dx.doi.org/10.14227/DT260219P48

Accelerated Dissolution Method to Facilitate In Vitro Evaluation of Risperidone-Containing Microspheres

Cheng Ma[1], Han Fu[2], Zhengwei Huang[1], Xiangyu Ma[3], Yang Liu[4], Wenhua Wang[1], Mingjun Wu[1], Xuejuan Zhang[1,5], Ying Huang[1*], and Chuanbin Wu[1]

[1]*School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou, China*
[2]*Shenzhen Center for Chronic Disease Control, Shenzhen, China*
[3]*College of Pharmacy, Molecular Pharmaceutics and Drug Delivery, The University of Texas at Austin, Austin, TX, USA*
[4]*Chongqing Medical and Pharmaceutical College, Chongqing, China*
[5]*Institute for Biomedical and Pharmaceutical Sciences, Guangdong University of Technology, Guangzhou, China*
Cheng Ma and Han Fu contributed equally to this paper.

e-mail: huangying3@mail.sysu.edu.cn

ABSTRACT

Microspheres have gained much interest because of their simple and controllable manufacturing process, sustained-release profile, and drug stabilization. However, the in vitro dissolution test of sustained-release microspheres is time consuming and impedes the development and quality control of microsphere products. In this study, an accelerated dissolution method was established to develop and evaluate microspheres in a timely and cost-effective manner. The effects of dissolution media temperature, pH, and ethanol content on the dissolution behavior were investigated to optimize the accelerated dissolution method. Media containing 25% ethanol with pH 7.4 and 37 °C was suitable for the microspheres. The correlation between the real-time and accelerated dissolution tests was established successfully with an R^2 = 0.9978, indicating potential to apply the accelerated dissolution method to the development and evaluation of microspheres.

KEYWORDS: Microspheres, in vitro evaluation, accelerated dissolution method, model fitting

INTRODUCTION

Advantages of microspheres (MS) include simple and controllable manufacturing processes, sustained release, and drug stabilization (*1–6*). MS have been successfully applied to treat many chronic diseases and cancers (*7–11*). With the rapid development of MS, the in vitro evaluation of MS is becoming more important to ensure the safety and efficacy of formulations containing MS (*12, 13*).

Real-time dissolution methods (e.g., extraction or dialysis) of MS is time consuming (e.g., several weeks to months) to extensively investigate the drug release over a long time period. This impairs the development and quality control of the products (*14*). Therefore, an reproducibly accelerated dissolution method is needed for MS drug development to assess the dissolution behavior of MS (*14, 15*).

An accelerated dissolution method should be able to simulate the real-time dissolution behavior (*16*). Many studies have attempted to establish predictive accelerated dissolution methods by adjusting only one factor (e.g., temperature, pH, or composition of dissolution media); however, a satisfying accelerated dissolution method should be established by considering all factors (*14, 15, 17–19*).

In our previous study, uniform and long-term risperidone-containing PLGA (RIS) MS (RIS-MS) were successfully prepared by a novel technology, ultra-fine particle processing system (UPPS); however, an 18-day dissolution test employed in the study was a heavy burden for product evaluation (*20*). Herein, an accelerated dissolution method is proposed by optimizing different factors. Moreover, correlation between the real-time and accelerated dissolution methods was established to confirm the predictability and reliability of the accelerated method.

MATERIALS AND METHODS

Material, Reagents, and Equipment

Self-prepared 30% drug-loading risperidone-containing MS with PLGA as matrix was fabricated by UPPS. The

*Corresponding author

preparation process and detailed characterization of RIS-MS has been published previously (*20*). First, phosphate buffer solution (PBS) media was prepared with sodium dihydrogen phosphate, then it was adjusted by phosphoric acid and sodium hydroxide to the required pH value. A high-performance liquid chromatography-ultraviolet (HPLC-UV) system employed a Luna 5-µm C_{18} column (4.6 mm × 150 mm, 5 µm) purchased from Guangzhou FLM Scientific Instrument Co., Ltd (Guangzhou, China). Methanol used in HPLC analysis was chromatographically pure and bought from Honeywell (Muskegon, MI). All the other regents were of analytical grade.

Solubility of Risperidone in PBS Media at Different pH Levels

The solubility of risperidone in PBS media (7 mL) at different pH levels (pH 2.0, 5.0, 5.8, 6.8, 7.4, and 7.9) at 37 ± 5 °C, 100 rpm, for 72 hours. The supernatant was withdrawn and detected by HPLC-UV to determine the solubility of risperidone in PBS media at each pH level.

Dissolution Test with Extraction Method

After determining solubility of risperidone in PBS media at different pH levels, the dissolution behavior of RIS-MS was detected with the extraction method. RIS-MS (5 mL) were dispersed with 4 mL of media: pH 7.4 PBS containing 0.02% Tween 80 to increase risperidone solubility in dissolution media and 0.05% clozapine as interior label, which met sink conditions. Then, the RIS-MS were held at 37 ± 5 °C and stirred at 100 rpm. Samples (4 mL) were withdrawn from tubes at 1 h, 4 h, 1 d, 2 d, 4 d, 6 d, 8 d, 10 d, 12 d, 14 d, 16 d, and 18 d, and replenished with fresh media. These samples were centrifuged at 4000 rpm for 5 min and filtered with a 0.22-µm micro-porous membrane (Tianjin Jinteng Experiment Equipment Co., LTD, Tianjin, China). The drug content of the filtered solution was determined with HPLC-UV. The accumulated release was calculated with Equation (1):

$$Q = \frac{V \times \sum_1^i C_i}{m \times \mathrm{LD}\%} \qquad \text{Eq. (1)}$$

where Q is the accumulated release, V is the volume of dissolution media, C_i is the drug concentration in media of i-th sample extraction, m is the total weight of MS, and LD% is the actual drug content in MS.

Dissolution Test with Dialysis Method

Dialysis is another common approach to detect real-time dissolution behavior of MS. Dialysis tubes with 8000 Da were applied to detect the dissolution behavior of RIS-MS. MS (5 mg) of MS were added into dialysis tubes, dispersed with 2 mL of dissolution media, pH 7.4 PBS, and the dialysis tubes were sealed with strings. Dissolution media (3 mL) was used to immerse dialysis tubes for 18 days under oscillation at 37 ± 5°C and 100 rpm. Samples were taken from outside of dialysis tubes at the same times and pretreated as described in the extraction method. The samples were assayed by HPLC-UV, and the accumulated release was calculated. Residual drug content in MS after the 18 day-dissolution test was also determined.

Model Fitting

The dissolution behavior of extraction method was fitted with Equations (2)–(5) to explore their dissolution mechanism:

Zero order model:

$$\frac{M_t}{M_\infty} = kt \qquad \text{Eq. (2)}$$

First order model:

$$\ln\left(1 - \frac{M_t}{M_\infty}\right) = -kt \qquad \text{Eq. (3)}$$

Higuchi model:

$$\frac{M_t}{M_\infty} = kt^{0.5} \qquad \text{Eq. (4)}$$

Korsmeyer-Peppas model:

$$\frac{M_t}{M_\infty} = kt^n \qquad \text{Eq. (5)}$$

where M_t and M_∞ represent the accumulated drug release at t and ∞ (total drug content) time, respectively; k is the kinetic constant, and n describes the kinetic index, which reveals the formulation dissolution mechanism. Fick diffusion is dominant when $n \leq 0.43$, and an integrated result attributed to drug diffusion and erosion are revealed when $0.43 < n \leq 0.85$. Dissolution is mainly driven by erosion when $n > 0.85$ (*21*). The model fitting results were obtained through DDSolver software (version 1.0, developed and published by China Pharmaceutical University) (*22*).

Dissolution Behaviors with Different Factors

Investigation of factors affecting dissolution behavior was performed based on the extraction method, except that samples were withdrawn at 1 h, 2 h, 6 h, 8 h, 1 d, 2 d, 3 d, 4 d, 5 d, 6 d, and 7 d. Temperature, pH value, and ethanol content in the dissolution media were explored as potential factors that influence RIS-MS dissolution behavior. Moreover, similarity factor, f_2, was used to evaluate the similarity between different samples to investigate the effect of each factor on RIS-MS dissolution behavior (*23, 24*). f_2 was calculated with Equation (6):

$$f_2 = 50 \log\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)\right] \times 100\right\}^{-0.5} \qquad \text{Eq. (6)}$$

where n is the number of samples, R_t and T_t are percentages of the dissolved drug for the reference and samples at each time interval, respectively. Results were compared to those from the extraction method

to investigate the effects of temperature, pH, and ethanol content) on dissolution behavior as follows.

Temperature: RIS-MS dispersed with media were oscillated at 37 °C, 45 °C, 55 °C, and 65 °C to investigate the effect of temperature on RIS-MS dissolution behavior.

pH value: RIS-MS were dispersed with 4 mL PBS media composed of sodium dihydrogen phosphate with adjusted pH levels of 4.0, 5.0, 6.0, 7.0, and 7.4 using phosphoric acid and sodium hydroxide to study the impact of pH value on RIS-MS dissolution behavior.

Ethanol content: Dissolution media containing 5%, 10%, 15%, 20%, and 25% ethanol (v/v) were applied to disperse RIS-MS and investigate the influence of ethanol on RIS-MS dissolution behavior.

Accelerated Dissolution Test

The accelerated dissolution method was optimized based on the above-mentioned factors. The accelerated dissolution method was established with the addition of 25% (v/v) ethanol in the media. Early, middle, and final phase (over 80% accumulated release) samples were extracted to compare with real-time dissolution test results according to the US Food and Drug Administration (FDA) guidelines for the application of f_2 (*25, 26*). Therefore, samples were extracted at 1 h, 4 h, 12 h, 1 d, 2 d, and 3 d in this accelerated method. The linear correlation between sample points of the accelerated dissolution behavior and real-time dissolution behavior (6 h, 24 h, 3 d, 6 d, 12 d, and 18 d) was established point by point in a time sequence, and the correlation coefficient (R^2) was calculated to analyze the similarity between the two dissolution profiles. The f_2 was also calculated to evaluate similarity between the two dissolution methods.

HPLC-UV Method

HPLC-UV was employed to detect the drug content from dissolution samples (10 µL) at 45 °C. The mobile phase consisted of methanol and 5 g/L ammonium acetate solution with a ratio of 50:50 (v/v) with a 1-mL/min flow rate. Detection wavelength was 278 nm.

Statistical Analysis

All studies were performed in triplicate unless otherwise stated. Data obtained were presented as mean ± SD, and one-way ANOVA with Student-Newman-Keuls (SNK) comparison by SPSS 19.0 software (IBM Corporation, Armonk, NY) was employed to analyze the data. A *p*-value less than 0.05 was considered statistically significant.

RESULTS

Dissolution Behavior with Extraction and Dialysis Methods

The solubility of risperidone in PBS media at different pH levels is shown in Table 1. The dissolution behavior using the extraction method and dialysis method are demonstrated in Figure 1A. The extraction method showed a higher total drug release (about 90%) than the dialysis method (about 70%) during the dissolution test. Moreover, a smaller standard deviation was recorded for the extraction method compared to the dialysis method.

Table 1. Solubility of Risperidone at Different pH Levels

Media (PBS)	Equilibrium Solubility (mg/mL)
pH 2.0	33.42
pH 5.0	8.48
pH 5.8	3.96
pH 6.8	0.97
pH 7.4	0.35
pH 7.9	0.20

Dissolution media was PBS with different pH adjusted by disodium phosphate and potassium dihydrogen phosphate containing 0.02% Tween 80 and 0.05% clozapine at 37 ± 5 °C for 72 h at 100 rpm. PBS, phosphate buffer solution.

Model Fitting of RIS-MS Dissolution Behavior

The results of model fitting for the RIS-MS dissolution test using the extraction method (real time) and accelerated method are displayed in Table 2. Based on the R^2 value, the best fit model for the real-time dissolution behavior was the Korsmeyer-Peppas model. The kinetic index (n) was 0.43 in this model, illustrating the contribution of drug diffusion to the RIS-MS dissolution behavior. The Korsmeyer-Peppas model also showed a better fit for the accelerated method with n = 0.39.

Table 2. Model Fitting for the Extraction (Real-Time) Method and Accelerated Dissolution Method of RIS-MS

	Zero Order Model	First Order Model	Korsmeyer-Peppas Model[1]	Higuchi Model
Real-Time Dissolution Method	$F = 0.0634 \times t$ $R^2 = 0.6725$	$\ln(1-F) = -0.1365 \times t$ $R^2 = 0.9760$	$F = 0.2674 \times t^{0.43}$ $R^2 = 0.9862$	$F = 0.2295 \times t^{0.50}$ $R^2 = 0.9856$
Accelerated Dissolution-Method	$F = 0.01351 \times t$ $R^2 = 0.4060$	$\ln(1-F) = -0.02461 \times t$ $R^2 = 0.8518$	$F = 0.1490 \times t^{0.39}$ $R^2 = 0.9926$	$F = 0.1024 \times t^{0.50}$ $R^2 = 0.9498$

[1]*Korsmeyer-Peppas Model was calculated with the data of t ≤ 16 d.*
RIS-MS, risperidone-containing PGLA microspheres; PLGA, poly(lactic-co-glycolic acid); F is the accumulated release.

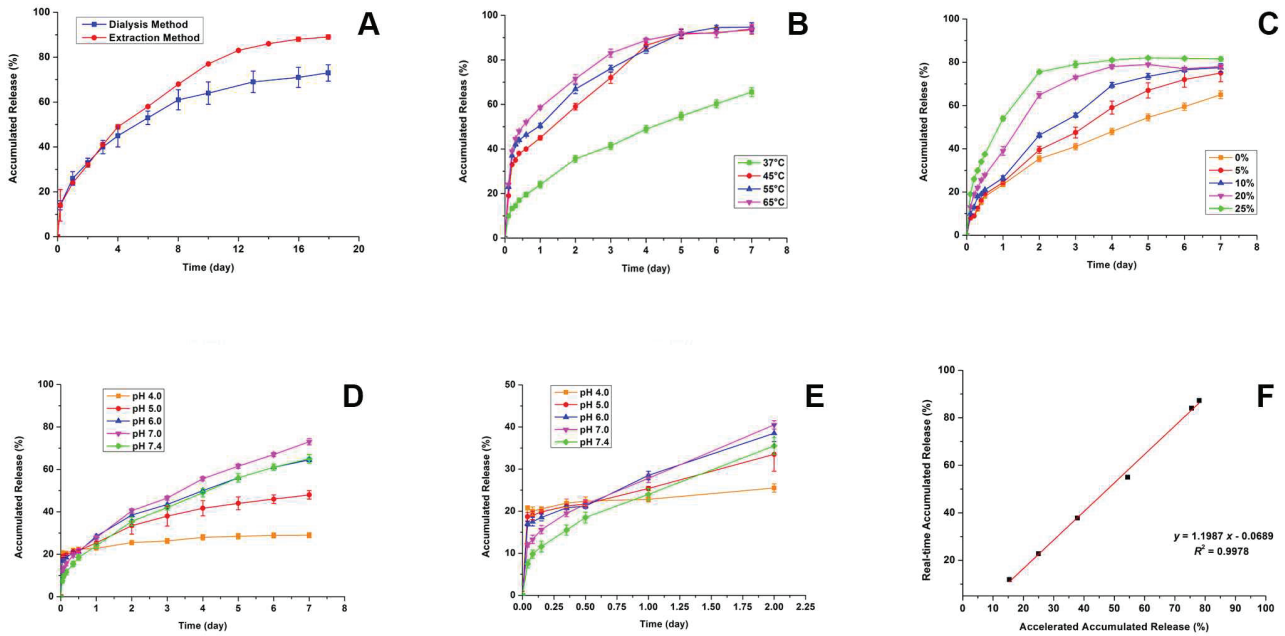

Figure 1. Dissolution behaviors of RIS-MS (mean ± SD accumulated drug release) under different conditions (n = 3): (A) extraction method vs dialysis method; effects of temperature (B), ethanol content (C), and pH (D and E); and correlation of accumulated release with the accelerated method vs real-time (extraction) method (F). RIS-MS, risperidone-containing PLGA microspheres; PLGA, poly(lactic-co-glycolic acid).

Dissolution Behavior of RIS-MS in Different Conditions

The f_2 of the dissolution behavior under different conditions are shown in Table 3. An increase in the dissolution rate was observed with increasing temperature, as shown in Figure 1B. The dissolution behavior at 45 °C, 55 °C, and 65 °C was not similar to the dissolution profile observed at 37 °C according to Table 3; however, similar dissolution behaviors were observed at 45 °C, 55 °C, and 65 °C.

Table 3. Similarity Factors (f2) for Dissolution of RIS-MS Using the Accelerated Method with Different Factors Groups

Temperature (Range)	f_2	Ethanol Content (Range)	f_2	pH (Range)	f_2
37 °C vs 45 °C	29.28†	0% vs 5%	64.53	4.0 vs 5.0	47.54†
37 °C vs 55 °C	27.67†	0% vs 10%	51.28	4.0 vs 6.0	35.14†
37 °C vs 65 °C	25.78†	0% vs 20%	38.49†	4.0 vs 7.0	30.81†
45 °C vs 55 °C	70.27	0% vs 25%	32.24†	4.0 vs 7.4	37.06†
45 °C vs 65 °C	57.02	5% vs 10%	65.23	5.0 vs 6.0	35.14†
55 °C vs 65 °C	69.24	5% vs 20%	42.39†	5.0 vs 7.0	30.81†
-	-	5% vs 25%	33.45†	5.0 vs 7.4	52.45
-	-	10% vs 20%	53.85	6.0 vs 7.0	66.01
-	-	10% vs 25%	38.07†	6.0 vs 7.4	66.78
-	-	20% vs 25%	54.05	7.0 vs 7.4	64.39

† *The dissolution behaviors of these two groups were not similar.*

The dissolution rate gradually increased with increasing ethanol content in media from 5% to 25%, as shown in Figure 1C, and the plateau of accumulated release occurred earlier with a higher ethanol content. Similar dissolution behaviors were shown between neighboring groups (0%-5%, 5%-10%, 10%-20%, and 20%-25%), and different behaviors were revealed between several interval groups (0%-20%, 0%-25%, 5%-20%, 5%-25%, and 10%-25%), as shown in Table 3. The accumulated release of the group with 25% ethanol achieved about 80% drug release within 3 days.

The effects of pH on the dissolution behavior are depicted in Figure 1D and 1E. As shown in Figure 1E, RIS-MS exhibited a burst release, which increased with increasing pH on the first day. The dissolution rate decreased as pH increased after 1 h, and higher accumulated release was obtained for higher pH levels except pH 7.4. As shown in Table 3, compared to dissolution behavior of pH 7.4, mimicking physical conditions, dissolution behavior was similar (above 50) at pH 4.0, 5.0, 6.0, and 7.0 according to f_2. The f_2 of pH 4.0 and pH 7.4 was 37.06 (dissimilar).

Correlation Between Accelerated and Real-Time Dissolution Behavior

The correlation coefficients of the accelerated method with different conditions compared to real-time

dissolution behavior are shown in Table 4. An accelerated dissolution method was established with the addition of 25% ethanol (v/v) in media. The correlation between dissolution behaviors in the accelerated method and conventional real-time method is depicted in Figure 1F. A linear correlation between the two dissolution methods (R^2 = 0.9978) was observed, and the accelerated dissolution method corresponded to its six-fold time point in real-time dissolution. Additionally, the f_2 between the accelerated and real-time dissolution behavior was 63.27, which demonstrated similar dissolution behavior in the two methods.

Table 4. Correlation Between Dissolution Behaviors of RIS-MS Using the Accelerated Method with Different Factors (3 Days) and the Real-Time (Extraction) Method (18 Days)

Factor	Correlation Equation	R^2
Temperature (45 °C)	y = 0.5848x + 15.3773	0.9440
Temperature (55 °C)	y = 0.6026x + 19.5064	0.9597
Temperature (65 °C)	y = 0.6718x + 20.8963	0.9602
pH (4.0)	y = 0.0743x + 19.3587	0.9559
pH (5.0)	y = 0.2441x + 14.0277	0.9422
pH (6.0)	y = 0.3456x + 10.6533	0.9651
pH (7.0)	y = 0.4353x + 5.6567	0.9832
Ethanol content (5%)	y = 0.5059x - 0.6125	0.9697
Ethanol content (10%)	y = 0.5742x + 0.1231	0.9592
Ethanol content (20%)	y = 0.7778x + 0.7180	0.9816
Ethanol content (25%)	y = 0.8048x + 8.4225	0.9983

DISCUSSION

Dissolution Mechanism for RIS-MS

The extraction method was selected as the real-time dissolution method due to its stable dissolution behavior (Fig. 1A). A higher dissolution rate and total accumulated release were obtained in the extraction method compared to the dialysis method, and a higher SD was observed in the dialysis method. Moreover, there was higher residual drug content in the dialysis method compared to the extraction method.

Differences between the extraction and dialysis dissolution methods might be related to their dissolution processes. As for dialysis dissolution method, risperidone released from MS accumulated in the dialysis tube first, then it passed through the dialysis membrane before entering the external dissolution media, as illustrated in Figure 2A. However, there was just a one-step diffusion process for risperidone to be released from MS to the external dissolution media in the extraction method. A more uniform dissolution solution could be achieved with the extraction method compared to the dialysis method, which contributed to the observed stable dissolution behavior. The extraction method was chosen as the real-time dissolution method to develop the accelerated method, and the dissolution mechanism of the extraction method was studied by model fitting.

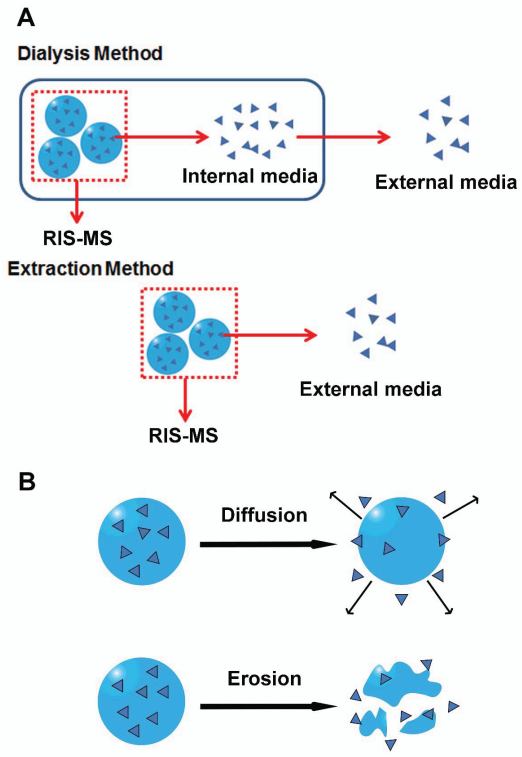

Figure 2. Dissolution process of RIS-MS by dialysis and extraction are shown in (A). Drug-releasing mechanisms, erosion and diffusion, are shown in (B). RIS-MS, risperidone-containing PLGA microspheres; PLGA, poly (lactic-co-glycolic acid).

Drug diffusion and skeleton erosion were two main mechanisms of the drug release from MS, as Figure 2B shows. The dissolution behavior of RIS-MS using the extraction method was mainly driven by drug diffusion according to model fitting (Table 2). The drug release can be analyzed with the Noyes-Whitney equation when dissolution is driven by diffusion:

$$\frac{dC}{dt} = k_d A(C_s - C_t) \qquad \text{Eq. (7)}$$

where dC/dt is the dissolution rate, k_d is the dissolution rate coefficient, A is drug surface area, C_s is saturated solubility of drug, and C_t is concentration of drug at time t. (*27*) Temperature, pH, and ethanol content can affect dissolution processes and alter the factors in this formula, which induced the accelerated dissolution behavior.

Effects of Different Factors on RIS-MS Dissolution Behavior

The selection of an accelerated dissolution method should be governed by the actual real-time dissolution mechanism and drug properties. The effects of temperature, pH, and ethanol content in media were investigated.

The increase of temperature to 45 °C, 55 °C, and 65 °C promoted RIS-MS release compared to 37 °C (Fig 1B). Higher temperatures accelerated drug release from RIS-MS by enhancing the drug solubility, C_s, which improved the drug release according to Equation (7). However, there were similar dissolution behaviors observed between different temperature groups, and it was difficult to obtain a suitable accelerated method corresponding to real-time dissolution behavior by only changing temperature.

A decrease in pH induced the increasing burst release at the initial time (Fig. 1D and 1E) . The accumulated release increased in the first 12 h as pH value decreased, because risperidone is a weak base, and its salt-dissociation can be influenced by the changes of pH, according to the Henderson-Hasselbalch equation:

$$\mathrm{pH} = \mathrm{p}K_a(A) + \lg\frac{C(\mathrm{A})}{C(\mathrm{HA^+})} \qquad \text{Eq. (8)}$$

where $pK_a(A)$ is the dissociation coefficient of the molecule, C(A) is the concentration of conjugated base of this molecule, and C(HA+) is the concentration of conjugated acid of this molecule (*28*). In this study, risperidone was a weak base, which can be ionized with decreasing pH when the total concentration of risperidone was constant. The ionized risperidone exhibited a higher C_s in water compared to its free base. According to Equation (8), increased C_s of risperidone improved the dissolution rate of risperidone.

Increasing ethanol content in media improved the drug dissolution rate mainly by enhancing the drug diffusion. The dissolution rate of RIS-MS increased with the content of ethanol in dissolution media (Fig. 1C). The addition of ethanol in dissolution media increased drug solubility, which increased C_s according to Equation (7) and increased the dissolution rate (*29*). In addition, different amounts of ethanol in media led to various dissolution behaviors, which demonstrated diverse accelerated dissolution behavior can be achieved through adjusting the ethanol content.

Correlation of dissolution behavior between different accelerated conditions (3 days) and real-time condition (18 days) are shown in Table. 4. The best correlation was found between the accelerated conditions with 25% ethanol in the dissolution media and the real-time condition, with the highest R^2 of 0.9983. Thus, an accelerated dissolution method was established with the addition of 25% ethanol in the dissolution media. Moreover, adding ethanol improved the dissolution rate by promoting drug diffusion through the whole dissolution process according to Table 2, which was governed by the identical real-time dissolution mechanism of RIS-MS.

Repeated dissolution tests under the real-time condition and accelerated condition with 25% ethanol were performed to verify the correlation. The correlation was established successfully with an R^2 = 0.9978 (Fig. 1F). The mechanism of this accelerated dissolution method was mainly driven by drug diffusion according to the model fitting of the accelerated dissolution in Table 2 (n = 0.39), which was same as the mechanism of the real-time dissolution behavior. This accelerated dissolution method was successfully optimized in different accelerated conditions, and it exhibited the same dissolution mechanism of MS. In summary, the accelerated dissolution method was able to simulate the real-time dissolution behavior, and it could significantly shorten the time for MS dissolution testing from 18 days to 3 days, which enhances the time and cost consumption for RIS-MS evaluation.

CONCLUSION

The effects and mechanisms of different factors, such as temperature, pH, and ethanol content in media, for the RIS-MS dissolution behavior were investigated in this study. An accelerated dissolution method with 25% ethanol in the dissolution media was established successfully to simulate the real-time dissolution behavior (R^2 = 0.9978). This accelerated dissolution method would substantially reduce the time consumption of dissolution tests compared to real-time dissolution tests for MS. This accelerated dissolution method is promising for MS product development and quality control, which are time and cost-effective.

ACKNOWLEDGEMENT

This work was supported by the Science and Technology Plan Projects of Guangdong Province under grant no. 2015B020232010 and by the Natural Science Fund Project of Guangdong Province under grant no. 2016A030312013.

CONFLICT OF INTEREST

The authors disclosed no conflicts of interest related to this article.

REFERENCES

1. Caine, M.; Carugo, D.; Zhang, X.; Hill, M.; Dreher, M. R.; Lewis, A. L. Review of the development of methods for characterization of microspheres for use in embolotherapy: translating bench to cathlab. *Adv. Healthc. Mater*. **2017**, *6*, 1601291. DOI: 10.1002/adhm.201601291.
2. Abadi, S. S.; Moin, A.; Veerabhadrappa, G. H. Review article: fabricated microparticles: an innovative method to minimize the side effects of NSAIDs in arthritis. *Crit. Rev. Ther. Drug Carrier Syst.* **2016**, *33*, 433–488. DOI: 10.1615/CritRevTherDrugCarrierSyst.2016016624.
3. Kelmendi-Doko, A.; Rubin, J. P.; Klett, K.; Mahoney, C.; Wang, S.; Marra, K. G. Controlled dexamethasone delivery via double-walled microspheres to enhance long-term adipose tissue retention. *J. Tissue Eng*. **2017**, *8*. DOI: 10.1177/2041731417735402.
4. Teekamp, N.; Van Dijk, F.; Broesder, A.; Evers, M.; Zuidema, J.; Steendam, R.; Post, E.; Hillebrands, J. L.; Frijlink, H. W.; Poelstra, K.; et al. Polymeric microspheres for the sustained release of a protein-based drug carrier targeting the PDGFβ-receptor in the fibrotic kidney. *Int. J. Pharm*. **2017**, *534*, 229–236. DOI: 10.1016/j.ijpharm.2017.09.072.
5. Zhang, H.; Wang, W.; Li, H.; Peng, Y.; Zhang, Z. Microspheres for the oral delivery of insulin: preparation, evaluation and hypoglycaemic effect in streptozotocin-induced diabetic rats. *Drug Dev. Ind. Pharm*. **2018**, *44,* 109–115. DOI: 10.1080/03639045.2017.1386197.
6. Desai, K. G. H.; Park, H. J. Preparation and characterization of drug-loaded chitosan-tripolyphosphate microspheres by spray drying. *Drug Dev. Res*. **2005**, *64*, 114–128. DOI: 10.1002/ddr.10416.
7. Yamakado, K.; Inaba, Y.; Sato, Y.; Yasumoto, T.; Hayashi, S.; Yamanaka, T.; Nobata, K.; Takaki, H.; Nakatsuka, A. Radiofrequency ablation combined with hepatic arterial chemoembolization using degradable starch microsphere mixed with mitomycin C for the treatment of liver metastasis from colorectal cancer: a prospective multicenter study. *Cardiovasc. Intervent. Radiol.* **2017**, *40*, 560–567. DOI: 10.1007/s00270-016-1547-3.
8. Parakh, S.; Gananadha, S.; Allen, R.; Yip, D. Cholecystitis after yttrium-90 resin microsphere radioembolization treatment: Clinical and pathologic findings. *Asian J. Surg*. **2016**, *39*, 144–148. DOI: 10.1016/j.asjsur.2015.04.002.
9. Brown, K. T.; Do, R. K.; Gonen, M.; Covey, A. M.; Getrajdman, G. I.; Sofocleous, C. T.; Jarnagin, W. R.; D'Angelica, M. I.; Allen, P. J.; Erinjeri, J. P.; Brody, L. A.; O'Neill, G. P.; Johnson, K. N.; Garcia, A. R.; Beattie, C.; Zhao, B.; Solomon, S. B.; Schwartz, L. H.; DeMatteo, R.; Abou-Alfa, G. K. Randomized trial of hepatic artery embolization for hepatocellular carcinoma using doxorubicin-eluting microspheres compared with embolization with microspheres alone. *J. Clin. Oncol*. **2016**, *34*, 2046–2053. DOI: 10.1200/JCO.2015.64.0821.
10. Chen, W.; di Carlo, C.; Devery, D.; McGrath, D. J.; McHugh, P. E.; Kleinsteinberg, K.; Jockenhoevel, S.; Hennink, W. E.; Kok, R. J. Fabrication and characterization of gefitinib-releasing polyurethane foam as a coating for drug-eluting stent in the treatment of bronchotracheal cancer. *Int. J. Pharm*. **2018**, *548,* 803–811. DOI: 10.1016/j.ijpharm.2017.10.026.
11. Zhang, M.; Ma, Y.; Li, R.; Zeng, J.; Li, Z.; Tang, Y.; Sun, D. RhBMP-2-loaded Poly(lactic-co-glycolic acid) microspheres fabricated by coaxial electrospraying for protein delivery. *J. Biomater. Sci. Polym. Ed*. **2017**, *28*, 2205–2219. DOI: 10.1080/09205063.2017.1390381.
12. Barhate, S.; Husain, M. Comparative in vitro dissolution testing of hydrophilic controlled-release venlafaxine matrix tablets and effexor XR using QbD. *Dissolut. Technol*. **2016**, *23*, 40–44. DOI: 10.14227/DT230316P40.
13. Tuszyński, P. K.; Polak, S.; Jachowicz, R.; Mendyk, A. From in vitro-in vivo relationship (IVIVR) towards in vitro-in vivo extrapolation (IVIVE): A case study of pulmonary delivery systems. *Dissolut. Technol*. **2017**, *24*, 32–35. DOI: 10.14277/DT240417P32.
14. D'Souza, S.; Faraj, J. A.; Dorati, R.; DeLuca, P. P. A short term quality control tool for biodegradable microspheres. *AAPS PharmSciTech.* **2014**, *15*, 530–541. DOI: 10.1208/s12249-013-0052-0.
15. D'Souza, S. S.; Faraj, J. A.; DeLuca, P. P. A model-dependent approach to correlate accelerated with real-time release from biodegradable microspheres. *AAPS PharmSciTech*. **2005**, *6*, E553–E564. DOI: 10.1208/pt060470.
16. Siewert, M.; Dressman, J.; Brown, C. K.; Shah, V. P. FIP/AAPS Guidelines to Dissolution/In Vitro Release Testing of Novel/Special Dosage Forms. *Dissolut. Technol*. **2003**; *4*, 6–15. DOI: 10.14227/DT100103P6.
17. Shameem, M.; Lee, H.; DeLuca, P. P. A short term (accelerated release) approach to evaluate peptide release from PLGA depot-formulations. *AAPS PharmSci*. **1999**, *1*, E7. DOI: 10.1208/ps010307.
18. Gaignaux, A.; Réeff, J.; De Vriese, C.; Goole, J.; Amighi, K. Evaluation of the degradation of clonidine-loaded PLGA microspheres. *J. Microencapsul.* **2013**, *30*, 681–691. DOI: 10.3109/02652048.2013.778905.
19. Tomic, I.; Vidis-Millward, A.; Mueller-Zsigmondy, M.; Cardot, J. M. Setting accelerated dissolution test for PLGA microspheres containing peptide, investigation of critical parameters affecting drug release rate and mechanism. *Int. J. Pharm*. **2016**, *505*, 42–51. DOI: 10.1016/j.ijpharm.2016.03.048.
20. Huang, Z.; Chen, X.; Fu, H.; Wen, X.; Ma, C.; Zhang, J.; Wu, C.; Huang, Y.; Pan, X.; Wu, C. Formation mechanism and in vitro evaluation of risperidone-containing PLGA microspheres fabricated by ultrafine particle processing system. *J. Pharm. Sci.* **2017**, *106*, 3363–3371. DOI: 10.1016/j.xphs.2017.07.010.
21. Su, Z.; Sun, F.; Shi, Y.; Jiang, C.; Meng, Q.; Teng, L.; Li, Y. Effects of formulation parameters on encapsulation efficiency and release behavior of risperidone poly(D,L-lactide-co-glycolide) microsphere. *Chem. Pharm. Bull. (Tokyo)* **2009**, *57*, 1251–1256. DOI: 10.1248/cpb.57.1251.

22. Zhang, Y.; Huo, M.; Zhou, J.; Zou, A.; Li, W.; Yao, C.; Xie, S. DDSolver: an add-in program for modeling and comparison of drug dissolution profiles. *AAPS J*. **2010**, *12*, 263–271. DOI: 10.1208/s12248-010-9185-1.
23. Huang, Y.; Yao, Q.; Zhu, C.; Zhang, X.; Qin, L.; Wang, Q.; Pan, X.; Wu, C. Comparison of novel granulated pellet-containing tablets and traditional pellet-containing tablets by artificial neural networks. *Pharm. Dev. Technol*. **2015**, *20*, 670–675. DOI: 10.3109/10837450.2014.910809.
24. Pan, X.; Huang, Y.; Dong, Y.; Wang, Z.; Zhu, C.; Li, G.; Chen, B.; Wu, C. Process investigation of a novel compaction technique with pellet-containing granules. *Ther. Innov. Regul. Sci*. **2013**, *47*, 593–601. DOI: 10.1177/2168479013495681.
25. Gohel, M. C.; Ramkishan, A.; Patel, T. M.; Pandya, R.; Suthar, V.; Koradia, H.; Madat, D. V.; Bariya, S.; Mehta, T. Nomogram for computing the value of similarity factor. *Indian J Pharm Sci.* **2014**, *76*, 245–251.
26. Burgess, D. J.; Crommelin, D. J.; Hussain, A. S.; Chen, M. L. Assuring quality and performance of sustained and controlled release parenterals: EUFEPS workshop report. *AAPS PharmSci*. **2004**, *6*, 100–111. DOI: 10.1208/ps060111.
27. Göke K, Bunjes H. Parameters influencing the course of passive drug loading into lipid nanoemulsions. *Eur. J. Pharm. Biopharm*. **2018**; *126*, 123-131. DOI: 10.1016/j.ejpb.2017.05.009.
28. Khames, A. Investigation of the effect of solubility increase at the main absorption site on bioavailability of BCS class II drug (risperidone) using liquisolid technique. *Drug Deliv*. **2017**, *24*, 328–338. DOI: 10.1080/10717544.2016.1250140.
29. Shukla, D.; Chakraborty, S.; Singh, S.; Mishra, B. Fabrication and evaluation of taste masked resinate of risperidone and its orally disintegrating tablets. *Chem. Pharm. Bull. (Tokyo)* **2009**, *57*, 337–345. DOI: 10.1248/cpb.57.337.