Original Research Paper

Effect of raw material variability of glipizide on the *in vitro* dissolution rate and *in vivo* bioavailability performance: The importance of particle size

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ABSTRACT

The objective of this study was to understand the impact of active pharmaceutical ingredients (API) particle size on a re-developed generic product of glipizide and to improve its formulation so that it exhibits bioequivalent to that of the reference listed drug (RLD). Two commercial batches of APIs (API-1 and API-2) with the same polymorphism and one batch of home-made APIs (API-3) with super-small particle size were used in the present study. The *in vitro* dissolution profiles of the tested formulations were compared with the RLD in a series of dissolution media. Then, the impact of particle size on *in vivo* absorption was evaluated in Beagle dogs. Compared with the RLD, formulation A with larger API size showed slower dissolution in pH 6.0 and 7.4 medium, resulting bioinequivalent with the RLD. Conversely, formulation B with smaller API size demonstrated similar *in vitro* dissolution profiles with the RLD and thus exhibited bioequivalent in the present study. Furthermore, formulation C with super small particle size still exhibited identical oral absorption although rapid dissolution was observed in the tested condition. Herein, it indicated that 2–5 µm might be defined as the "inert size range" of glipizide for ensuring the bioequivalence with the RLD. The results in the present study might help to obtain a better understanding of the variability in raw materials for oral absorption, develop a bioequivalent product and thus post-market quality control.

1. Introductions

Glipizide, an oral antidiabetic drug of the second-generation sulfonylurea, is used to control blood glucose in patients with non-insulin-dependent diabetes mellitus [1,2]. It promotes insulin release via blocking ATP sensitive potassium channel and thus reducing blood glucose levels [2]. Generally, glipizide can be classified as a typically Biopharmaceutical Classification System (BCS) II class drug with weakly acidic character

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(pK$_a$ 5.9) [3,4]. Similar with other class II drugs, the solubility of glipizide in gastrointestinal tract limits the dissolution, and thus oral absorption and bioavailability *in vivo* [5]. Previous studies demonstrated that several strategies including particle size reduction [4], solid dispersion and cyclodextrin complexation [6] facilitated to improve the *in vitro* dissolution and thus oral bioavailability of glipizide *in vivo*.

Clinical studies indicated that the absorption of glipizide *in vivo* was formulation dependent. Notably, the formulation prepared with non-micronized active pharmaceutical ingredients (APIs) led to slow and incomplete absorption with large inter-individual variation [7]. Similar variation in oral absorption stemmed from the particle size of APIs was also observed for glyburide, another second-generation sulfonylurea, highlighting the key role of particle size distribution in the development of a suitable product for sulfonylurea [8].

In recent years, drug developers and manufacturers are encouraged to utilize the achieved product and process understanding to address the sources of variation to product quality and to obtain the appropriate control strategies to identify the risk area [9]. Considering the potential raw material variability due to the change in either manufacturing route or their production sites, the relationship between material variability and product quality needs to understand [10]. Although previous studies indicated the critical role of particle size in the quality attribute of glipizide, few studies investigated the impact of APIs variability upon the final product quality [11]. Specially, the particle size of APIs provided by even the same manufacturer but varied batches might result in a quality attribute [12]. Herein, the present study aimed to understand the particle size of glipizide impacting *in vitro* dissolution and then *in vivo* oral absorption. Accordingly, we designed three identical formulations except for the particle size of glipizide. Since the dissolution test has been widely used to serve as an indicator to predict the quality of products, our study also aimed to evaluate the power of *in vitro* dissolution test in predicting *in vivo* performance of formulations. Such understanding is in favor of improving quality control in the production changes.

2. Materials and methods

2.1. Materials

Glipizide tablets chosen as the reference (containing 5 mg glipizide in total weight of 200 mg, RLD) were purchased from Pfizer Co., Ltd. (Dalian, China). Two batches of glipizide were obtained from Weihai Disu Pharmaceuticals Co., Ltd. (Shandong, China). Reference standards of glipizide (99.5% purity) and gliclazide used as internal standard were obtained from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). Lactose monohydrate was purchased from Jiangsu DaoNing Pharmaceutical Co., Ltd. (China). Microcrystalline cellulose, starch and colloidal silicon dioxide were gifted by Anhui Sunhere Pharmaceutical Excipients Co., Ltd.(China). Stearic acid was provided by Huzhou Zhan Wang Pharmaceutical Co., Ltd. (China). Methanol, acetonitrile and formic acid, all with HPLC grade were procured from Fisher (USA). Ammonium acetate

(HPLC grade) was purchased from Dikma (Richmond Hill, NY, USA). Deionized-distilled water was used throughout the study.

2.2. Preparation of glipizide power with reduced particle size

A PM planetary ball mill (Nanjing Chishun Science & Technology Co., Ltd., Nanjing, China) was used to prepare glipizide power with further reduced particle size. Zirconium dioxide beads (0.4–0.5 mm of diameter) were served as the milling media. The coarse suspension (3% glipizide, w/v) was dispersed in water and the mixture then was directly transferred to the planetary ball mill with the equal volume of beads. The milling was performed at a rotation speed of 35 Hz for 2 h. The resulting suspensions were then lyophilized using FDU-1100 freeze-drier (EYELA, Tokyo Rikakikai Co., Ltd, Japan). Briefly, lactose (10%, w/v) were added into the freshly prepared suspensions as lyoprotectants.

2.3. Particle size analysis

The particle distribution of the APIs from the manufacturers were measured directly by the laser diffraction beam as an aerosolized dry powder with the RODOS dry powder accessory (Sympatec HELOS Compact, R1 helium-neon laser GmbH, Windox Software 5.0, Clausthal-Zellerfeld, Germany). The measurement was conducted at air pressure of 3.5 bar. On the other hand, the particle size and polydispersity index of home-made glipizide power were measured by ZS-90 nanoparticle size analyzer (Malvern Instruments Ltd., UK). The power was dispersed with distilled water and each sample was determined in triplicate.

2.4. Scanning electron microscopy (SEM)

The morphology of the APIs was observed by SEM (Hitachi SU8010, Hitachi LTD., Tokyo, Japan). Briefly, the APIs were uniformly distributed on the surface of insulated double-sided carbon tape and the analysis was carried out at an acceleration voltage of 10 KV. Sputter coating was not needed. Micrographs were recorded at the required magnification.

2.5. X-Ray powder diffraction (XRPD)

XRPD patterns of the samples were determined using a D\MAX- 2400 Powder X-ray Diffractometer (Rigaku, Japan). The instrumental conditions were set as follows: CuK$_\alpha$ source at a generator power of 40 kV and 30 mA; divergent beam (2 mm); scanning range set from 0 to 60°.

2.6. Formulations

Three tested formulations of glipizide were used for *in vitro* dissolution testing and *in vivo* bioequivalence study. The reference formulation of glipizide was MINIDIAB® tablet (5 mg, Pfizer, Dalian, China). The test formulations containing API-1, API-2 or home-made API-3 were prepared via the wet granulation compression method by the following steps: lactose monohydrate, microcrystalline cellulose and glipizide were

mixed uniformly for 5 min, passing through an 80 mesh screen for 5 times and then were added with 10% starch paste to obtain the soft material. Soft materials were then passed through a 20 mesh screen to be uniform granules which allowed to be dried at 60 °C for 1 h. After arranging dry granules through 20 mesh screen, 2% colloidal silicon dioxide and 0.5% stearic acid were added and then blending uniformly to tablet. Consequently, three identical formulations were prepared except the difference of the particle size of APIs. Formulation A, B or C was made of API-1, API-2 or API-3 respectively. The developed test products contained qualitatively the same ingredients as the reference product (data not shown).

2.7. Dissolution tests

The *in vitro* dissolution tests were conducted according to the USP Apparatus 2 (Paddle method, RC806D, TDTF) in different media at 37 ± 0.5 °C. Paddles were rotating at 50 rpm and 900 ml of dissolution medium was used. 5 ml of samples were withdrawn at 5, 10, 15, 20, 30, 45 and 60 min and then filtered through 0.22 μm filter. The same amount of fresh medium was replaced at predetermined time intervals. Dissolution for each formulation was carried out in triplicate ($n = 3$) and the average of the values was calculated. The content of glipizide in withdrawn samples was measured by UV spectrophotometer at 220 nm. The similarity factor (f_2) was calculated to compare the dissolution profiles between the test and reference formulations.

2.8. Design of the bioavailability studies

Both of *in vivo* pharmacokinetic studies were conducted with ethical permission, which was permitted by Ethical Committee in China and was processed in accordance with the Guide for the Care and Use of Laboratory Animals [13].

2.8.1. Study 1
A single center, randomized, single dose, three-period, three-treatment, crossover study in nine healthy male Beagle dogs weighing 10 ± 1.5 kg, with a washout period between doses of one week was used. The investigated products (formulation A, B and RLD) as tablet containing 5 mg glipizide with 100 ml warm water were orally administrated under fasting condition. The animals were provided with a standard lunch 4 h after dosing. Blood samples (3 ml) were collected by venipuncture into a heparinized blood collection tube at pre-dose, and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 and 24 h post-administration in each study period. Blood samples were immediately centrifuged at 3500 rpm for 10 min and stored in polypropylene tubes at -80 °C till further analysis. All plasma samples were thawed at room temperature before preparing for HPLC/MS/MS analysis [14].

2.8.2. Study 2
A single center, randomized, single dose, two-period, two-treatment, crossover study in six healthy male Beagle dogs weighing 10 ± 1.5 kg, with a washout period between doses of one week was used. The investigated products (formulation C and RLD) as tablet containing 5 mg glipizide with 100 ml warm water were orally administrated under fasting condition. The blood samples were taken at predetermined time and the plasma were prepared as described above.

2.8.3. Bioanalytical method
The glipizide plasma concentrations were determined by a high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method after protein precipitation extraction. An aliquot of 50 μl IS solution (gliclazide, 100 ng/ml) was pipetted into a 1.5 ml eppendorf micro centrifuge tube. Afterwards, 100 μl of plasma and 50 μl of diluent (methanol/water = 10/90, v/v) solution were added. The sample was vortex-mixed for 1 min. And then 300 μL of methanol was added. The mixture was mixed for 1 min again. After being centrifuged at 13 000 rpm for 10 min, the supernatant liquid was for direct injection to the HPLC-MS/ MS system. The chromatography was carried out on the Acquity UPLC^TM system (Waters Corp., Milford, MA, USA) with a cooling autosampler. Chromatographic separation was achieved on a Phenomenex C_{18} column (50 mm × 2.1 mm, 2.6 μm) with a guard column (C_{18}, 4 mm × 3 mm, Phenomenex Ltd). The mobile phase A consisted of methanol and mobile phase B was composed of 10 mmol/l ammonium acetate and 0.2% formic acid (A: B = 60:40, v/v). The flow rate was set at 0.2 ml/ min. The injection volume was 10 μl. Mass spectrometric detection was performed on a tandem quadrupole detector equipped with an electron spray ionization (ESI) source. The ESI source was set in positive mode. The two compounds were detected using multiple reaction monitoring (MRM) of the transition of m/z 446.3→321.1 for glipizide and 324.1→127.1 for IS, with a scan time of 0.20 s per transition. The optimal MS parameters were set as follows. The capillary voltage was 3.2 kV, the cone voltage was 30 V for glipizide and 30 V for IS, the source temperature was kept at 120 °C and the desolvation temperature was kept at 500 °C. The optimized collision energy was 16 eV for glipizide and 20 eV for IS. Nitrogen was used as the desolvation and cone gas with a flow rate of 550 and 50 l/h respectively.

2.8.4. Plasma concentration data processing and statistical analyses
All data collected were carried out by using the Masslynx^TM NT 4.1 software with the QuanLynx^TM program (Waters Corp., Milford, MA, USA). Pharmacokinetic parameters which using non-compartmental model analysis to calculate, including the maximum concentration (C_{max}) and the time of the maximum plasma concentration (T_{max}), were obtained directly from the measured data. The formula $T_{1/2} = 0.693 k_e$ was used for calculating the elimination half-life ($T_{1/2}$). The linear regression of the terminal points in the semi-log plot of plasma concentration against time was for the purpose of calculating the elimination rate constant (k_e). The linear trapezoidal rule was used for the area under the plasma concentration–time curve (AUC_{0-t}) to the last measurable plasma concentration (C_t). The formula $AUC_{0-\infty} = AUC_{0-t} + C_t/k_e$ was used for figuring the area under the plasma concentration–time curve to time infinity ($AUC_{0-\infty}$). The paired t tests were used on the logarithm-transformed data for the C_{max} and AUC to evaluate whether the 90% confidence intervals were within the range of 80–125%.

Table 1 – Results for particle sizing.				
	D_{10} (μm)	D_{50} (μm)	D_{90} (μm)	VMD* (μm)
API-1	0.72	2.09	11.70	11.66
API-2	0.6	1.74	5.65	2.58
* VMD: volume median diameter.				

3. Results and discussion

3.1. Particle size analysis of glipizide

The dry powder laser particle size analyzer was used to analyze the particle distribution of the APIs from the manufactory. As shown in Table 1, API-1 had wider particle size distribution due to the existence of large particles as evidence that D_{90} and volume median diameter (VMD) of API-1 were 11.70 and 11.66 μm respectively. Indeed, the size distribution showed the powder of API-1 presented two different populations (Fig. S1), one for individual particles and one for agglomerates. On the contrary, API-2, which had a D_{90} value of 5.65 μm and a VMD value of 2.58 μm, was more uniform and smaller compared to API-1.

To avoid the interference of lactose in the determination by dry powder laser particle size analyzer, the home-made API-3 was initially dispersed in suspension and then analyzed. As also shown in Fig. S1, the average particle size of fresh prepared suspensions was 2 μm with a PDI of 0.202, indicating uniform distribution of small API particles.

3.2. SEM analysis

SEM was used to determine the surface morphology and particle size of APIs. As shown in the Fig. 1 all of APIs were rod-like shaped with a smooth surface, which was consistent with the previous reports [15]. Consistently with the results from laser particle size analyzer, API-1 had larger particle size with around 10 μm in length, whereas API-2 was smaller with
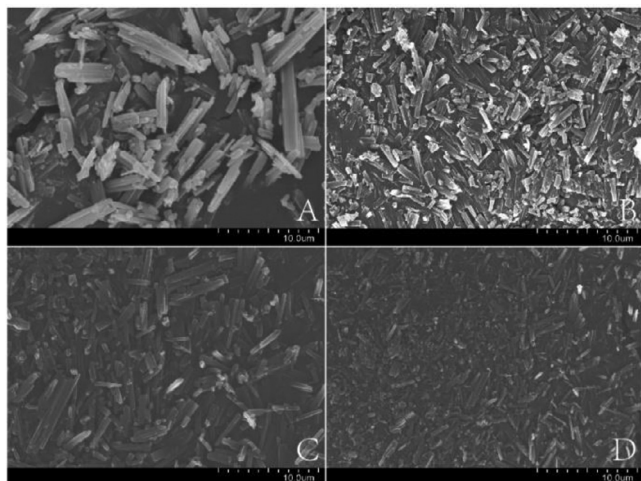

Fig. 1 – SEM images of APIs of glipizide (A): API-1; (B): API-2; (C): API-3 before ball mill; (D): API-3 after ball mill.
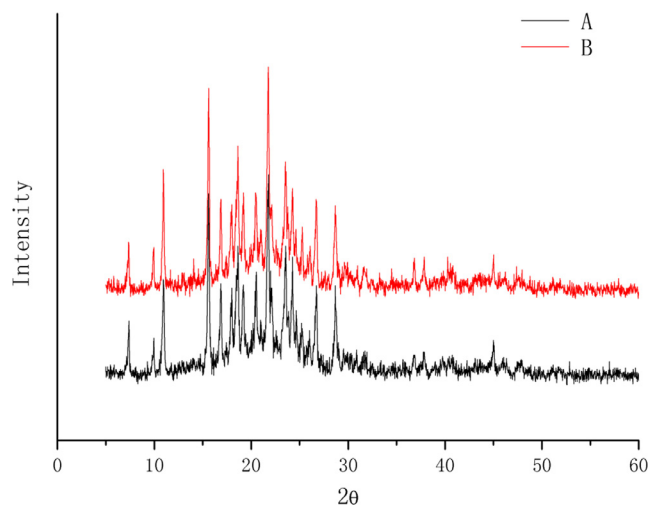

Fig. 2 – XRPD studies of APIs of glipizide (A: API-1; B: API-2).

around 5 μm in length. Furthermore, home-made API-3 exhibited super-small particles as compared with API-1 and API-2.

3.3. X-ray diffraction studies

XRPD was used to determine the identity of crystalline forms presenting in two commercial batches of APIs. The XRPD results exhibited no distinguishable differences in the diffraction patterns between API-1 and API-2 (Fig. 2), indicating the same crystalline form. Compared with the previous reports, the identical peaks and their strength in the diffraction patterns were consistent with those of the most stable crystalline form [15]. PXRD analysis of API-3 and the RLD failed in the present study due to rather low dose of glipizide and abundant crystalline stated lactose in the powder of API-3 and MINIDIAB®.

3.4. In vitro dissolution and in vivo pharmacokinetic study

To address the risk of APIs raw material, we designed two runs to identify the differences in the formulations. The first run employed two commercial batches of glipizide from one supplier to prepare the test products with the aim to investigate the impact of raw material variability on regarding quality in practice. The second one utilized the home-made APIs with super-small particles size to determine the controlled safety range of raw material for a robust formulation. Accordingly, the comparison of the dissolution profiles and their corresponding bioavailabilities were performed to consider the

Table 2 – The characterizations of RLD and self-made tablets.

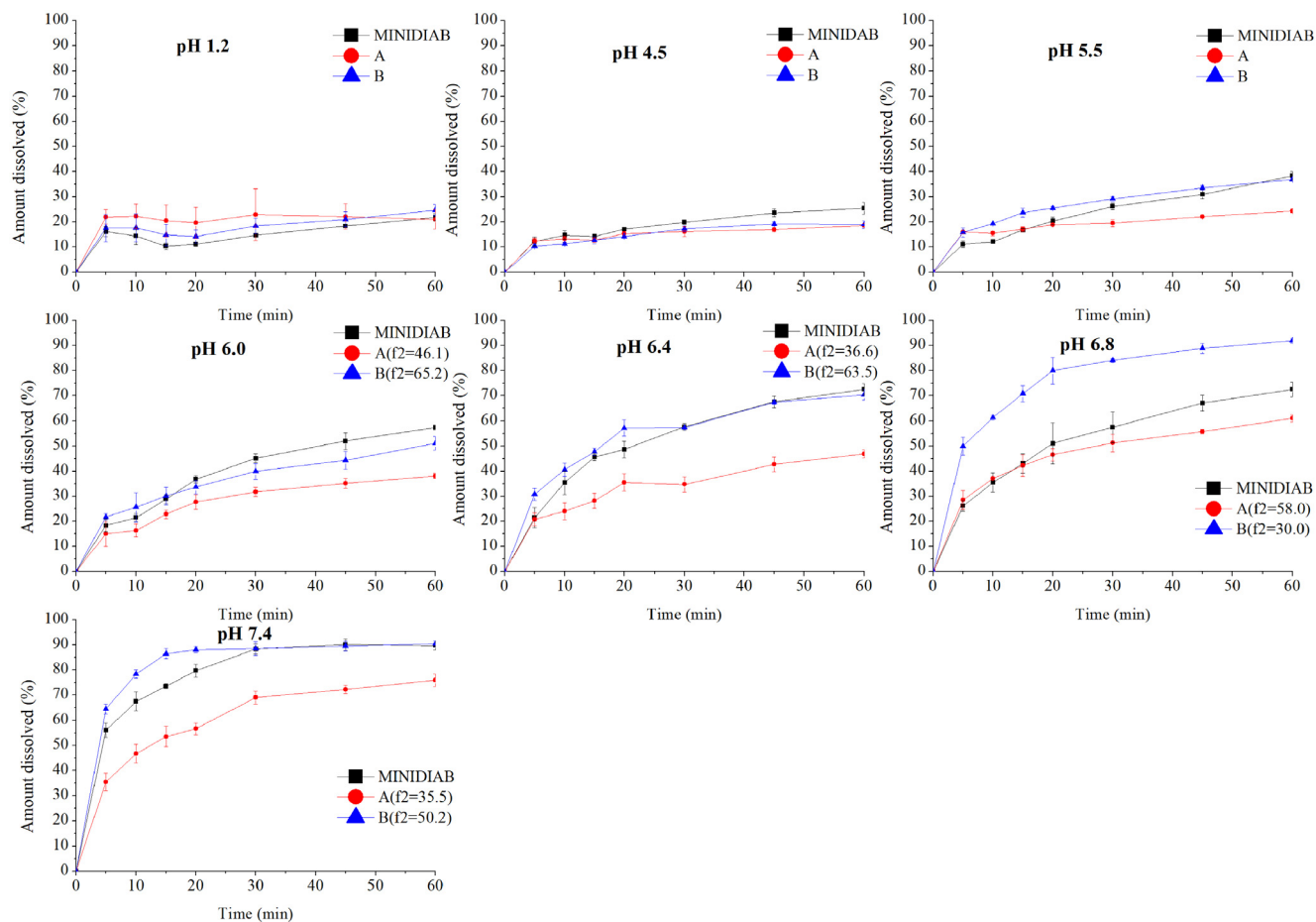|	RLD	Formulation A	Formulation B	Formulation C
Disintegration time (s)	75.7 ± 3.3	95.8 ± 4.9	101.3 ± 4.7	89.7 ± 6.5
Hardness (kg/mm^2)	5.0 ± 0.4	5.3 ± 0.4	5.5 ± 0.4	5.7 ± 0.3

Fig. 3 – The dissolution profile comparisons of formulation A and B with the reference tablets (MINIDIAB®) in buffered media.

relevant properties in the re-development of formulations. To further evaluate the *in vitro* and *in vivo* performance of the developed formulations, the hardness and disintegration time of tablets were compared and the results were shown in Table 2. Consequently, all of the tested formulations exhibited rapid disintegration (< 2 min) and no significant difference ($P > 0.05$) between either of test tablets was observed in the hardness.

3.4.1. Study 1

The dissolution profiles from the test formulations and the RLD (MINIDIAB® tablet) were evaluated in four recommended dissolution media (pH 1.2, 4.5, 6.8 and 7.4) and several dissolution media with pH values around pKa of glipizide (5.9). The dissolution results were shown in Fig. 3. As shown in Fig. 3, the dissolution profiles of glipizide strongly depended on its solubility. Our preliminary study revealed that the solubility of API initially slowly increased with pH until its pKa, where a sharp

enhancement was observed. Consequently, it demonstrated that glipizide released from either of the tested formulations with a slow dissolution rate in the buffered media below pH 6.0, as the evidence of less than 40% of the total amount dissolved within 60 min (Fig. 3A–C). The difference of dissolution rate among formulations in these conditions was not prominent because of rather low solubility of API. In contrast, formulation B showed a significantly higher dissolution rate compared with the formulation A when the media buffered media above pH 6.0 were used, probably due to small particle size of APIs used in formulation B. In order to assess the similarity between the test formulation and RLD, the f_2 was calculated and the results were also shown in Fig. 3. As depicted in Fig. 3, glipizide dissolved from formulation A with a lower dissolution rate as compared with that from MINIDIAB® in pH 6.0, 6.4 and pH 7.4 buffer media, exhibiting less than 50 of calculated f_2. On the other hand, the formulation B exhibited similar dissolu-

Table 3 – Pharmacokinetic parameters obtained from the Glipizide formulations including MINIDIAB® (RLD), formulation A and B after single oral administration in beagle dogs. (data were shown as mean ± SD, $n = 9$).

PK parameters	R	A	B
C_{max} (ng/ml)	3139.88 ± 964.07	2255.2 ± 629.74	2803.22 ± 833.23
T_{max} (h)	2.83 ± 0.75	3.56 ± 1.10	4.56 ± 1.74
$t_{1/2}$(h)	5.22 ± 2.15	3.25 ± 0.48	4.49 ± 1.76
AUC_{0-t} (ng·h/ml)	27691.59 ± 10106.13	23149.12 ± 6818.19	28417.33 ± 11042.82
$AUC_{0-\infty}$(ng·h/ml)	29901.42 ± 11305.96	23435.39 ± 6841.66	29671.33 ± 10992.36
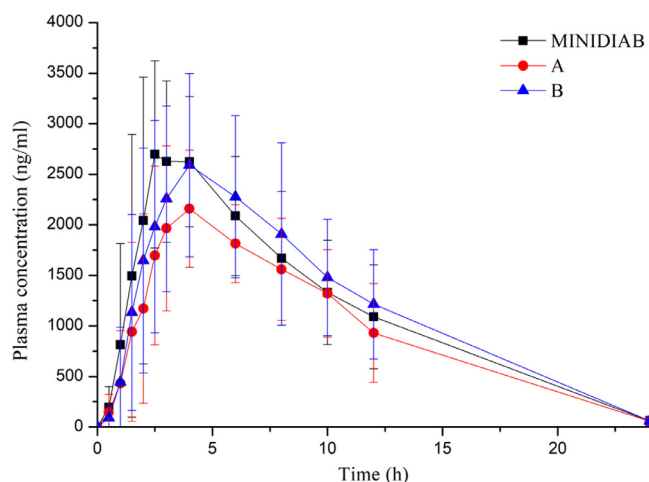
Fig. 4 – Mean plasma concentration–time curves of glipizide after oral administration of formulations A, B or MINIDIAB®.

tion profiles of MINIDIAB® in pH 6.0, 6.4 and pH 7.4 buffer media with the calculated f_2 above 50. However, the dissolution rate of API from the formulation B (∼90% dissolved in 60 min) was significantly higher than that of MINIDIAB® (∼70% dissolved in 60 min) in the dissolution medium of pH 6.8, resulting in 30.0 of f_2. On the contrary, the difference of dissolution rate between the formulation A and MINIDIAB® was reduced in PBS 6.8 (f_2 of 58.0), especially for the initial 20 min.

Based on above *in vitro* dissolution results, both of test formulations were then evaluated their bioequivalence with RLD in Beagle dogs. The mean concentration-time profiles of glipizide after single oral administration of either of test formulations or RLD were demonstrated in Fig. 4 and the involved pharmacokinetics parameters were showed in Table 3. As shown in Fig. 4, the oral administration of formulation A gave a lower plasma concentration of glipizide than that of RLD or formulation B over 24 h. As a result, both of AUC_t (23149.12 ± 6818.19 ng·h/ml) and C_{max} (2255.2 ± 629.74 ng/ml) were lower for formulation A than those for RLD (27691.59 ± 10106.13 ng·h/ml and 3139.88 ± 964.07 ng/ml). On the contrary, the formulation B with small particle size of API demonstrated comparable AUC_t (28417.33 ± 11042.82 ng·h/ml) and C_{max} (2803.22 ± 833.23 ng/ml) of RLD.

According to the guideline, the test formulation is believed bioequivalent with the reference when the 90% confidence interval (CI) of the mean ratios (test/reference) for the log-

transformed C_{max}, $AUC_{0-\infty}$ and AUC_{0-t} are within the range of 80.0%–125.0%. The two one-sided t tests and 90% CIs results of AUC and C_{max} were summarized in Table 4. As exhibited in Table 4, the 90% CIs of C_{max} and AUC_{0-t} for formulation A were 65.5%−81.0% and 76.4%−95.0%, respectively, indicating bioinequivalent with the RLD. Conversely, the 90% CIs of C_{max} and AUC_{0-t} for formulation B were 81.0%−100.3% and 91.3%−113.5%, respectively, within the range of 80.0%−125.0%, indicating bioequivalent with the RLD (Table 5).

3.4.2. Study 2

Although formulation B was assessed as bioequivalent, the absorption rate of glipizide administrated was still a bit lower when compared with RLD (2803.22 ± 833.23 ng/ml *vs* 3139.88 ± 964.07 ng/ml, P > 0.05). Then formulation C with super-small particle size of APIs was then used in the second run. Fig. 5 demonstrated the comparative dissolution profiles in the similar conditions of the first run. As estimated, formulation C exhibited faster dissolution rate in almost all the investigating mediums. The f_2 of the dissolution profiles for the test formulation vs. RLD at pH 6.0, 6.8 and 7.4 were 46.5, 20.5 and 32.7, respectively. The pharmacokinetic study, which compared formulation C to the RLD, however, indicated similar AUC and C_{max} (P > 0.05) with after oral administration (Fig. 6 and Table 6). Since high within-subject variability in AUC and C_{max} were obtained in Study 2, it was not suitable to proceed into the bioequivalent assessment due to a rather small sample of beagle dogs.

3.5. Discussion

The present study indicated that the variability of APIs can severely impact *in vitro* dissolution and *in vivo* absorption of glipizide. Accordingly, it is crucial to address the sources of variation to set up a controlled strategy for reliable product quality by a design of robust formulation and process. If possible, the evaluation of API variability needs to be identified in an early stage of formulation development to mitigate risk for the final product quality [16].

Notably, several parameters of APIs could be used to character physicochemical properties of raw material and then their impacts on processability and drug product quality, such as the particle size, polymorphism, agglomeration profile and specific surface area [9,17]. Among these parameters, two main independent variables (the particle size and polymorphism) could be routinely used to understand the physicochemical properties of raw materials. Generally, the analysis of particle size distribution would mirror the agglomeration

Table 4 – Two one-sided *t*-test of main parameters between formulation A and the RLD.

Parameters	t_1	t_2	$t_1 - 0.05(14)$	90% confidence
AUC_{0-t}	1.009	6.186	$t_1 < t_1 - 0.05(14), t_2 > t_1 - 0.05(14)$	76.4%–95.0%
$AUC_{0-\infty}$	0.104	7.135	$t_1 < t_1 - 0.05(14), t_2 > t_1 - 0.05(14)$	72.2%–89.7%
C_{max}	−1.551	8.933	$t_1 < t_1 - 0.05(14), t_2 > t_1 - 0.05(14)$	65.5%–81.0%

Table 5 – Two one-sided *t*-test of main parameters between formulation B and the RLD.

Parameters	t_1	t_2	$t_1 - 0.05(14)$	90% confidence
AUC_{0-t}	3.883	3.312	$t_1 > t_1 - 0.05(14), t_2 > t_1 - 0.05(14)$	91.3%–113.5%
$AUC_{0-\infty}$	3.568	3.672	$t_1 > t_1 - 0.05(14), t_2 > t_1 - 0.05(14)$	89.4%–111.1%
C_{max}	1.972	5.410	$t_1 > t_1 - 0.05(14), t_2 > t_1 - 0.05(14)$	81.0%–100.3%
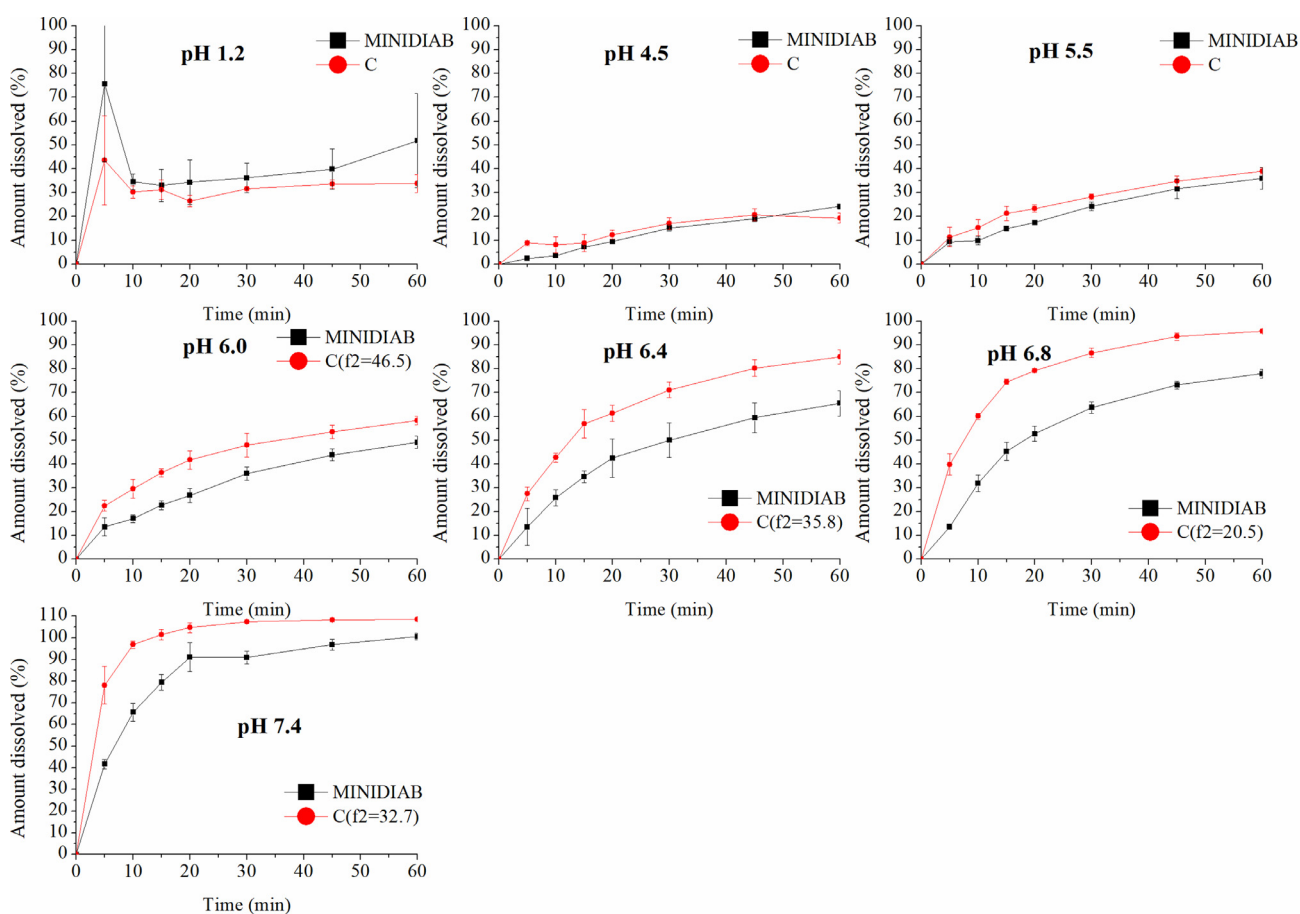
Fig. 5 – The dissolution profile comparisons of formulation C with the reference tablets (MINIDIAB®) in buffered media.

profile of API provided the consistency of production. In the same way, specific surface area of raw materials is highly depended on the crystal habit and the particle size of APIs. As showed in Fig. 1, all tested APIs exhibited similar morphology, which was in accordance with the previous results founded in the most stable polymorphism of glipizide. Accordingly, the particle size would be the key factor to address the controlled strategy of API as long as the same polymorphism of glip-

izide has been employed in the preparation of products. To avoid the misinterpretation of multivariate parameters, a single material parameter (particle size) was allowed to explore the influence of API variabilities on drug product quality in the present investigation. The reason was because that API variation in particle size was considered as the representative and regular "batch to batch" variability [18]. On the other hand, the solid state of APIs is another key determining factor for their
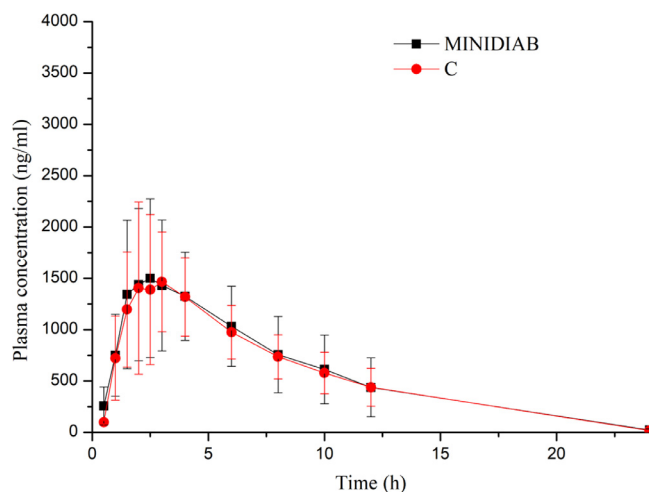
Fig. 6 – Mean plasma concentration–time curves of glipizide after oral administration of formulations C or MINIDIAB®.

Table 6 – Pharmacokinetic parameters for Glipizide formulation including MINIDIAB®, formulation C (data were shown as mean ± SD, n = 6).

PK parameters	RLD	C
C_{max} (ng/ml)	1710.00 ± 461.39	1638.83 ± 573.92
T_{max} (h)	2.42 ± 0.86	2.67 ± 0.75
$T_{1/2}$ (h)	3.98 ± 1.71	5.06 ± 1.78
AUC_{0-t} (ng·h/ml)	13053.33 ± 6637.92	12699.73 ± 4459.71
$AUC_{0-\infty}$ (ng·h/ml)	14015.88 ± 7383.07	13902.92 ± 5285.58

basic physicochemical properties, including solubility, stability and dissolution rate [15,19]. In this respect, the most stable polymorphism of glipizide was used in the preparation process of the test formulations. We did not observe any change in the performance characteristics of dosage form, such as power flow, tablet compressibility and mechanical strength, when the APIs from different batches was introduced in the formulation.

Being a weak acid ($pK_a = 5.9$), glipizide is better absorbed in basic medium, whereas in very acidic media, the solubility of glipizide is minimal [20,21]. Herein, the chosen dissolution medium aimed to mimic the *in vivo* gastrointestinal conditions has been widely used in the *in vitro* dissolution investigation. Consequently, the evaluation of the generic formulations at pH 1.2, 4.5 and 6.8 reflects roughly the pH conditions in the gastrointestinal tract, however, the comparative dissolution in relevant media may not distinguish significant changes in the composition and manufacturing process. For example, the dissolution profiles of formulation A in pH 1.2, 4.5 and 6.8 conditions were assessed as similar with the RLD, whereas the product failed in the bioequivalence test. Conversely, the formulation B exhibited faster dissolution of API in the regarding conditions, while proving the bioequivalence to RLD. It indicated that the discriminatory power of the classical dissolution criteria was not suitable for the product quality evaluation of glipizide. Therefore, the comparative dissolution studies were further conducted at pH 6.0, 6.4 and 7.4 in the present study with the aim to cover the middle and high solubility regions of API. As anticipated from the solubility evaluation, rapid and complete dissolution for formulation B and RLD was observed at pH 7.4 (f_2 of 50.8), while formulation A with coarse particle size distribution led to a slow and incomplete dissolution. In the middle solubility region of API, formulation A and B also maintained slow and comparable dissolution rate respectively when compared with RLD. Specially, the run-to-run variability in the dissolution of RLD was not observed at pH 5.5, 6.0 and 6.4, where the pH condition was most close to pK_a of API. Overall, the dissolution medium with pH 6.0 was considered to be robust enough to tolerate slight changes (± 0.4 pH) in laboratory conditions to provide reproducible dissolution profiles of API [22]. Therefore, the dissolution medium with pH 6.0 and 7.4 were initially proposed to possess suitable discriminatory nature.

The second run of comparative dissolution and pharmacokinetic study was conduct to seek the acceptable lower limit of APIs' particle size. The obtained results suggested overdiscriminating dissolution tests in pH 6.0 and 7.4. Specially, formulation C exhibited faster dissolution rate ($f_2 < 50.0$), however, it showed comparable oral absorption extent and rate when compared with the RLD. We speculated that one possible reason for this phenomenon was less sensitivity of oral absorption of glipizide given the particle size of APIs in the range of 2–5 μm. Therefore, 2–5 μm might be defined as the "inert size range" of APIs for ensuring the bioequivalence with the RLD [23]. Our preliminary studies indicated that the granularity of glipizide could be reduced to~4 μm (D_{90}, measured by dry powder laser particle size analyzer) by the jet grinding mill. The powder of APIs obtained from the relating process might be used for the development of generic products.

4.   Conclusion

In conclusion, the present study highlighted that the particle size distribution of glipizide was an important factor to the *in vitro* dissolution and thus *in vivo* bioavailability of redeveloped oral tablets. The dissolution profiles in pH 6.0 and 7.4 mediums offered discriminatory nature in understanding the impact of API's particle size. The formulations with API's particle sizes in the range of 2–5 μm appeared to exhibit similar oral absorption when compared with the reference products. In the near further, the clinical trials will be conducted to assess the bioequivalence between the re-developed formulation and the RLD.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ajps.2018.06.005.

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