

Dissolution profile study on the novel doxycycline hydrochloride sustained-release capsules

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Abstract: In present study, a novel doxycycline hydrochloride (DC) sustained-release capsule was prepared with the new manufacturing technology, extrusion-spheronization method. The release studies were performed using marketed sample as a reference and data were analyzed in terms of cumulative release amounts as a function of time. Results demonstrated that our developed sample was similar to reference preparation in release characteristics *in vitro*. The *in vitro* release characteristics of different batches of preparations were quite similar with each other, the total release proportions of DC from sustained-release capsule reached higher than 90 % within 4 h. Similarity factors f_2 of two preparations were all higher than 50, the release mechanism of drugs from capsules fitted to non-Fichian diffusion. The developed sustained-release preparation may be a promising alternative dosage form for treatment of related diseases.

Keywords: Doxycycline hydrochloride; sustained-release; *in vitro* release; dissolution; similarity factors; extrusion-spheronization.

INTRODUCTION

Doxycycline hydrochloride (DC), 4-(dimethylamino)-1, 4, 4a, 5, 5a, 6, 11, 12a-octahydro-3, 5, 10, 12, 12a-pentahydroxy-6-methyl-1, 11-dioxo-2-naphthacene carboxamide monohydrate, is one of the tetracycline derivation antibiotics obtained by modification for the oxytetracycline molecule, which has a wide range of (1999). It has a broad spectrum of activity against a wide variety of microorganisms, and exerts a bacteriostatic effect by inhibiting protein synthesis process of chlamydiae, rickettsiae, mycoplasmas and aerobic, anaerobic Gram-positive or Gram-negative bacteria (Attia *et al.*, 2011). DC has been used for about fifty years in treatment for skin, soft tissue, respiratory and genitourinary infections. Compared with other members of the tetracycline antibiotic, a major advantage of doxycycline is its high lipophilicity, which increase its distribution and tissue penetration largely, prolongs its half-life *in vivo*, all of these will lead to its improved antimicrobial activity (Sun *et al.*, 2002 and Satinsky *et al.*, 2005). In addition, this drug has fewer side effects and is relatively inexpensive. Therefore, it was arousing more and more interest and research in antibacterial clinical practices.

Conventional orally administered drug formulations do not usually provide rate-controlled or targeting release. In most cases, conventional drug delivery means rapid and sharp increases of plasma drug concentration at potentially toxic levels. Following a relatively short period at or above the therapeutic level, drug concentration in blood and/or tissues rapidly drops off

until re-administration (Freiberg *et al.*, 2004 and Shibata *et al.*, 2010). Recently, several new methods of drug delivery system to bring about sustained-release or controlled-release are possible. Delayed drug delivery systems (DDS), are now the focuses of oral controlled-release solid dosage forms for researchers, for instance, the new sustained-release capsules. These systems normally consist of a core and a coating. The core is coated with different barriers by film or compression, and the coating can prevent drug quick-release from the core until the shell is completely swollen or eroded by liquid substance *in vivo* (Ross *et al.*, 2000 and Li *et al.*, 2004). These new pharmaceutical preparations can exhibit drug release constantly at a steady rate, the slow and sustained release of the active compounds is beneficial to patients to maintain sustainable levels in blood, thus will bring out better compliance to those need long-term and continuous therapy (Liu *et al.*, 2012).

The aim of this study is to develop a novel DC sustained-release dosage form by applying extrusion-spheronization method. *In vitro* dissolution testing is frequently used to determine the release characteristics of the pharmaceutical products over time. On this basis, a dissolution assay method was established and validated to the *in vitro* release study for sustained-release capsules. The dissolution tests are performed in different media, the dissolution profiles of the commercial and self-made ones are compared by similar factors method, to evaluate the drug release performance of the developed formulation.

MATERIALS AND METHODS

Chemicals and Reagents

The reference substances of DC (purity>99.8%) was

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obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Potassium dihydrogen phosphate and sodium hydroxide were provided by Qinjiahong chemical reagent Co., Ltd (Zhengzhou, China). Hydroxy-propyl methyl cellulose (HPMC), microcrystalline cellulose (MCC) and ethyl cellulose (EC) were provided by Beijing huajinsheng technology co., Ltd. The commercial product (Razadyne ER) was purchased from the market. Chemicals of analytical grade were provided by Nanjing chemical reagent Co., Ltd (Nanjing, China). Water was distilled and purified using a Milli-Q System (Millipore, MA, USA).

Preparation of DC sustained-release capsules

Sustained-release capsules were prepared by extrusion-spheronization method (Di Pretoro *et al.*, 2010 and Han *et al.*, 2013). In brief, an appropriate quantity of DC was weighed and mixed with MCC, followed by adding a solution of HPMC in water; pellets were prepared by extruding and rolling. Immediate-release pellets were prepared by using spray-coating technique with the solution of HPMC on fluidized bed bottom to envelop the sealing coat; some small samples were taken for spraying to coat with EC water dispersion on fluidized bed bottom similarly, to produce sustained-release pellets. Finally, the immediate-release and sustained-release ones were encapsulated to capsules proportionally and packed, yielded the products.

Development of assay method for DC

Linearity

The developed samples were 40 mg standard in doxycycline, the total volume of release medium was 750 ml while determining in hydrochloric acid solution, and was 950 ml while determining in buffer solution, thus the concentrations as the whole drugs in capsules were dissolved and released were 16 and 16.8 $\mu\text{g}\cdot\text{ml}^{-1}$ in the above two medium. Linearity testing was performed in acid solution and buffer solution, respectively.

The appropriate amounts of DC was weighed precisely and dissolved in acid solution and buffer solution, to prepare the testing solutions of different concentrations. Absorbances were determined at 345 nm for all the samples. The calibration curve samples were assayed in triplicate, using concentration (C) as abscissa and absorbance value (A) as ordinates.

Recovery

Absolute recovery of analytes was evaluated by QC samples, and data were determined by comparing the mean amounts obtained from the excipients solution spiked with reference solution with that of the neat standard samples. Three different concentration levels of analytes were evaluated by analyzing the three samples at each level.

Precision and accuracy

Precision was assessed by determining the replicate QC samples of 100% concentration level in recovery determination experiment on the same day (intra-day precision) and three consecutive days (inter-day precision). Accuracy was described by relative error and precision was evaluated by intra- and inter-day relative standard deviation (RSD).

Stability

The stability of DC was evaluated using the samples at the concentration of 16 $\mu\text{g}\cdot\text{ml}^{-1}$. The samples were analyzed at 0, 1, 2, 4, 6 and 8h after conditioning at room temperature, respectively, both in acid solution and buffer solution.

Dissolution assay method for sustained-release capsules

The oar method for dissolution test was applied to determine dissolution of DC from sustained-release capsules. 900 ml release medium was taken to dissolution glass at predetermined temperature; release medium was agitated by stirring blades at the rotation speed of 75 $\text{r}\cdot\text{min}^{-1}$ and sampled at the scheduled time after initiating experiment. 10ml sample was collected and filtered through a 0.45 μm membrane, filtrate was selected to determine as testing solution.

DC content at each time point was determined by absorbance assay. Meanwhile, the proper amounts of reference substance was dissolved and diluted quantitatively by release medium to the final concentration of 16 $\mu\text{g}\cdot\text{ml}^{-1}$, which was used as standard solution for the total drug amounts (W). The above solutions were analyzed by external standard method, accumulative release amounts and release percent were calculated according to the formula:

$$Q_n = C_n \cdot N_0 + \sum_{i=1}^{n-1} C_i \cdot N_i \quad \text{вспомогательные величины в формуле (6)} = Q_n \cdot \frac{M}{M_0} \times 100\%$$

Noting: Q_n was the accumulative release amounts at each time point, C_n was the measured concentration at each time point, V_0 was the bulk volume of release medium, V_i was the sampling volume, C_i was the measured concentration at time point i , W was the total drug amounts in capsules.

Release sensitivity of DC in different medium

The *in vitro* release feature of DC was assessed in purified water, hydrochloric acid solution (0.1M HCl), phosphate buffer (PBS, pH 6.0), PBS (pH 6.8) and acetate buffer (pH 5.5), respectively. The solution samples were taken for dissolution determination at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 16 and 24h following the assay procedures described above in the purified water medium, and only sampled until 6h for the other medium. Filtrate was taken determine the accumulative release amounts at each time point and draw the release curve.

Comparative analysis of dissolution in different medium

The developed formulation and marketed product were both taken for dissolution determination with the above five solution used as release medium, respectively. Accumulative release percents were calculated for both the preparations and release performance in each medium was contrastively evaluated.

Drug release study and statistical analysis for release data

According to the sustained-release capsule assay provided by Dissolution Methods for Drug Products and the characteristic of product, 0.1 mol·L⁻¹ hydrochloric acid solution was used as release medium in the first two hours after experimenting. Subsequently, 200 ml potassium dihydrogen phosphate solution (0.2 mol·L⁻¹ PBS) containing sodium hydrate was added and the pH value was adjusted to about 6.0 in the new medium. Therefore, drug dissolution test was performed in acid solution and buffer solution successively. Sampling time was reset at 0.5, 1, 2, 2.5, 3 and 4 h.

According to the guideline for bioavailability and bioequivalency of orally administered solid drugs, similarity (f_2) measuring was applied to evaluate the closeness between the two dissolution profiles. The f_2 was calculated according to the equations given below:

$$f_2 = 50 \times \lg \left[\frac{1 + Q/n}{1 + 1/2 \times 100} \right] \quad Q = \sum_{t=1}^n (R_t - T_t)^2$$

where n is the number of time points, R_t and T_t are the percentages of the reference and testing drug release at each time point t , respectively. In order to consider the release profiles similar, the f_2 values should be close to 100. In general, f_2 value of the two drug release profiles is between 50 and 100, and then these two drug release characteristics are similar, whereas value below 50 indicates differences between the release profiles (Liu *et al.*, 2012).

RESULTS

Method validation

Linearity

The calibration curves were prepared at the concentration levels of 1.6-19.2 and 1.6-22.4 µg·ml⁻¹, in acid solution and buffer solution, respectively. The typical curve equations were constructed with a weight of 1/x² and described as $A=0.0337C+0.0006$, $A=0.0349C-0.017$, with the correlation coefficients (r) higher than 0.999 both for the two medium.

Recovery

Absolute recovery of DC was determined by comparing the contents of three-level QC samples incorporated with excipients to that of the standard solutions, which were directly diluted by release medium. In acid solution, The recovery were 100.51±0.41%, 100.39±0.50% and

100.98±0.48 % at low, middle and high QC concentrations, and the corresponding values were 100.86±0.26%, 100.91±0.40% and 101.06±0.36% while determining in buffer solution. All these results showed that the absolute recoveries were high enough for the analysis of in preparation.

Precision and accuracy

The results of precision and accuracy were assessed at low, median and high levels. The RSD values of intra- and inter-day precision were within 1% and accuracy results extended from 95% to 105%.

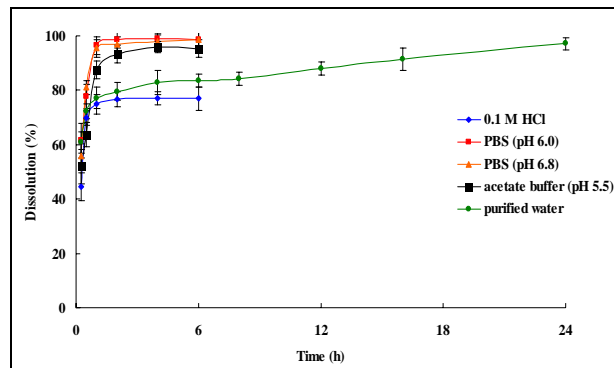


Fig. 1: Effects of different mediums on the release of DC in sustained-release capsules. Each point represents average ± standard deviation (n=6).

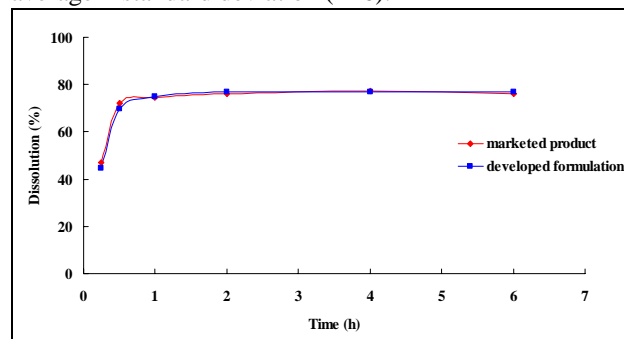


Fig. 2: The release curve of the developed testing and marketed reference sustained-release samples, with 0.1 M HCl as the release medium at the rotation speed of 75 r·min⁻¹.

Stability

The room temperature stability results of DC with the RSD values lower than 2% showed that the testing samples were stable under storage conditions and routine analysis for release study.

Release sensitivity of DC in different medium

The release curve of DC in five medium was shown in fig. 1. It can be drawn that the accumulative release percents calculated in different medium were different. In hydrochloric acid solution (0.1 M HCl), the drug release amounts were no longer increased and remained constant at about 2 h (about 75%). In other three buffer solutions, the drugs can be fully released and the release percents all

reached above 95%. Considering that the dynamic process of orally administered drugs *in vivo* and the pH value in superior extremity in intestine was about 6.0, hydrochloric acid solution and phosphate buffer (PBS, pH 6.0) were selected as release medium in the former two hours and the latter part, respectively.

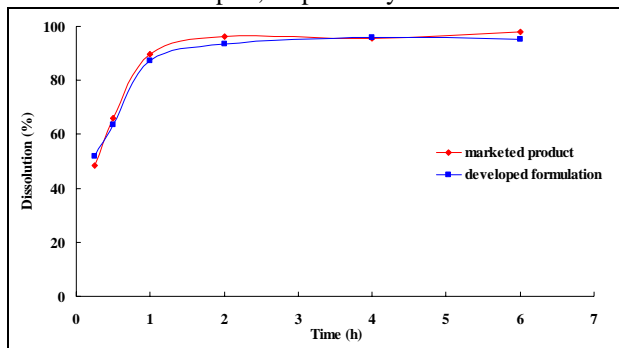


Fig. 3: The release curve of the developed testing and marketed reference sustained-release samples, with acetate buffer (pH 5.5) as the release medium at the rotation speed of 75 r·min⁻¹.

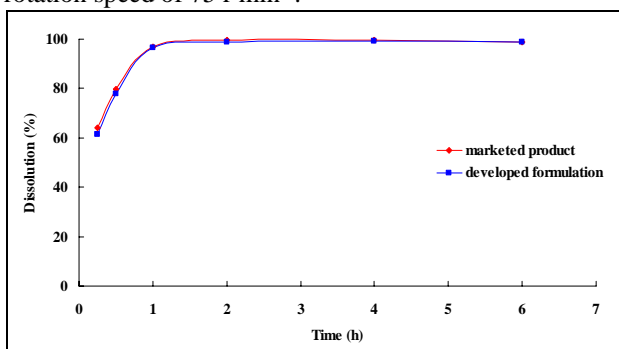


Fig. 4: The release curve of the developed testing and marketed reference sustained-release samples, with PBS (pH 6.0) as the release medium at the rotation speed of 75 r·min⁻¹.

Comparative analysis of dissolution in different medium

The dissolution of developed formulation and marketed product were evaluated in the five-release medium to investigate the drug release consistency *in vitro* for both the preparations. The release curves of the developed formulation and marketed product were shown in figs. 2-6. We can conclude that DC in the two preparations was released with nearly the same property, and dissolution increased continuously along with the time. The total release amounts and release rates were all consistent with each other between the developed and commercial samples, indicating that there were little effects from release medium on the release performance of sustained-release formulations.

Drug release study

The dissolution curve of developed formulation and marketed product in hydrochloric acid solution and phosphate buffer was shown in fig. 7. The similarity

factors (f_2) were calculated for the formulations using the release profile with the marketed product as the reference. The f_2 values of three batches were all higher than 50 (91.2, 85.7 and 88.9, respectively), suggesting that their release profiles were quite similar to that of the reference.

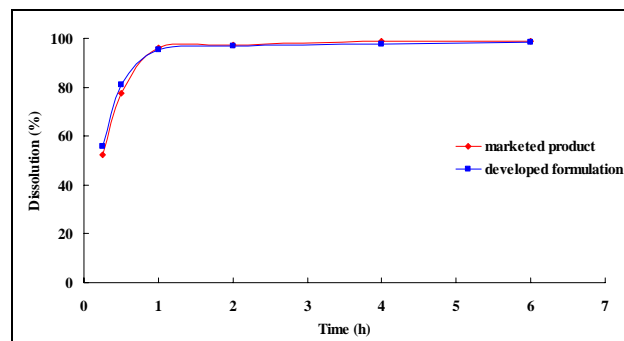


Fig. 5: The release curve of the developed testing and marketed reference sustained-release samples, with PBS (pH 6.8) as the release medium at the rotation speed of 75 r·min⁻¹.

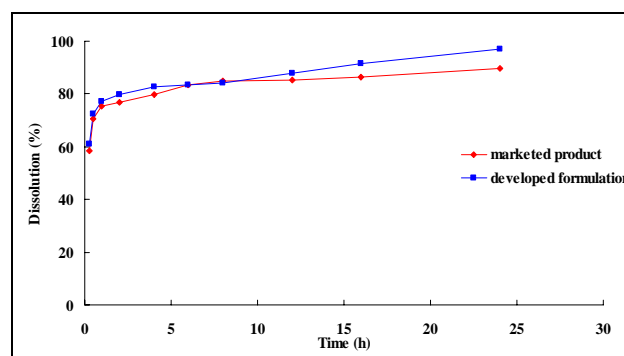


Fig. 6: The release curve of the developed testing and marketed reference sustained-release samples, with purified water as the release medium at the rotation speed of 75 r·min⁻¹.

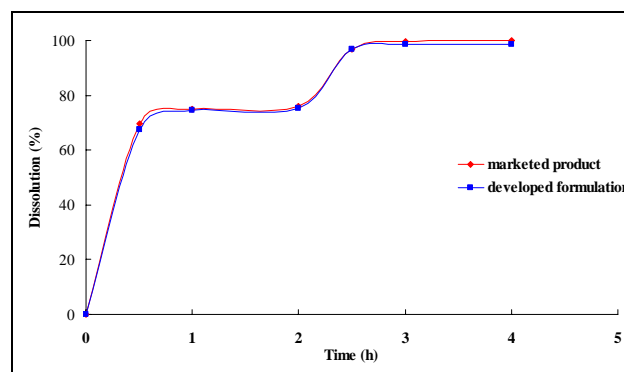


Fig. 7: The dissolution curve of the developed testing and marketed reference sustained-release samples, with 0.1 M HCl and PBS (pH 6.0) as the release medium in the different stages in experiment.

According to the guiding principles of quality standards for sustained-release, controlled release and delayed

Table 1: Release rate determination results of DC sustained-release capsule. (n = 3). Data represent average \pm standard deviation.

Time	45 min in 0.1 M HCl ($\geq 55\%$)	2 h in 0.1 M HCl ($\leq 85\%$)	45 min in PBS (pH 6.0) ($\geq 90\%$)
Batch 1	69.9%	75.4%	95.8%
Batch 2	71.1%	72.2%	98.2%
Batch 3	73.4%	75.0%	99.2%
Reference	72.2%	74.4%	99.0%

release preparations, 45 min, 2h in 0.1M HCl solution and 45 min in PBS (pH 6.0) were selected as sampling time points, the total release amounts for each capsule should attain about 55%, 85% and higher than 90% corresponding to the three time points, respectively. The dissolution contrast results were summarized in table 1.

DISCUSSION

In the dissolution determination, in total two solutions, 0.1 M HCl solution and PBS (pH 6.0) were selected as release medium, thus sink condition experiment for medium were performed in the two solutions, respectively. In HCl solution, 333 mg DC standard sample was precisely weighed and dissolved into the 750 ml 0.1 M HCl (7 folds of raw material drug amounts equivalent to standard amount be dissolved in the same volume of release medium) (Pradhan *et al.*, 2014 and Singh *et al.*, 2000). In PBS buffer, 750 ml HCl was taken into glass, 200 ml PBS solution ($0.2 \text{ mol}\cdot\text{L}^{-1}$) was added and the pH value was adjusted to about 6.0 by HCl or sodium hydrate solution, 333 mg DC standard was precisely weighed and dissolved into the new medium. The experimental results showed that DC could well be dissolved in these two-release medium; therefore, the mixed solvents can meet the release determination requirement of drugs in sustained-release capsules.

For the drug release mechanism in matrix formulations, it can be decided by the formula: $Q=Kt^n$, where n is the release exponent value. Practically speaking, in cylindrical formulations, n value lower than 0.45 suggesting the drug release was corresponding to the Fichian diffusion, higher than 0.89 suggesting the drugs were released by matrix corrosion pathway. Whereas the characteristic parameter n is between 0.45 and 0.89, the main release mechanism of drugs from solid preparations was non-Fichian diffusion (Ritger *et al.*, 1987 and Ritger *et al.*, 1987). In this study, the n value for both the developed formulation and marketed product were 0.74 and 0.67, respectively, indicating that the drug release mechanism followed the non-Fick diffusion, which was affected by the drug diffusion and matrix corrosion.

In conclusion, we prepared galantamine hydrobromide sustained-release capsules using the new extrusion-spheronization preparative method, and developed an

analysis method for the quantification and dissolution study of DC. The results showed this assay method had highly convenience and reliability for the rapid quantitative determination of DC contents in high-throughput release characteristic studies. Furthermore, the drugs could be well released from sustained-release carriers within the specified time limit, and the release characteristics of the developed formulation and commercial samples were quite consistent with each other.

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