# Research on *in vitro* release of Isoniazid (INH) super paramagnetic microspheres in different magnetic fields

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**Abstract**: To explore *in vitro* release rules of isoniazid (INH) when Isoniazid Super paramagnetic Iron Oxide Microspheres (ISPIOM) are subject to no external magnetic field, applied mechanical magnetic field and scillating magnetic field. ISPIOM was prepared by using the spray drying method; Isoniazid contented in the microspheres was determined, the drug loading capacity and encapsulation efficiency were calculated. Release of isoniazid in the microspheres was determined respectively under the effect of no external magnetic field, applied mechanical magnetic field and oscillating magnetic field, to explore the release rules. In solution with pH=7.4 PBS, microspheres featured 8-hour sustained release under the effect of magnetic field, the released rate of the microspheres is accelerated,. In solution with pH=3 PBS, microspheres release faster and could realize the fastest completion of release in 2 hours under the effect of oscillating magnetic field. To join the external magnetic field in different point time to can't affect the release, under pH=3 of medium, ISPIOM release faster; under the effect of magnetic field, the released rate of the microspheres is accelerated, and the longer effect of magnetic field, the faster release. Oscillating magnetic field can make ISPIOM within a certain period of time, get ideal release curve, so as to achieve good control release effect.

**Keywords**: Isoniazid, microsphere, super paramagnetism, magnetic field, release characteristics.

## INTRODUCTION

Tuberculosis (TB) has a growing morbidity in recent years, with a long treatment course, many adverse reactions and serious drug resistance, so it is even fatal to human beings (Phillips *et al*., 2012; Miyata *et al*., 2012).

Super paramagnetic iron oxide (SPIO) is an ideal magnetic targeting drug carrier. According to the studies (Jain *et al*., 2008; Li *et al*., 2013), SPIO participates in hemoglobin myoglobin synthesis after having metabolism or is discharged through energy metabolism, resulting in hard accumulation of SPIO, so it is easy and simple to prepare SPIO by means of the coprecipitation method commonly used in the laboratory to obtain small-sized SPIO. Dames *et al*. (2007) has gotten favorable feasibility of guiding SPIO with the magnetic field for targeted therapy of TB of animal models.

Microspheres is the particle dispersoid formed by drug dispersing or being absorbed in the macromolecule or polymer matrix. It is prepared mainly by means of the crosslinking-emulsion method, drying-in-liquid method, spray drying process, etc., among which the spray drying process is used safely and simply, with a high encapsulation efficiency, so as to generate good-form microspheres with even size distribution (Yang *et al*., 2013; Wei, 2008). Verma *et al*. (2008) has pointed out that pulmonary delivery preparations for microspheres can increase the pulmonary drug concentration and decrease the toxic and side effect.

Combining the advantages of magnetic targeting and microspheres, this experiment has prepared the isoniazid super paramagnetic iron oxide microspheres (ISPIOM) via the spray drying process, and taking the releasing rate as an investigation indicator, has conducted a preliminary investigation for factors impacting the release of ISPIOM (external magnetic field intensity, external magnetic field added at different time, action time of the magnetic field, pH value of dissolution medium and type of external magnetic field), laying a foundation for improving the bioavailability of isoniazid, reducing the toxic and side effect of TB drugs and implementing further targeted experiments.

## MATERIALS AND METHODS

### *Reagents*

isoniazid (sigma), F68 (sigma), SPIO (laboratory-made), chitosan (food-grade), L-leucine and NaOH are all

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analytically pure domestically. Scientz-IID ultrasonic cell disputer (Chengdu Shengke Instrument Co., Ltd.), cts24 Hall Effect magnetometer (Shanghai No.4 Electricity Meter), RY-1500 spray dryer (Shanghai Ruiyuan Mechanical Equipment Co., Ltd.), oscillating magnetic field (School of Electric Engineering of Chongqing University), magnetic field (School of Electric Engineering of Chongqing University), AR1140 electronic scales (Ohaus), uv756crt ultraviolet spectrophotometer (Shanghai Yoke Instrument Co., Ltd.) and ZRS-8G dissolution tester (Tianjin Tianda Tianfa Pharmaceutical Testing Instrument Manufacturer)

***ISPIOM preparation***

Spray drying process: a right amount of isoniazid, F68, leucine, SPIO and other accessories are dissolved into the chitosan liquid, stirred for 30 minutes at the speed of 1,000 r/min and kept a continuous 30s reaction with certain spray drying parameters to get the microspheres.The plastic bags specifically for the experiment are used to collect ISPIOM and then the collected ISPIOM is stored in a closed glass jar with the silica-gel desiccant inside. The formulation screening is conducted with the grain size and encapsulation efficiency as indicators; 0.0250 g ISPIOM is weighted precisely and put into a 100mL volumetric flask; the acid PBS buffer solution whose pH value is 3 is added till a scale; then the flask is shaken violently; ISPIOM goes through 30-minute ultrasonic dissolution in the Scientz-IID ultrasonic cell disputer and is filtered via a 0.22μm millipore filter, then its UV absorption value is determined with a wavelength of 263nm via the UV method and its encapsulation efficiency and drug loading capacity are calculated. The formula for encapsulation efficiency is: Encapsulation efficiency=drug amount in the microspheres /input drug amount ×100%; the formula for the drug loading capacity is: drug loading capacity=mass of drug in the microspheres/mass of microspheres ×100%.

***Determination and specificity of testing wavelength***

A certain amount of isoniazid standard substance is weighted and dissolved in the PBS solution with a pH value of 7.4 to generate the 20ug/ml solution (Zhou *et al*., 2010) and its maximum absorption wavelength is determined to be 263nm after being scanned via an ultraviolet spectrophotometer within a wavelength range from 190nm to 400nm. The microspheres and blank microspheres are prepared into solution with the same concentration and then scanned within a wavelength from 190 nm to 400 nm.

***Preparation of standard curve***

20 mg isoniazid is weighted precisely, put into a 100mL volumetric flask and diluted to the scale with the PBS solution whose pH value is 7.4 and then the isoniazid mother solution of 200μg/mL is gotten; 0.05mL, 0.1mL, 0.2mL, 0.3mL, 0.4mL, 0.5mL, 1.0mL, 1.5mL, 2.0mL, 3.0mL and 10mL isoniazid solution is respectively put into a 10mL volumetric flask and diluted to the scale with PBS solution; then the absorbance (a) value is determined under a wavelength of 263nm with PBS as a blank (Pan, 2009).

***Recovery***

An appropriate amount of microspheres and blank microspheres is taken and prepared into three groups of solution respectively with the high, medium and low concentration (2μg/mL, 10μg/mL and 20μg/mL) with PBS solution whose pH value is 7.4; then, the isoniazid content in each group of solution is determined via the UV sampling method, three times for each, and the average recovery of each is calculated.

***RSD***

An appropriate amount of microspheres and blank microspheres is taken, and prepared into three groups of solution respectively with the high, medium and low concentration (2μg/mL, 10μg/mL and 20μg/mL) with PBS solution whose pH value is 7.4; then, the isoniazid content in each group of solution is determined via the UV sampling method, three times for each. Isoniazid content is determined via the method for each group of solution with five sample introductions and then the within-day precision is calculated. Isoniazid content and within-day RSD are respectively determined for each group of solution with sample introduction for consecutive five days.

***Test of releasing rate***

Applied-magnetic-field-free group: the stirring basket method (Xie, 2012; Xie, 2009) is selected in the experiment. The unit is linked according to Appendix XD of *Chinese Pharmacopoeia 2010*, and 500mL PBS solution is respectively added to six dissolution flasks and degassed by heating and stirring (Guo *et al*., 2012); till the medium temperature of 37°C±0.5°C is constant, six groups of 0.0300g isoniazid or 0.3750g ISPIOM are weighted, wrapped with double gauzes and respectively put into 6 dry stirring baskets; when the sample surfaces definitely show no bubble, the instrument is started at the speed of 100r/min separately for 30min, 1h, 2h, 4h, 6h, 8h and 24h. 5mL solution is taken every time, then 5mL dissolution medium at the temperature of 37°C±0.5°C is supplemented timely; the UV absorption values are determined and recorded with a wavelength of 263 nm by UV method after filtration via the 0.22μm millipore filter. The process from sampling to filtration should be completed within 30 seconds.

Mechanical magnetic field and oscillating magnetic field group: the method is seen in the releasing rate experiment for the magnetic-field-free group. 10mL dissolution medium is taken from the dissolution flask 1h, 2h or 4h

later or at a certain time and put into a plastic bottle, then the microspheres acts in the mechanical magnetic field and the oscillating magnetic field for several minutes and the UV absorption values are determined before and after the action. Then, the solution is put back to the dissolution flask, the releasing experiment is continued for 24 hours and finally the curve is collected by the origin software.

***Single factor study***

A preliminary investigation is conducted for the factors impacting the release of ISPIOM (external magnetic field intensity, action time of the magnetic field, external magnetic field added at different time, pH value of dissolution medium and mode of action of different external magnetic fields), with the releasing rate as an investigation indicator. And, the similarity of release curves and each factor's influence on releasing are evaluated according to the similarity factor of the release curves f2=50×lg ((1+Q/n)-0.5×100) (Q is the sum of variations of average dissolution rate of the experimental medicines and the control medicines): if f2 >50, the curves are similar, and the bigger f2 is, the higher similarity of both curves is.

***Data processing***

All the data comparisons should be collected and analyzed by use of SPSS 19.0 software and represented with mean ± standard deviation ( $\bar{x}\pm s$).

## RESULTS

***Structural characterization of ISPIOM***

It can be seen from fig. 1 that under microscope the sizes of ISPIOM are relatively even, which are mostly in the range of 1-5μm, in the shape of circle or oval with good dispersity. According to formula, the encapsulation efficiency is 99% and the drug loading capacity is 8%-10%.

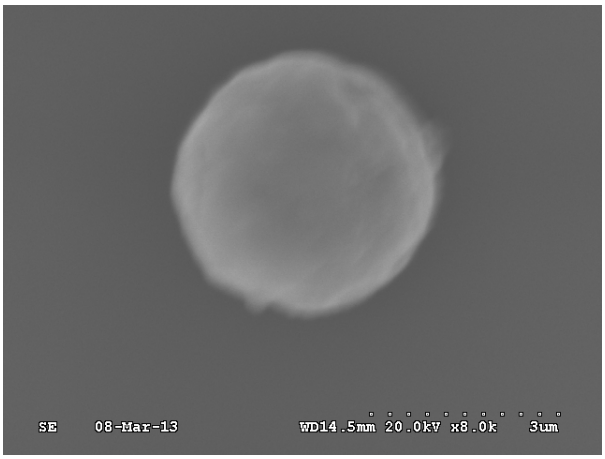


**Fig. 1**: SPIO under SEM

***Determination and specificity of testing wavelength***

At the wavelength of 263 nm, isoniazid has the maximum absorption peak. And there is no supplementary material interference.

***Standard curve drawing***

Using concentration C to calculate the linear regression of absorbance A, the standard curve equation A = 0.03009C+ 0.02425 is acquired, r=0.99972, (n=10). If P<0.0001, there is a good linearity whose range is 2μg/mL-60μg/mL.

***Recovery***

Average recovery rate is 100.25%, RSD=0.36% (n=9), then the result shows that this method has good accuracy.

***Relative standard deviation***

The within-day RSDs of low, medium and high concentration are respectively 0.16%, 0.19% and 0.15%, then the total RSD is 0.56% (n=15). The day to day RSDs are respectively 0.22%, 0.35% and 0.20%, the total RSD is 0.98% (n=15), which is much less than 5%. It is thus clear that this method has good RSD.

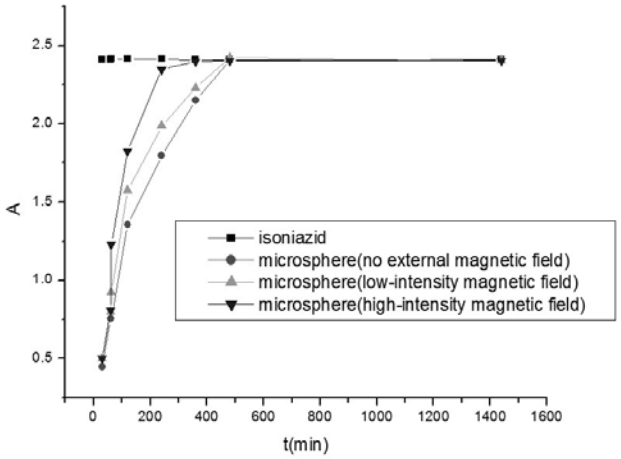


**Fig. 2**: The Influence of different magnetic Field Intensities on releasing rate

***Single factor investigation***

*The Influence of Different Magnetic Field Intensities on Releasing Rate*

Determined the releasing rate of isoniazid, microspheres, microspheres after the effect of low-intensity magnetic field and microspheres after the effect of high-intensity magnetic field in pH=7.4 PBS using the method described in Article 1.2.6. Repeat the steps for 3 times to observe the influence of magnetic field intensity on the releasing of isoniazid. fig. 2 indicates that isoniazid is released rapidly in medium. Microspheres experimental group without applied magnetic field is completely released in 8h. Microspheres after the effect of high-intensity magnetic field is released more that 90% in 6h. F2(isoniazid /microspheres) =11<50, the curves are not similar, which indicates that isoniazid is not slowly released. Microspheres's slow-release effect in-vitro experiment is 8h. F2 (low-intensity magnetic field/high-intensity magnetic field)=49, which indicates that increasing magnetic field intensity can accelerate microspheres's releasing. Repeat the inspection for 3 times to see the influence of different join time of

magnetic fields on releasing". Fig. 3 indicates that, the four curves are similar. By calculation, F2 values of every two groups of data are all above 50, which indicates that in this experiment, different join time of magnetic fields doesn't have much influence on releasing.

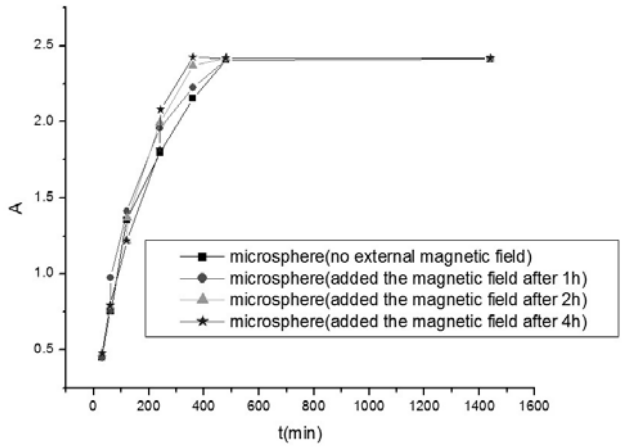


**Fig. 3**: Influence of different time of join magnetic field on releasing rate.

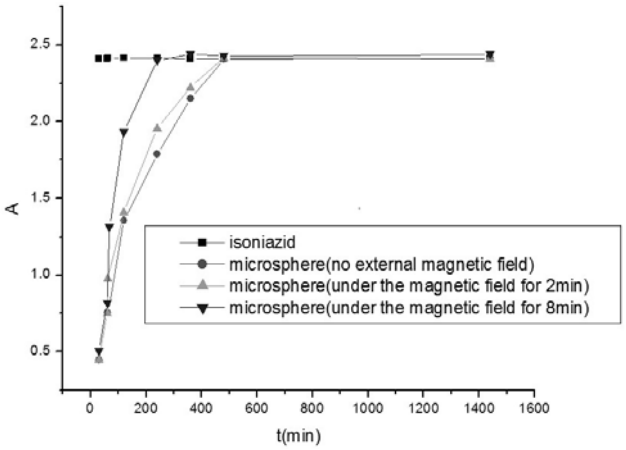


**Fig. 4**: Influence of different action time of magnetic field on releasing rate.

***Influence of different action time of magnetic field on releasing rate***

Inspect the releasing rate of isoniazid, microspheres without external magnetic field, microspheres which is added the effect of magnetic field after 1h for 2min and microspheres which is added the effect of magnetic field after 1h for 8min in pH=7.4 PBS using the method described in Article 1.2.6. Repeat the inspection for 3 times to see the influence of the length of action time of magnetic field on releasing. fig. 4 indicates that the release curve of the 8min group is remarkably higher than that of the 2min group, F2(2min/8min)=37, which indicates that increasing the action time of magnetic field will accelerate microspheres releasing.

***Influence of different pH release medium on releasing rate***

Inspect the releasing rate of isoniazid, microspheres without external magnetic field and microspheres after the effect of strong oscillating magnetic field in PBS of pH=3, pH=7.4 and pH=10using the method described in Article 1.2.6. Repeat the inspection for 3 times to see the influence of pH value of medium on releasing rate. fig. 5 indicates that curves of pH=7.4microspheres group and pH=10microspheres group nearly coincide, F2 (pH=7.4/ pH=10)=98; pH=3microspheres group's curve is remarkably higher than the former two groups, F2(pH=3/ pH=7.4)=46; F2 (pH=3oscillating group/ pH=7.4 oscillating group)=49, which indicates that releasing medium of acidic pH will accelerate microspheres releasing, while that of alkaline pH has no influence on microspheres releasing.

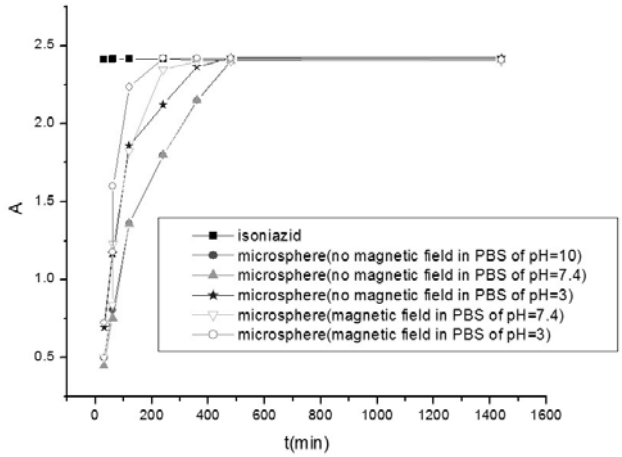


**Fig. 5**: Influence of different pH release medium on releasing rate.

***Influence of different external magnetic fields on releasing rate***

Inspect the releasing rate of microspheres, microspheres after effect of mechanical magnetic field and microspheres after effect of oscillating magnetic field in medium of pH=3 PBS and pH=7.4 PBS. Set the intensity of both mechanical magnetic field and oscillating magnetic field as 0.1570 T. Repeat the inspection for 3 times to seethe influence of different external magnetic fields on releasing. fig. 6 indicates that both curves of oscillating magnetic field groups are higher than that of mechanical magnetic field group, F2(pH=7.4oscillating / pH=7.4mechanical)=49, F2(pH=3oscillating / pH=3 mechanical)=54, which indicates that oscillating magnetic field has a larger influence on microspheres releasing, but its influence is reduced in releasing medium of pH=3.

## DISCUSSIONS

In this experiment, the external spray drying method is used to prepare ISPIOM with SPIO as supplementary material. The encapsulation efficiency is 99%. The

obtained microspheres are in good shape and evenly distributed size. ISPIOM's slow release time in PBS is 8h, indicating its good slow release effect which is remarkably prolonged compared with former isoniazid microspheres (Wu *et al*., 2009; Zhou *et al*., 2010). This provided certain theoretical basis for slow release preparation.

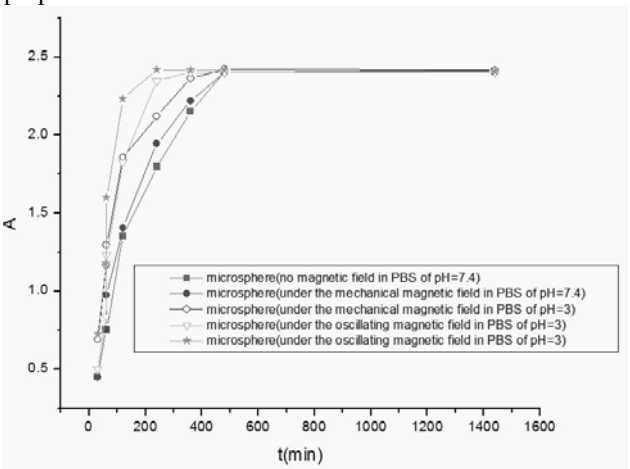


**Fig. 6**: Influence of different external magnetic fields on releasing rate.

This experiment, for the first time, inspected the releasing situation of microspheres's isoniazid in three conditions without applied magnetic field, with applied mechanical magnetic field and with applied oscillating magnetic field from the angle of *in vitro* drug releasing. This experiment carried out initial exploration and screening for factors that influence ISPIOM releasing (external magnetic field intensities, different join time of external magnetic field, length of action time of external magnetic field, PH of dissolution medium and different external magnetic field), conducted curve fitting to the releasing degree of the group of microspheres without applied magnetic field: y=2.43976522592 (1-e-6.20751595092t), standard deviation S=0.0602393; related coefficient r=0.9980250, and confirmed that different join time of external magnetic field does not influence releasing, the releasing of ISPIOM will accelerate in acidic pH, the releasing of ISPIOM will accelerate with effect of external magnetic field and the larger the intensity is and the longer the action time is, and the releasing will be quicker. This experiment laid a foundation for following experiments of sustained releasing of new type ISPIOM.

All mechanical and oscillating magnetic fields used in the experiment are produced by the School of Electrical Engineering of Chongqing University with the application for patents for inventions, among which, the oscillating magnetic field consists of three parts of high-power targeted oscillating magnetic field generator, electromagnet, storage battery (tree sections in total) and adopts technologies of single storage battery, two storage battery in series and three storage battery in series. Under the effect of bipolar oscillating magnetic field, through adjusting oscillating magnetic field's frequency and intensity, the gradient oscillating magnetic field is formed and thus the super paramagnetic particles are gathered in a three dimensional scope. While reaching concentration, oscillating magnetic field will drive nanomagnets's self-motion and (or) drive the motion of the whole magnetic drug-loaded particle and facilitate releasing or diffusion of drugs in, on or around magnetic drug-loaded particle, so as to reach the purpose of promoting or partially controlling drug releasing. Moreover, oscillating magnetic field can use the magnetism or super paramagnetism of nanoscale magnetic drug-loaded particle to give drugs partial target and certain function of releasing control. This method is simple, safe and repeatable.

For the first time, this experiment compares mechanical and oscillating magnetic fields. With the same intensity, mechanical magnetic field's influence is less than that of oscillating magnetic field. The possible reason is that mechanical magnetic field gives just simple mechanical movement, while oscillating magnetic field presents magnetic field of bipolar pulse current with strong amplitude of variation and constant changes of magnetic pole directions, and thus the oscillating phenomenon is very remarkable (Luo *et al*., 2008), which leads to a more obvious releasing rate influence. Magnetic field intensity and action time can be adjusted in oscillating magnetic field. In experiment of ISPIOM releasing, through adjusting magnetic field intensity and action time of the oscillating magnetic field, a more ideal releasing curve will be obtained and thus a better effect of releasing control will be achieved.

Due to the limitation of ultraviolet instrumentation itself, in next experiment, we will use HPLC to further verify the results of this experiment, in order to provide basis for ISPIOM's application in animal experiment under mechanical and oscillating magnetic fields and the R&D of long-acting new type ISPIOM and to lay a foundation for the development of new formulation of isoniazid drug and the improvement of clinical treatment effect of TB.

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