the solution, and the reaction rate increases (Li, Zhou, 2012; Guan *et al.*, 2010). Due to the large particle size of the self-mad resin (the average particle size is about 190 μm), the resin is crushed and passed through a 120-mesh screen, and the resin particle size is not more than 125μm.

Characterization of drug-resin complexes

The blank resin after crushing was basically the same as the PAZ-Na-DRC, and the surface of the resin was smooth and there was no drug adhesion after drug loading (fig. 4). The resin basically has no crystalline characteristic peaks, and there are obvious sharp peaks between PAZ-Na at 10°~35° (fig. 5). It shows that PAZ-Na is crystalline.

From the spectrum of the physical mixture of PAZ-Na resin, there is still a crystallization peak of PAZ-Na, but the strength is reduced. It shows that its crystal structure has not changed but is a simple physical mixture. However, the characteristic peak of PAZ-Na-DRC completely disappeared, indicating that the drug existed in an amorphous form after drug loading.

Coating process of PAZ-Na-DRC

To achieve its efficacy, PAZ-Na needs to be transformed into active ingredients in a highly acidic environment in the lumen of parietal cells, so it needs to be absorbed intact through the stomach and reach the duodenum (Cheer *et al.*, 2003; Sachs *et al.*, 2003). Although studies have shown that after the proton pump inhibitor drug is combined with the resin, the drug will be dispersed in the polymer skeleton system of the resin, reducing its chance of bonding with external water and air, further improving the stability of the drug, and also has certain moisture resistance and acid resistance (Hansson and Kristensen, 2017). However, considering the properties of the drug itself and the stability and safety of subsequent preparations, it is necessary to enteric coating the drug resin to achieve the extended-release effect.

The coating process is widely used in the pharmaceutical industry, and the coating of drugs helps to improve the therapeutic effect and market value of specific drugs. When coating pantoprazole sodium drug resin, the coating layer needs to be evenly distributed on its surface while maintaining good mechanical properties. Combined with the preliminary research basis of the research group, this chapter selects fluidized bed for coating, the main advantage of which is that the uniformity of the coating film is high, and the quality of the coating film can change with the adjustment of the process conditions.

Preparation of PAZ-Na delayed release suspension

Because oral liquid preparations are suitable for a wide range of people and have greater clinical needs, they are also an important development direction of pharmaceutical preparations in the future. However, from the current clinical use point of view, there is a relatively short variety of oral liquid preparations in China, and some commonly used drugs with high demand do not have suitable oral liquid preparations (Chen *et al.*, 2021). Compared with conventional dosage forms, PAZ-Na-MC are made into suspensions, which are easier to take, can be flexibly adjusted in dose, and have a wide range of applicability.

The suspension aids of different types of suspensions (xanthan gum, HPMC, CMC-Na) were investigated with reference to the evaluation index (F and RI). Among them, HPMC has a general suspension effect, and the microcapsule is easy to settle and has poor stability. Xanthan gum has a good suspensing effect. CMC-Na has obvious suspension effect at high concentrations, but the coated microcapsules are easy to adhere and have poor redispersibility. Xanthan gum was finally selected for use in combination with CMC-Na as a suspension aid, and the prescription of the relevant patent was shown in table 2.

In vivo and pharmacokinetic studies

The existing dosage forms of PAZ-Na for the treatment of peptic ulcer in China are mainly tablets, capsules and injections. Considering the operability of the follow-up gavage experiment, pantoprazole sodium enteric-coated pellet capsules were selected as a control to evaluate the effect of the PAZ-Na delayed release suspension *in vivo*. Compared with commercially available PAZ-Na enteric-coated pellet capsules, the PAZ-Na delayed release suspension has lower C_{max}, larger T_{max}, and a smoother drug-time curve and a certain delaying effect (fig. 14). The T_{max} of the PAZ-Na delayed release suspension was delayed from 2h to 4h compared with the PAZ-Na enteric-coated pellet capsules. Similarly, the C_{max} was reduced from 6.162μg/mL to 3.244μg/mL with the concentration-time curve being gentler compared with the commercially PAZ-Na enteric-coated pellet capsules (table 4). The relative bioavailability of the two is 112.67%, which can be considered equivalent in both dosage forms. It shows that the effect of the PAZ-Na delayed release suspension is good. *In vitro* experiments, the drug suspension was released at a slower rate and longer than the immediate-release capsule. *In vivo* experiments, the blood concentration rises slowly, the peak decreases and the half-life is long. The results of drug release *in vitro* and *in vitro* were consistent.

CONCLUSION

In this study, a novel PAZ-Na delayed release suspension was prepared. Using self-pharmaceutical grade anion

exchange resin as the carrier, PAZ-Na drug-resin complex was prepared by the ion exchange process. On this basis, the composite was coated with enteric-coated materials by fluidized bed coating method, and coated microcapsules with good enteric-coated and extended-release effects were obtained. After that, suitable auxiliary materials are mixed to make a relatively stable suspension. *In vivo* studies also showed that compared with commercially available PAZ-Na enteric-coated pellet capsules, the PAZ-Na delayed release suspension had lower C_{max} and larger T_{max} and the drug duration curve was smoother and had a certain delaying effect. Therefore, this study demonstrated the release effect of the PAZ-Na delayed release suspension. The relative bioavailability of commercially available PAZ-Na enteric-coated pellets and the PAZ-Na delayed release suspension was as follows: the AUC_{0-24} of the PAZ-Na delayed release suspension was 19.578μg·h·mL^{-1} and the AUC_{0-24} of commercially available PAZ-Na enteric-coated pellet capsules was 17.388μg·h·mL^{-1} and the relative bioavailability of the two was 112.67%, which was within the range of 80-125%. So, PAZ-Na enteric-coated pellets and the PAZ-Na delayed release suspension were bioequivalent.

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