Chinese Chemical Letters 32 (2021) 1071–1076

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Communication

Formulation optimization and *in vitro* antibacterial ability investigation of azithromycin loaded FDKP microspheres dry powder inhalation

Qiyue Wang[a,1], Liang Ge[a,1], Lu Wang[a], Ying Xu[b], Si Miao[a], Guiping Yu[c,*], Yan Shen[a,*]

[a] *Center for Research Development and Evaluation of Pharmaceutical Excipients and Generic Drugs, Department of Pharmaceutics, China Pharmaceutical University, Nanjing 210009, China*
[b] *College of Pharmacy, Jiangsu University, Zhenjiang 212013, China*
[c] *Department of Cardiothoracic Surgery, The Affiliated Jiangyin Hospital of Southeast University Medical College, Jiangyin 214400, China*

ARTICLE INFO

Article history:
Received 5 February 2020
Received in revised form 20 March 2020
Accepted 23 March 2020
Available online 2 April 2020

Keywords:
Azithromycin
FDKP
Formulation optimization
In vitro antibacterial efficiency
Streptococcus pneumoniae

ABSTRACT

Azithromycin loaded fumaryl diketopiperazine (FDKP) dry powder inhalation was designed and prepared for the treatment of community-acquired pneumonia. The solubility of FDKP and stability of azithromycin solution was investigated. Formulation of azithromycin loaded FDKP microparticle was investigated and optimized by the single factor experiment. High-pressure homogenization and spray drying conditions were also optimized to prepare the particles by spray drying azithromycin dissolved FDKP microparticle suspension at pH 4.5. The *in vitro* antibacterial efficiency and *in vitro* dispersion performance was also investigated to confirm the antibacterial efficiency, dispersion and deposition behavers. FDKP/azithromycin mass ratio (3:2) was the optimized formulation of azithromycin loaded FDKP microparticle with the maximal drug loading efficiency. High-pressure homogenization and spray drying conditions were also optimized. The *in vitro* antibacterial results indicated that only with the antibiotic concentration higher than mutant prevention concentration could totally inhibit the reproduction of bacteria. *In vitro* dispersion performance of azithromycin loaded FDKP microparticles (AZM@FDKP-MPs) also shows remarkable improvement of dispersion and deposition behavers of AZM. AZM@FDKP-MPs dry powder inhalation as a targeting delivery route has better potential for lung infection treatment.

© 2020 Chinese Chemical Society and Institute of Materia Medica, Chinese Academy of Medical Sciences. Published by Elsevier B.V. All rights reserved.

The pulmonary infection has become the third most fatal disease in the world and pneumonia which responsible for over 4 million deaths every year has become one of the major contributors to hospitalizations and deaths based on the lung infection [1,2]. Community-acquired pneumonia (CAP), pneumonia acquired outside a hospital, was major caused by the infection of bacterial, virus, chlamydia and mycoplasma which especially by the Gram-positive bacteria like *Streptococcus pneumoniae*, *Mycobacterium tuberculosis*, *Staphylococcus aureus* and so on [3–5]. Cephalosporins and macrolides were the most commonly used antibiotic on the CAP treatment, and quinolones might be combined to treat the infection with *Pseudomonas aeruginosa*.

Azithromycin, a semi-synthetic 9-*N*-methylation derivative of erythromycin, was typically recommended as a first-line outpatient treatment for the CAP. The antibacterial spectrum of azithromycin was similar to erythromycin but shows more powerful antibacterial activity on the *Streptococcus pneumoniae*, *Legionella pneumophila*, *Haemophilus influenza* and increased acid stability [6–10]. For the CAP treatment, only azithromycin injection and orally tablets (like Zithromax®, Sumamed®) were developed as commercial products, which drug directly absorbed into circle system with low pulmonary accumulation and easily lead to a side effect of the gastrointestinal tract such as nausea, vomit and diarrhea, *etc*. From the theory of mutation selection window (MSW), bacterial co-culture with antibiotic with concentration between minimum inhibitory concentration (MIC) and mutant prevention concentration (MPC) will selectively enrich of one-step mutant bacteria reproduction and lead to the antibiotic resistant [11–13]. Therefore, the new delivery routes were urgently needed to improve the treatment efficiency and avoid the antibiotic resistant mutation of bacteria.

Dry powder inhalation has already investigated for the local therapeutic such as pulmonary cancer, asthma, pulmonary infection for a long time [14,15]. As dry powder inhalation is

* Corresponding authors.
E-mail addresses: xiaoyuer97103@163.com (G. Yu), shenyan19820801@126.com (Y. Shen).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.cclet.2020.03.062
1001-8417/© 2020 Chinese Chemical Society and Institute of Materia Medica, Chinese Academy of Medical Sciences. Published by Elsevier B.V. All rights reserved.

driven by the breathing behavior of patients, the satisfied atomization ability of powders was necessary. For the higher pulmonary drug deposition efficiency, different kinds of evaluation need to be done on the characterization which could affect the aerodynamics behavior and aerosol generation such as particle size, particle shapes, surface morphology, charges and water content [16–19]. Different manufacture technologies like fluid energy milling, spray drying, spray freeze-drying and supercritical fluid technology was used to reduce the particle size and modify the particle properties such as smoothness, surface composition and particles shapes [20–22]. Spheroidal particles with a smoother surface and less active groups shows better flowability and *in vitro* deposition when particle's aerodynamics diameter between 1 μm and 5 μm. Delivery azithromycin by dry powder inhalation could significantly increase the antibiotic concentration in local infection site with longer residence time. Low enzyme activity and neutral pH environment also increase the stability of azithromycin by avoiding degradation in the digestive system.

Even though so many technologies could be used to optimize the deposition property of dry powders, the limited numbers of inhalable excipients severely hinder the development of dry powder inhalation. Only several pharmaceutical excipients until now were approved for the pulmonary delivery such as lactose, mannitol, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) [23,24]. Recently, fumaryl diketopiperazine (FDKP) was approved by the FDA and used for the pulmonary delivery of insulin [25]. FDKP shows satisfied safety with special solubility character which could dissolve in the neutral and alkaline environment but precipitated and then formed microparticle at the acidic environment. With negative charges on the surface, FDKP microparticles could load proteins and small molecules with positive charges by electrostatic adherence and used as pulmonary delivery carrier.

In this study, an azithromycin dry powder inhalation was designed and prepared for the community-acquired pneumonia treatment caused by *Streptococcus pneumoniae*. The materials, detailed synthesis processes, the methods of formulation optimization and antibacterial activity are provided in Supporting information. Special dissolution property of FDKP was investigated and confirmed. Stability of azithromycin aqueous solution was also investigated with different pH, buffer salt and ionic strength. The formulation and preparation technology of azithromycin loaded FDKP microparticles (AZM@FDKP-MPs) were designed and optimized by the single factor experiment and orthogonal experiment design which utilize particle size and flowability parameters as a major index. MIC and MPC of azithromycin on *Streptococcus pneumoniae* were measured and the in vitro antibacterial efficiency was further investigated. *In vitro* dispersion of AZM@FDKP-MPs was further investigated by next generation impactor (NGI) to the simulating aerodynamic deposition behavior.

Azithromycin as one of macrolides antibiotics shows degradability in both acidic or alkaline conditions, which glucosides bond easily hydrolyzed with proton and lactone rings easily open with alkali. Therefore, the preparation process of azithromycin loaded FDKP microparticles should be avoided to expose azithromycin in the strong acid or alkaline environment. Stability of azithromycin aqueous solution was investigated with several influence factors such as different pH environment, different buffer salt and its concentration and different ionic strength. As shown in Fig. 1A, the hydrolysis of azithromycin follows the first-order kinetics. By regression analysis the logarithm of remaining azithromycin and the time, the apparent rate constant (k_{obs}) for degradation of azithromycin was calculated which value was the slope of regression curve (Fig. 1E). The k_{obs} shows the smallest value at pH 6.47 which confirms pH 6.4 was the most stable pH of azithromycin in aqueous solution. Moreover, the significant increase of k_{obs} was observed when pH increased up to 8 or decreased to 4, which confirmed the degradability of azithromycin in both acidic or alkaline aqueous solution. Whereas, considering the FDKP could only be precipitated and forming microparticles at acidic environment with pH < 5.5, which could be used to load azithromycin or other drugs with negative charges. Therefore, the preparation pH should be high enough to reduce the degradation rate of azithromycin but also not to lead to the dissolution of FDKP microparticles.

Azithromycin aqueous solution stability was also investigated on the effects of different buffer salts and its concentrations (Figs. 1B and C). At pH 4.5, the degradation rate of azithromycin in phosphate buffer was lowest compared to in citrate buffer and acetate buffer. Whereas, the difference of remaining azithromycin in a short time period (1 h) was not significant. Different concentration of buffer salt shows a similar result that higher concentration of buffer salt could increase the degradation rate slightly but not significantly in a short time period. Higher ionic

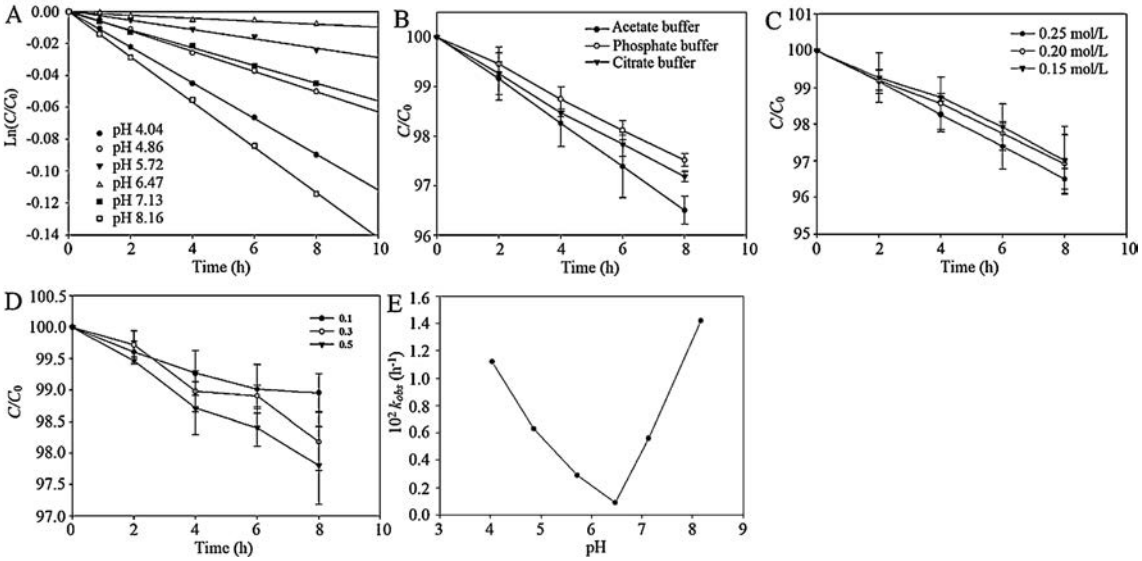

Fig. 1. Effects of different pH (A), buffer salts (B), buffer concentration (C) and ionic strength (D) on the stability of azithromycin and the apparent dissociation constant of azithromycin at different pH (E).

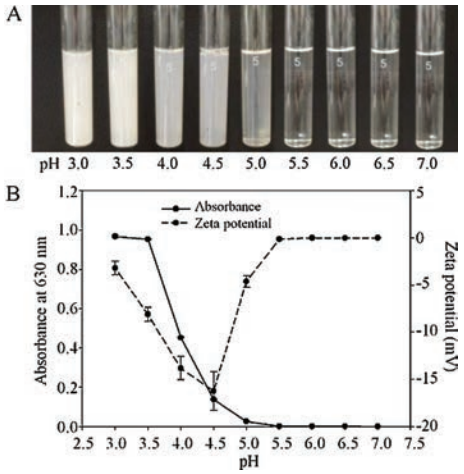

Fig. 2. (A) The photographs of FDKP suspension at different pH. (B) Absorbance at 630 nm and zeta potential of FDKP suspension at different pH.

strength also will increase the degradation rate of azithromycin whereas the remaining azithromycin was not significantly changed in a short time period (Fig. 1D). According to the law of Debye-Huckel [26], the k_{obs} was positively correlated with ionic strength which means the charges on azithromycin (positive) and attack ions were the same, therefore, it was speculated that the azithromycin degradation was mainly caused by hydrion-related catalysis. The ether bond in 15-member aglycone ring was attacked by the hydrion and leading to the cleavage of azithromycin.

FDKP has two carboxyl groups on the side chain which lead to the specifically pH sensitive dissolution characteristics. In neutral or alkaline environment, carboxyl groups could rapidly ionization and increased the solubility of FDKP. Whereas the dissolution of carboxyl group decreased gradually with pH value decreased which lead to FDKP precipitating from solution. Therefore, the absorbance of FDKP solutions at 630 nm at different pH was investigated. As shown in Fig. 2A, FDKP could dissolve rapidly and form clear solution with pH range higher than 5.5 while the white precipitation was appeared gradually with pH lower than 5.0. The absorbance of the FDKP solution also shows similar change trend with absorbance increased from 0 to over 0.8 starting at pH 5.0. After the pH lower than 3.5, the absorbance was kept stable which due to FDKP has totally precipitated from the solution and form suspension. Zeta potential was also measured to confirm the formation of FDKP-MPs. FDKP could precipitate in the pH range from 3.0 to 5.0 with one of the carboxyl group dissociated, which lead to negative charges appearing on the FDKP-MPs. As shown in Fig. 2B, zeta potential of FDKP-MPs was increased from pH 5.5 and reach the maximum value at pH 4.5 with potential −15 mV and down again, which coincided with variation of absorbance curve. As most negative charges on the surface, FDKP shows highest drug absorb potential ability with positive charges on pH 4.5.

Oven drying, freeze-drying and spray drying were all utilized to evaporating the water and collect FDKP microparticle dry powders. As the difference in drying mechanism, the microparticles powder produced by different process might have different characters which could affect the inhalability. The morphology of FDKP microparticles was observed by optical microscopy (UB200i, Nan Jing Ji Fei Technology Co., Ltd., China). The FDKP could self-assembly as spherical particles at pH 4.5 with particle size about 20 μm and the size could decrease to about 5 μm with uniform spherical structure and size after the high-pressure homogenization (Figs. 3A and B). The effects of drying method on morphology and size of FDKP-MPs were further investigated. As shown in Fig. 3C, the particles drying by oven show irregular size and aggregation which hard to separated and lead to the large particles size with D_{90} 17.73 ± 2.16 μm (Table S1 in Supporting information). Major of freeze-drying particles shows separated spherical morphology while the particles size was still unsuitable with some particles adherent with each other and lead to D_{90} reached 11.36 ± 1.42 μm (Fig. 3D and Table S1). Only spray drying microparticles shows small and uniform particle size (D_{90} was 7.292 ± 0.583 μm) with a most spherical morphology which indicated the spray drying method were most suitable for preparing FDKP-MPs (Fig. 3E and Table S1). The SEM images of spray drying FDKP-MPs show a flower-like rougher surface with a small molecular size which self-assembled by the FDKP microcrystal formed by participating of FDKP molecule (Fig. 3F). Flowability of FDKP-MPs prepared by freeze drying and spray drying show better flowability with repose angle smaller than 30°. Considering the particles size was the most important factors effect on the deposition, the spray drying method was used to preparing FDKP-MPs.

As FDKP shows different dissociation degree at pH range from 3.0 to 5.0, FDKP shows absorbability on drugs with positively charged in the pH range. While the different dissociation degree might affect the size of FDKP microcrystal and further affect the self-assemble behavior, the preparing pH environment on FDKP-MPs characters should be further investigated. The microparticles preparing at different pH were spray dried and then observed by an optical microscope (Fig. S1 in Supporting information). All FDKP-

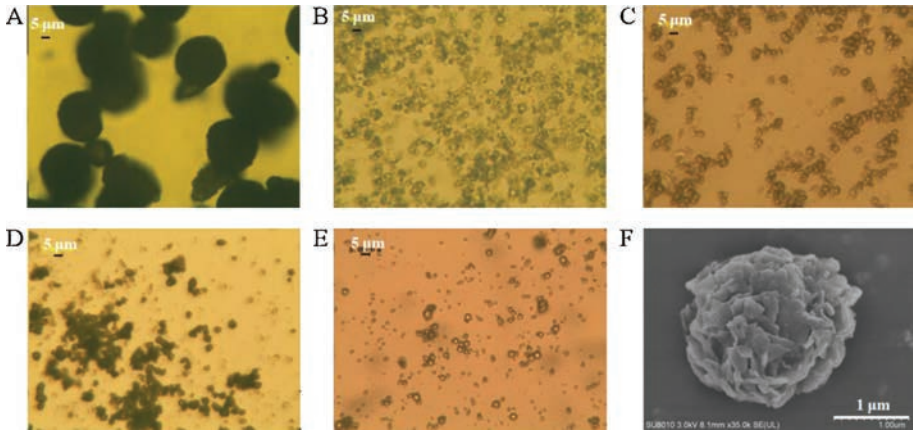

Fig. 3. Structure of FDKP-MPs suspension observed by light microscope: Before (A) and after (B) homogenization. Structure of FDKP-MPs dry powder by oven drying (C), freeze drying (D) and spray drying (E). (F) The SEM image of spray dried FDKP-MPs.

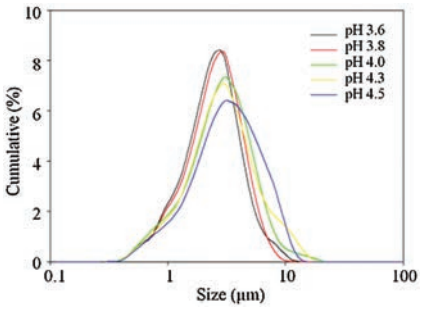

Fig. 4. Size and distribution of FDKP microparticles prepared at different pH conditions.

MPs shown uniform particle size and was excellent dispersion with spherical morphology at different pH value. The particle size of FDKP-MPs indicated there was a positive correlation between pH and particle size. With the increase of pH value, the particle size of the microspheres has a slight increase which D_{90} increase from 4.340 ± 0.0185 μm up to 6.762 ± 0.0191 μm (Fig. 4, Table S2 in Supporting information). The difference on the particle size might be caused by the different charge carried on the microcrystal [27]. As the dissociation of carboxyl group was different during the variation of pH, FDKP could precipitate and form microcrystal with negative charges and the amount of charge varies with pH value. The self-assemble process of FDKP micro crystal could be affected by the electrostatic repulsion. With higher repulsive force at pH 4.5, the FDKP microparticle shows looseness and large particle size. After weighing the advantages and disadvantages between particle size and stability of azithromycin, pH 4.5 preparing condition was selected and used for preparing azithromycin loaded microparticles.

The investigation of drug/carrier dosing ratio on the character of azithromycin loaded FDKP microparticles have been done with azithromycin/FDKP mass ratio at 1:3, 2:3 and 3:3, respectively (Table S3 in Supporting information). The drug loading efficiency was measured by HPLC and the actual drug loading efficiency was just little lower than theoretical loading efficiency (23.68% ± 0.12% *vs*. 25%, 38.56% ± 0.48% *vs.* 40%, 48.31% ± 0.32% *vs.* 50%) which indicated that almost all the azithromycin can be adsorbed on the surface of FDKP microparticles. Also, the acid preparation environment had not significantly decreased the amount of azithromycin which proved that short time exposes in the acidic environment was acceptable for preparing azithromycin loaded dry powder inhalation.

Particle size and repose angle were then investigated. With the increment of azithromycin amount, the particle size shows slightly increased as the azithromycin all covered on the surface of microparticles which increased the thickness of the drug layer. These also lead to the poor flowability of dry powder inhalation with repose angle increased from 35.67° ± 0.58° to 46.67° ± 0.58°. As the limitation of flowability base on the U.S. Pharmacopeia is repose angle < 40° which was the highest value will not affect the manufacturing process, the azithromycin/FDKP mass ratio should be chosen as 2:3 with the maximized drug loading efficiency without compromising flowability.

Emulsifier was used to avoid particle size of FDKP microparticles getting too large which lead to FDKP participating. Polysorbate 80 was a nonionic surfactant with small molecule and good *in vivo* safety. With 0%, 0.03% and 0.06% polysorbate 80 incorporated, there was no significant difference observed on particle size and repose angle (Table S4 in Supporting information). With acceptable aerodynamics diameter and flowability, polysorbate 80 will not be used in the formulation to prepare azithromycin loaded FDKP microparticles.

High-pressure homogenization was used to reduce and uniform the particle size and the homogenizing pressure and time were the two factors affect the particle characters. The particles size and aerodynamic diameter decreased following the increment of the homogenization pressure from 100 bar to 180 bar (Table S5 in Supporting information). With particles size and the aerodynamic diameter larger than 10 μm and 5 μm respectively, the dry powder could majorly be deposited in the oral and throat which hardly reached the deep infect location. While the higher homogenization pressure also leading to the poor flowability with repose angle 43.67° ± 1.53° caused by the smaller particles size, which could hardly meet the requirement of manufacture process.

The particle size also affected by the homogenization time which significantly decrease with prolonging the homogenization time. After 10 min homogenization, the particle shows poor flowability with repose angle 42.33° ± 1.53° which unsuitable for the manufacturing process (Table S5 in Supporting information). Therefore, the homogenized condition was decided as 8 min homogenization with 140 bar compress pressure.

The spray drying condition was also optimized by the orthogonal experiment. The inlet temperature and aspirator efficiency could affect the drying rate by increase or decrease the evaporating rate. The air input speed and feed rate could affect the particle size of dry powders. Therefore, all spray drying parameters should be investigated and optimized. The results were shown in Table S6 (Supporting information). Considering the multi index play key role on the inhalability and deposition behaviors, a comprehensive Z score were used to aggregate all *t*-statistics across all individuals [28] and analyzed the results and optimize the spray drying conditions. According to Equations S1 and S2 (Supporting information), Z score was calculated and used in the range analysis which shown in Table S7 (Supporting information), the optimized spray drying condition was: the inlet temperature of 150 °C, aspiration pump rate 100%, air atomization flow rate 600 L/h and feed rate 2.45 mL/min.

Minimum inhibitory concentration (MIC) is the lowest concentration of antibiotics which prevents visible growth of a bacterium and was used as an indicator of the antibacterial ability [29–31]. When the concentration of antibiotic increase to the MIC, the CFU of bacterial would decrease rapidly and then remain stable at a low level. With the further culture, antibiotic-resistant mutation could be occurred on the survived bacterium to form the one-step mutations bacterial which then further reproduce the bacterial population. The concentration of antibiotic was further increasing until could complete inhibiting of one-step mutations bacterial reproduction which was called the mutation preventive concentration (MPC) and the concentration range between MIC and MPC were then defined as mutation selection window (MSW).

The MIC and MPC of azithromycin on *Streptococcus pneumoniae* were shown in Figs. 5A and B. With concentration of azithromycin decreasing to 0.25 μg/mL, optical density shows significantly increase which represents the reproduction of bacterium could not be inhibited at this concentration. Therefore, the MIC of azithromycin on *Streptococcus pneumoniae* was 0.5 μg/mL. The MPC measurement method was similar with MIC treatment method with the difference in the number of inoculated bacteria as the antibiotic resistance one-step mutation frequency was 10^{-6}∼10^{-8}. After 72 h incubation, optical density shows significantly increase with the concentration of azithromycin lower than 1.25 μg/mL, which was the MPC of azithromycin on *Streptococcus pneumoniae*.

To investigate if the spray drying process could affect the antibacterial efficiency, the azithromycin raw materials and spray dried microparticles were used to measure their MIC on *Streptococcus pneumoniae*. As shown in Fig. 5C, there was no significant difference between MIC of raw material and spray dried

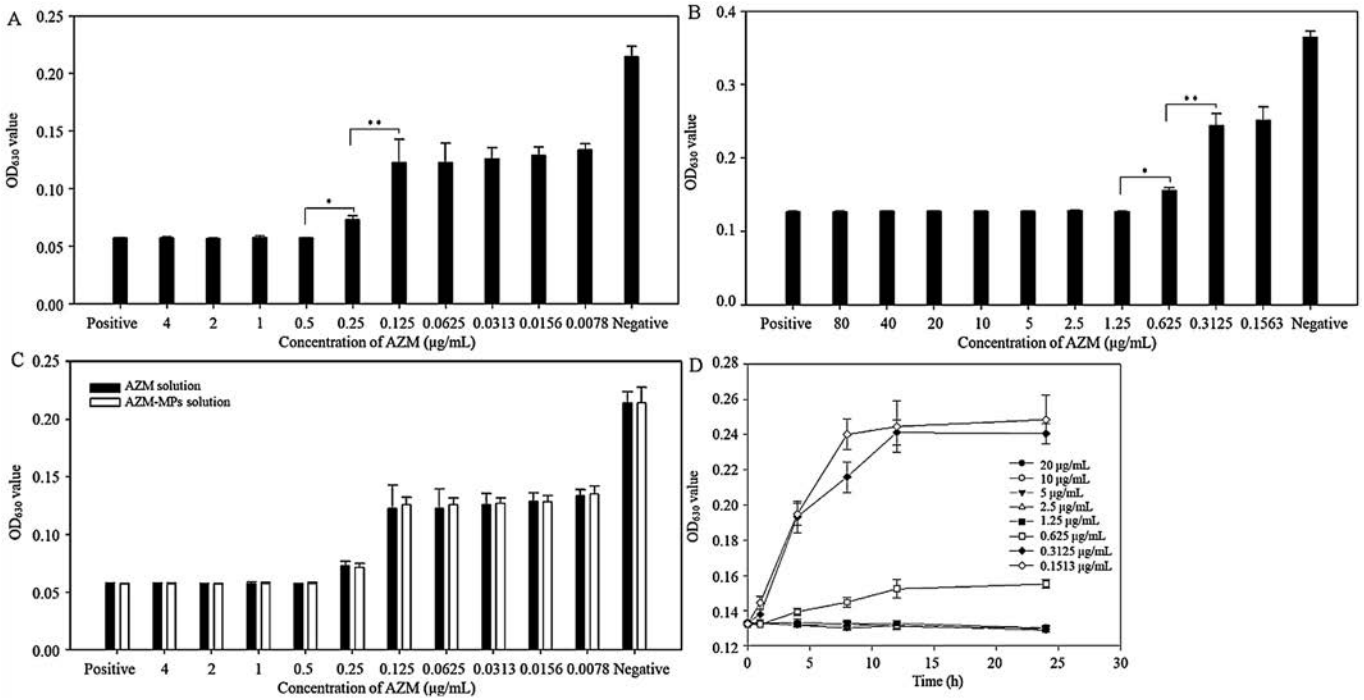

Fig. 5. (A) MIC and (B) MPC of azithromycin on *Streptococcus pneumoniae* measured by microdilution method; (C) Effect of spray-drying on the MIC of azithromycin on *Streptococcus pneumoniae*; (D) Antibacterial activity of azithromycin dry powder inhalation.

microparticles (both were 0.5 μg/mL) which indicated azithromycin expose in short-time heating process during the spray drying still could maintain the same antibacterial ability.

The *in vitro* antibacterial activity of azithromycin loaded dry powder inhalation was shown in Fig. 5D. With azithromycin concentration not smaller than MPC (from 1.25 μg/mL to 20 μg/mL), the optical density was kept stable in 24 h which indicated the reproduction of *Streptococcus pneumoniae* was inhibited. While optical density was significantly increased only 4 h after incubated with azithromycin concentration lower than MIC. A short time period inhibition of *Streptococcus pneumoniae* was observed with incubated azithromycin concentration located in the MSW, whereas still shows increased bacterial reproduction 12 h after incubation.

The short time period inhibition of bacterial might could be explained by the MSW theory which the slowly bacterial reproduction was caused by the one-step mutation of *Streptococcus pneumoniae*. Azithromycin concentration in the range of MSW could only slow down the reproduction rate in the short time but could not completely inhibit the growth of bacteria. The survived one-step mutation bacterial were selectively enriched and then amplified again. After the second mutation process, the antibiotic-resistant bacterial will produced and lead to the antibiotic treatment failure.

Due to the low possibility of spontaneous bacterial resistance mutation, the antibiotic-resistant bacteria only could produce when the concentration of bacteria with 10^{12}–10^{14} CFU/mL which do not be existed in human bodies (highest concentration was 10^{10} CFU/mL). Therefore, increase and maintain the antibiotic concentration higher than MPC could avoid the antibiotic-resistant of bacteria. Azithromycin administrated by the dry powder inhalation could increase the drug concentration in the lung by targeting delivery which could significantly higher than MPC and overcomes the drawback of other delivery routes with lower lung distribution.

Next generation impactor was used to investigate the depositional distribution of AZM-MPs and AZM@FDKP-MPs. A large number of AZM-MPs were deposited in throat which leading to lower FPF (Fig. 6, Table S8 in Supporting information). With FDKP as a carrier, AZM@FDKP-MPs show better dispersion performance with mainly deposition at stage 2 to 4. The higher FPF (55.92% ± 0.97%) and lower MMAD (3.85 ± 0.07 μm) indicated the AZM@FDKP-MPs could significantly improve the deposition of AZM *in vitro* suggesting that AZM accumulation in the lung tissues could be highly improved over AZM-MPs alone.

The dispersion and deposition behavers of microparticles (MPs) are affected by several factors such as surface properties and interparticulate forces (including van der Waals, electrostatic, and capillary forces). AZM-MPs was prepared by spray drying AZM solutions, which had high particle surface free energy due to the high amount of hydrogen donors and acceptors provide by AZM, therefore, could easily adhere to each other followed by particle aggregation and poor re-dispersibility *via* strong interparticulate interactions. Incorporate FDKP in the formulation was hypothesized to improve the dispersion of MPs by decreasing the intermolecular hydrogen bonding between MPs as FDKP has less hydrogen bond donors or acceptors and could reduce the surface free energy. Based on Fig. 6 and Table S8, EF, FPF and MMAD were

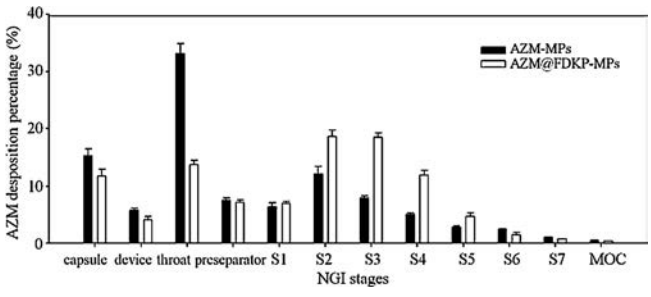

Fig. 6. AZM dispersion performance of AZM-MPs and AZM@FDKP-MPs.

significantly improved in AZM@FDKP-MPs, which consist with the expectation.

In this study, an azithromycin dry powder inhalation was designed for community-acquired pneumonia caused by *Streptococcus pneumoniae*. Characterization of FDKP was investigated to confirm the drug loading ability based on electrostatic adsorption. The formulation was investigated and optimized by the single factor experiment which result indicated that FDKP/azithromycin mass ratio (3:2) could satisfy the flowability with the maximal drug loading efficiency (38.56% ± 0.48%). The preparation conditions (high-pressure homogenization and spray dry) were also optimized by the single factor experiment and orthogonal experiment design, which result indicated the suitable particle characterization will be acquired by homogenizing (140 bar for 8 min) and spray drying (the inlet temperature of 150 °C, aspiration pump rate 100%, air atomization flow rate 600 L/h and feed rate 2.45 mL/min) the suspension at pH 4.5 environment. The *in vitro* antibacterial efficiency was further investigated with the concentration range from lower than MIC to higher than MPC and the result indicated that only with the antibiotic concentration higher than MPC could totally inhibit the reproduction of bacteria. *In vitro* dispersion performance of AZM@FDKP-MPs shows remarkable improvement of AZM dispersion and deposition behavior with FPF 55.92% ± 0.97% and MMAD 3.85 ± 0.07 μm, which suggest the dry powder inhalation (targeting delivery route) has better potential on lung infection treatment.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the Ministry of Science and Technology of China (No. 2017ZX09101001-005-003), the National Natural Science Foundation of China (Nos. 81501579, 81673364 and 81972892), the Natural Science Foundation of Jiangsu Province (No. BK20150702), the Science and Technology Development Fund of Nanjing Medical University (No. 2016NJMU105), Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (No. KYCX17_0674).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cclet.2020.03.062.

References

[1] J.P. Mizgerd, PloS Med. 3 (2006) e76.
[2] S. Jain, D.J. Williams, S.R. Arnold, et al., N. Engl. J. Med. 372 (2015) 835–845.
[3] T.M. File, Lancet 362 (2003) 1991–2001.
[4] D.M. Musher, A.R. Thorner, N. Engl. J. Med. 371 (2014) 1619–1628.
[5] S.M. Huijts, M.W. Pride, J.M. Vos, et al., Eur. Respir. J. 42 (2013) 1283–1290.
[6] C.J. Dunn, L.B. Barradell, Drugs 51 (1996) 483–505.
[7] W. He, C. Yang, X. Zhao, Y. Wang, Bioorg. Med. Chem. Lett. 27 (2017) 4576–4577.
[8] R. Li, H. Wang, Q. Shi, et al., PloS One 12 (2017) e0181690.
[9] F. Teng, P. Deng, Z. Song, et al., J. Colloid Interface Sci. 496 (2017) 16–25.
[10] X. Liu, Z. Li, X. Wang, et al., Int. J. Nanomed. 11 (2016) 6781–6794.
[11] W. Ni, X. Song, J. Cui, Eur. J. Clin. Microbiol. Infect. Dis. 33 (2014) 385–389.
[12] F. Vassilara, I. Galani, M. Souli, et al., Diagn. Microbiol. Infect. Dis. 88 (2017) 276–281.
[13] K. Nakase, H. Nakaminami, Y. Toda, N. Noguchi, Chemotherapy 62 (2017) 94–99.
[14] M. Hoppentocht, P. Hagedoorn, H.W. Frijlink, A.H. de Boer, Eur. J. Pharm. Biopharm. 86 (2014) 23–30.
[15] T.S. Patil, A.S. Deshpande, S. Deshpande, P. Shende, J. Drug Targeting 27 (2019) 12–27.
[16] P. Muralidharan, M. Malapit, E. Mallory, D. Hayes, H.M. Mansour, Nanomedicine 11 (2015) 1189–1199.
[17] A.H.L. Chow, H.H.Y. Tong, P. Chattopadhyay, B.Y. Shekunov, Pharm. Res. 24 (2007) 411–437.
[18] J. Schulze, S. Kuhn, S. Hendrikx, et al., Small 14 (2018) 1701810.
[19] Z. Wang, S.A. Meenach, Pharm. Res. 33 (2016) 1862–1872.
[20] P. Gabrielle, A. Karim, Int. J. Pharm. 392 (2010) 1–19.
[21] H. Yu, J. Teo, J.W. Chew, K. Hadinoto, Int. J. Pharm. 499 (2016) 38–46.
[22] P.C.L. Kwok, A. Grabarek, M.Y.T. Chow, et al., Int. J. Pharm. 491 (2015) 367–374.
[23] J. Weers, Adv. Drug Delivery Rev. 85 (2015) 24–43.
[24] D.E. Geller, J. Weers, S. Heuerding, J. Aerosol Med, Pulm. Drug Delivery 24 (2011) 175–182.
[25] J.P. Cassidy, N. Amin, M. Marino, et al., Pharm. Res. 28 (2011) 2157–2164.
[26] A.N. Martin, Kinetics, in: A.N. Martin, P. Bustamante, A.H.C. Chun (Eds.), Phyiscal Pharmacy: Physical Chemical Principles in the Pharmaceutical Sciences, 4th ed., Lea and Febiger, Philadelphia, 1993, pp. 284–323.
[27] K.A. Oberg, J. Sulner, M.L. Grant, Patent, US 9717689, 2017.
[28] P.M. Dechow, M.R. Huson, R.G. Sloan, Acc. Rev. 69 (1994) 138–156.
[29] X.Y. Zheng, Q. Cao, Q. Cao, et al., Chin. Chem. Lett. 31 (2020) 413–417.
[30] Y.H. Zhang, R. Wang, T. Zhang, et al., Chin. Chem. Lett. 30 (2019) 653–655.
[31] L.H. Li, A. Wang, B. Wang, et al., Chin. Chem. Lett. 31 (2020) 409–412.