International Journal of Pharmaceutics 410 (2011) 175–179

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical Nanotechnology

Controlled antisolvent precipitation of spironolactone nanoparticles by impingement mixing

Yuancai Dong[a,*], Wai Kiong Ng[a], Shoucang Shen[a], Sanggu Kim[a], Reginald B.H. Tan[a,b,**]

[a] Institute of Chemical and Engineering Sciences, 1 Pesek Road, Jurong Island, Singapore 627833, Singapore
[b] Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 119260, Singapore

ARTICLE INFO

Article history:
Received 1 November 2010
Received in revised form 21 February 2011
Accepted 7 March 2011
Available online 11 March 2011

Keywords:
Spironolactone
Nanoparticles
Antisolvent precipitation
Impingement mixing
Oral bioavailability

ABSTRACT

Continuous antisolvent precipitation of spironolactone nanoparticles were performed by impingement mixing in this work. In the range of Reynolds numbers (Re) 2108–6325 for the antisolvent water stream and 1771–5313 for the solvent stream, i.e. acetonic drug solution, 302–360 nm drug nanoparticles were achieved. Increasing drug concentration from 25 to 50 and 100 mg/ml led to a significant size increase from 279.0 ± 2.6 to 302.7 ± 4.9 and 446.0 ± 17.3 nm, respectively. "Two-step crystallization" was first observed for spironolactone in the water/acetone system: the drug was precipitated initially as spherical cluster, which rearranged into ordered cuboidal nanocrystals finally. The nanoformulation showed faster dissolution rate in comparison with the raw drug. By combining the impingement mixing and an on-line spray drying, a fully continuous process may be developed for mass-production of dried drug nanoparticles.

1. Introduction

Nanoformulation is one of the effective dosage forms to enhance the dissolution rate of poorly water-soluble drugs and overcome their low and variable oral bioavailability (Merisko-Liversidge et al., 2003; Rabinow, 2004; Kesisoglou et al., 2007). Significant therapeutic benefits of nanoformulations have been seen from the marketed products, such as Rapamune®, Emend®, Triglide®, etc. To make nanoparticulate drugs, "top-down" approach is mainly used in the pharmaceutical industry, which breaks large bulk particles down to less than one micron by milling or high pressure homogenization (Keck and Muller, 2006; Junghanns and Muller, 2008). It must be acknowledged, however, "top-down" approach is time and energy consuming process. Also, the size and morphology of the processed nanoparticles cannot be well controlled. Alternatively, some "bottom-up" techniques, i.e. precipitation of drug nanoparticles from its molecular solution, are energy and time effective; in addition, it offers flexibility in controlling the size and morphology of the final products (Horn and Rieger, 2001). Liquid antisolvent precipitation is one of such "bottom-up" techniques to make drug nanoparticles and has great potential for scaling up. For a typical liquid antisolvent precipitation, drug is dissolved in water-miscible organic solvents and mixed with the antisolvent, i.e. water. High supersaturation is thus generated, which leads to fast nucleation rate and results in the precipitation of drug particles in the nanometer (1–1000 nm) range (Horn and Rieger, 2001; Matteucci et al., 2006). To achieve high supersaturation, a rapid and homogenous mixing of the solvent and the antisolvent prior to precipitation is essential. That is, the characteristic micromixing time, τ_m, needs to be less than the characteristic precipitation time τ_{precip}, i.e. $\tau_m < \tau_{precip}$. To meet this requirement, one should shorten τ_m by specific mixing techniques or extend τ_{precip} by stabilizers. Mixing in a conventional stirred tank is basically inhomogeneous and has a relatively large τ_m, which is obviously not suitable for antisolvent precipitation of drug nanoparticles. Indeed, for industrial and continuous antisolvent precipitation of drug nanoparticles, some mixing-intensification equipments have been studied, such as submerged or confined jet impingement mixer (Mahajan and Kirwan, 1996; Johnson and Prud'homme, 2003), rotating packed bed (Chen et al., 2006), multi-inlet votex mixer (Liu et al., 2008), static mixers (Gassmann et al., 1994; Douroumis and Fahr, 2006; Dong et al., 2010), Y or T-mixer (Beck et al., 2010) and microfluidics (Ali et al., 2009), etc. All these mixers are able to realize rapid and homogeneous mixing of two miscible fluids and suitable for a continuous precipitation process. Among them, jet impingement mixer is the simplest one both in principle and in practice: the two streams are impinged on each other directly at certain angle to realize the rapid and thorough mixing. Some excellent work has demonstrated that

* Corresponding author. Tel.: +65 67963864; fax: +65 63166183.
** Corresponding author at: Institute of Chemical and Engineering Sciences, 1 Pesek Road, Jurong Island, Singapore 627833, Singapore. Tel.: +65 67963855; fax: +65 63166183.
E-mail addresses: dong_yuancai@ices.a-star.edu.sg (Y. Dong), reginald_tan@ices.a-star.edu.sg (R.B.H. Tan).

0378-5173/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2011.03.005

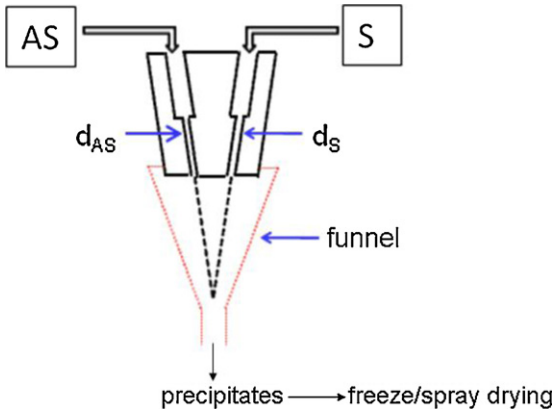

Fig. 1. Schematic impingement mixing process for antisolvent precipitation of drug nanoparticles.

impinging is able to achieve small τ_m and thus effective to obtain nanoparticles (Liu and Fox, 2006; Marchisio et al., 2006; Gavi et al., 2007a, 2007b; Zhao et al., 2007; Lince et al., 2009).

Spironolactone is a steroidal API to treat a series of diseases, such as edema, cirrhosis of the liver, and hypokalemia, etc. It is a typical BCS class II drug with low aqueous solubility and high permeability. The slow dissolution rate renders spironolactone with a variable and incomplete oral bioavailability (Levy, 1962; Clarke et al., 1977). Therefore, size reduction approach is used in the commercial product to expedite the dissolution rate of spironolactone by enlarging the surface area (McInnes et al., 1982; China Pharmacopeia, 2005). In the recent years, nanosizing spironolactone has gained much attention due to the higher dissolution rate offered in comparison with micronization (EI-Shabouri, 2002; Langguth et al., 2005; Dong et al., 2010).

Our previous work described a static mixing process for making nanoparticles of poorly water-soluble drugs (Dong et al., 2010). In this contribution, free impingement mixing (i.e. non-submerged and non-confined) is employed for continuous liquid antisolvent precipitation of spironolactone nanoparticles. In comparison with the confined impinging jet reactor used in the literature (Marchisio et al., 2006; Gavi et al., 2007a, 2007b; Lince et al., 2009), this is an alternative impinging device and has not been reported, to our knowledge. The scheme is shown in Fig. 1. The stream inlet and outlet channels were drilled in a stopper as illustrated and a funnel was fitted below to direct the flow the precipitated particles suspension. Some advantages of this free impinging mixer may be derived from: (1) it is convenient to connect the exit to an industrial spray dryer through a tube to realize the fully continuous process, i.e. from drug solution to the final dried drug nanoparticles, which is the ultimate goal of our work. (2) Upon mixing of the solvent and antisolvent flow, the formed film goes downward (without back mixing with the solvent and antisolvent) to the tube and spray dryer continuously. Therefore, the solvent/antisolvent ratio can be maintained constant guaranteeing the homogeneity of the precipitated particles and (3) the mixer is simple including the fabrication and operation. In this work, effects of the antisolvent precipitation process parameters, such as Reynolds number (Re) and flow rate of the solvent/antisolvent streams and drug concentration on the size of the precipitated particles were investigated. The size was measured by the dynamic laser light scattering techniques. Evolution of the morphology of the precipitates was visualized by cryo-field emission scanning electronic microscopy (cryo-FESEM). Crystallinity of the nanoformulation and raw drug was analyzed by Differential Scanning Calorimetry (DSC). Finally, the dissolution behaviors of nanoformulation and raw drug were compared.

2. Materials and methods

2.1. Materials

Spironolactone was obtained from Wuhan Hezhong Biochemical Manufacture Co. Ltd., China. Hydroxylpropylmethyl cellulose (HPMC), sodium dodecyl sulfate (SDS) and mannitol were purchased from Sigma. Acetone was from Fisher Scientific.

2.2. Controlled antisolvent precipitation of drug nanoparticles by impingement mixing

Spironolactone was dissolved in acetone at 25–100 mg/ml as solvent (S). Water containing 0.275–1.1 mg/ml HPMC and 0.275–1.1 mg/ml SDS was used as antisolvent (AS). The diameter of outlet channel was 0.5, 1 or 1.5 mm for solvent flow (d_S) and 1.5, 3 or 4.5 mm for antisolvent flow (d_{AS}), respectively. In brief, the solvent and antisolvent were pumped to the fabricated impingement device at 25–100 and 225–900 ml/min, respectively. The impingement of the solvent and antisolvent took place upon leaving the outlet channels. The flow rate ratio of solvent to antisolvent was maintained at 1:9. Reynolds number (Re) of the solvent and antisolvent streams was adjusted by varying the diameter of outlet channels at certain flow rate. To obtain the dried nanoformulation, the water stream containing 9.9 mg/ml mannitol, together with 0.55 mg/ml HPMC and 0.55 mg/ml SDS was impinged on the acetone stream containing 50 mg/ml drug. The resulting fresh precipitates were immediately frozen in the liquid nitrogen and then freeze dried for 5 days. The lyophilized samples were used for DSC and dissolution studies.

2.3. Particle size measurement

Size of the freshly precipitated drug particles was determined by dynamic laser light scattering technique (Nano-Zetasizer, Malvern). Before measurement, the drug suspension was diluted by saturated aqueous drug solution to ca. 0.2 mg/ml. The mean *z*-average size and polydispersity index (PDI) of triplicate measurement were reported.

2.4. Morphology

Morphology of the precipitated drug particles in the suspension was characterized by cryo-field emission scanning electron microscope (cryo-FESEM, JEOL JSM-6700F). At different time after precipitation, i.e. 0, 15, 30 and 60 min, a drop of precipitate suspension was sampled and frozen in liquid nitrogen for cryo-FESEM observations.

2.5. DSC

The thermograms of the raw drug and freeze dried nanoformulation were analyzed by Diamond DSC Calorimeter (PerkinElmer). The samples were equilibrated at 20 °C for half hour and then heated to 220 °C at 10 °C/min in N_2 atmosphere.

2.6. Dissolution measurement

Drug dissolution was carried out according to the USP XXV type II (paddle) method (VK 7010, VARIAN). The nanoformulation tested was the freeze dried powder containing drug nanoparticles, mannitol, HPMC and SDS. Freeze-dried raw drug was also prepared as a control, which contained raw drug and same excipients in the same ratio as the nanoformulation. The rotation speed of paddle was 100 rpm. 900 ml 0.1 M HCl containing 0.1% SDS was used as the dissolution medium and maintained at 37 °C during studies. At 2.5,

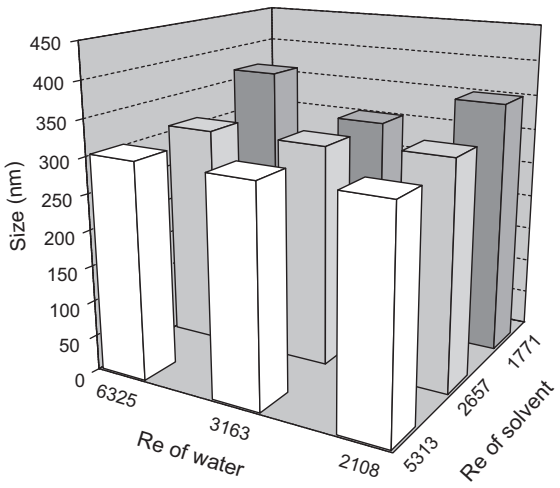

Fig. 2. Effect of Reynolds number on the size of the precipitated particles (the standard deviation in the figure for all the particles is less than 5% of the mean size; the flow rate was 50 and 450 ml/min for the solvent and water stream, respectively; different Re was investigated here for sake of convenience but actually velocity was being changed as the main controlling parameter: $Re = vd/u$, v: fluid velocity d: outlet channel diameter u: kinematic viscosity (water was taken here)).

5, 7.5, 10, 15, 20, 30, 40 and 60 min, 0.5 ml aliquot of the dissolution medium was taken out, filtered (pore size: 0.22 μm) and directly injected to the HPLC system (Agilent 1100) for drug concentration analysis. The column used was Agilent Eclipse XDB-C18 column (5 μm, 4.6 mm × 250 mm) and the mobile phase was the mixture of 50% acetonitrile and 50% Millipore water (v/v) with a flow rate of 0.8 ml/min. The drug was detected at wavelength 238 nm and the retention time of the drug was ~12 min. The experiment was conducted in triplicate.

2.7. Statistical analysis

Statistical analysis of the results was performed with the Package for Encyclopaedia Medical Statistics (PEMS). p-Values less than 0.05 were considered to be statistically significant. All statistical tests were two-tailed.

3. Results and discussion

3.1. Antisolvent precipitation of spironolactone nanoparticles by impingement mixing

Impingement mixing is the simplest mixing technique in principle, i.e. the two miscible fluid streams are mixed by being impinged on each other, but with the smallest τ_m in comparison with other mixing approach (Johnson and Prud'homme, 2003). In general, at constant flow rate, the higher the Re of the solvent and antisolvent streams, the smaller the τ_m (Matteucci et al., 2006). In this study, the flow rate of solvent and antisolvent streams was kept constant to be 50 and 450 ml/min; their Re was adjusted by varying the outlet channel diameter, and thus the fluid jet velocity, which is actually the main controlling parameter. For sake of convenience, however, effect of the Re of the solvent and antisolvent streams on the size of the precipitated particles was examined in this work and the results are illustrated in Fig. 2. As can be seen, when the Re of the solvent stream was 5313 or 2657, the precipitated spironolactone particles, achieved by being impinged on the water stream with the Re of 2108, 3163 or 6325, had a size of 302–318 nm with a polydispersity index of 0.05–0.15. There is no clear dependence of size on Re in this range. When the Re of the solvent stream was reduced to 1771, the particles precipitated by impingement with the water stream with a Re of 3163 had a size of 309.7 nm with a polydispersity index of 0.087 ± 0.029, which was similar as the aforementioned particles; while impingement with the water stream of lower Re (2108) or higher Re (6325) both resulted in a relatively larger particles, i.e. ~360 nm. This may be explained by the fact that the impingement of the solvent stream of lowest Re with the water stream of lowest RE would generate a lower mixing intensity comparatively and relatively larger particles precipitated could thus be expected, while for impingement of the solvent stream of lowest Re with the water stream of highest Re, the diameter of the water stream, in this case, is smaller than the solvent stream. Therefore, the solvent cannot be well engulfed by the water upon impingement, which led to a relatively poor mixing. Effect of even lower Re for both water and solvent stream was not investigated as the two streams cannot be impinged on each other in that case. Our results demonstrate that the spironolactone nanoparticles can be produced in a wide range of Re at a constant flow rate. Indeed, this is encouraging in terms of a large scale production, since the precipitation process can be operated in a more mild and easy-to-control mode (i.e. low Re) without sacrificing the productivity (i.e. flow rate) and the size of the obtained nanoparticles.

For antisolvent precipitation, increasing drug concentration (solvent) leads to higher supersaturation and a faster nucleation rate; increase in drug concentration, however, promotes agglomeration as well (Matteucci et al., 2006). Therefore, the size variation of the precipitated particles caused by drug concentration increase depends on which mechanism prevails. In this work, increasing drug concentration from 25 to 50 and 100 mg/ml caused a significant size increase of the precipitated nanoparticles from 279.0 ± 2.6 to 302.7 ± 4.9 and 446.0 ± 17.3 nm ($p < 0.05$), respectively. In addition, the drug nanoparticles precipitated from 100 mg/ml drug solution had a polydispersity index of 0.370 ± 0.015, which was the largest ($p < 0.05$) in comparison with the nanoparticles precipitated from 25 (PDI: 0.156 ± 0.028) and 50 mg/ml drug solution (PDI: 0.098 ± 0.046). This indicates that the agglomeration caused by increasing drug concentration plays a significant role in determining the size of the precipitated particles. Another reason is that, when the drug concentration is increased more nuclei are produced in the solvent–nonsolvent interface resulting in the inefficient mixing of solvent and antisolvent and therefore larger particles.

Keeping the diameter of the outlet channels to be constant, i.e. 0.5 mm for the solvent stream and 1.5 mm for the water stream, the size of the particles precipitated at the flow rate 25/225, 50/450 and 100/900 ml/min was 338.0 ± 2.0, 302.7 ± 4.9 and 333.0 ± 1.0 nm and the corresponding polydispersity index was 0.091 ± 0.040, 0.098 ± 0.046 and 0.106 ± 0.056, respectively. It is evident that medium flow rates 50/450 generates the best mixing and results in the smallest particles ($p < 0.05$). The possible reason is that, at lower flow rates 25/225 ml/min, the impingement generates lower mixing intensity leading to relatively larger precipitated particles finally; while at higher flow rate, i.e. 100/900 ml/min, the impingement is too strong, which causes overspray of the fluid leading to a relatively poor mixing.

3.2. Morphology

Ostwald ripening is a significant feature for most of the drug nanoparticles achieved by liquid antisolvent precipitation due to the presence of the solvent, which may cause the growth of nanoparticles in size and/or change in crystallinity (Liu et al., 2007). Therefore, the freshly precipitated drug nanoparticles need to be stabilized by (1) homogenization, e.g. NANOEDGE® technique, which is actually similar as an "annealing" process to maintain the size by converting the precipitated particles into low energy crystalline state (Keck and Muller, 2006), or (2) freeze/spray drying to form dried particles. In this work, the morphology evolution

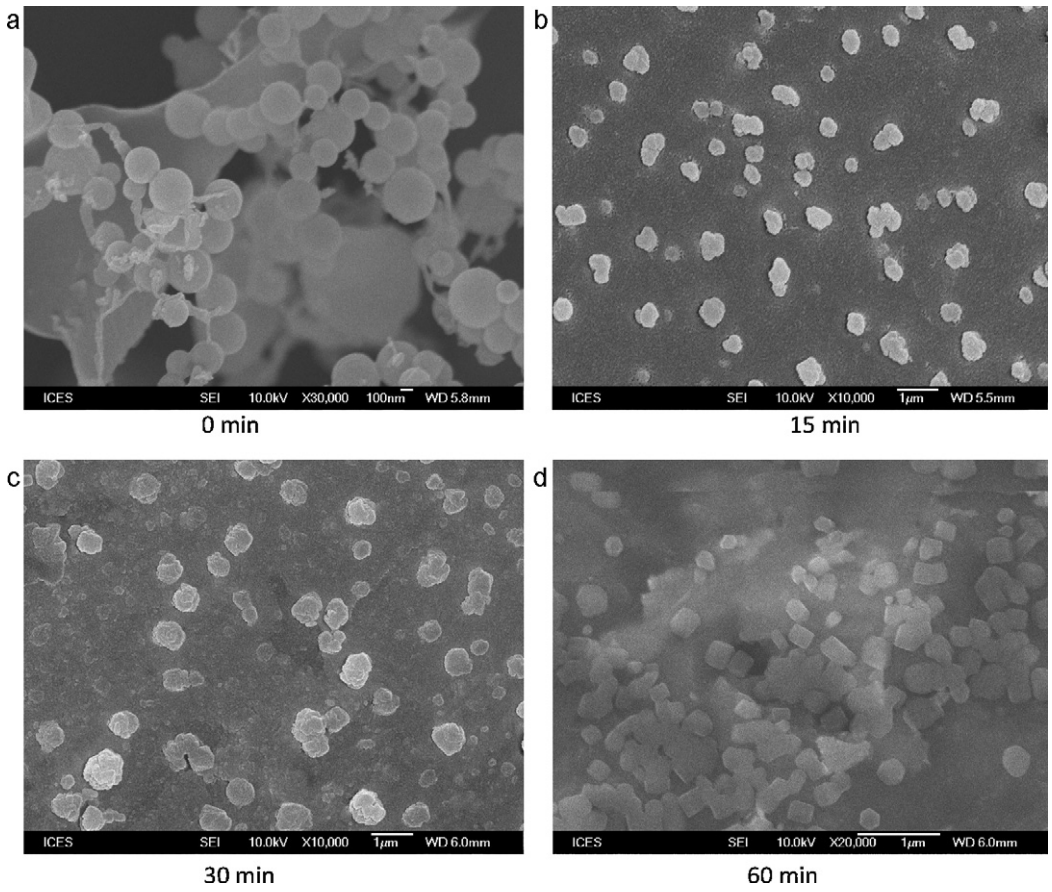

Fig. 3. Cryo-FESEM of the precipitated particles.

of the freshly precipitated drug particles in the slurry with time was monitored by cryo-FESEM. The initial drug precipitates, as can be seen from Fig. 3(a), were perfect spherical particles with the size of approximately 300 nm. After 15 min (Fig. 3(b)), some spherical particles became more angular and adopted irregular shape. At 30 min, a relatively cuboidal morphology began to emerge (Fig. 3(c)). After 1 hr, the particles were predominantly cuboidal in shape (Fig. 3(d)). It seems that, rather than the classical nucleation model, antisolvent crystallization of spironolactone in the water/acetone system follows a "two-step crystallization model", i.e. a spherical cluster was formed first, followed by rearrangement of the spheres into ordered nanocrystals (Erdemir et al., 2009). This, to the best of our knowledge, is the first evidence for "two-step crystallization" in the antisolvent crystallization of pharmaceutical nanoparticles.

3.3. DSC

The DSC thermograms of the raw drug, freeze-dried raw drug and freeze-dried nanoformulation are shown in Fig. 4. As can be seen, raw spironolactone exhibited a sharp melting peak at 208.9 °C (curve a) with an enthalpy of 55.90 J/g. In comparison, freeze-dried raw drug showed a decreased melting point (194.7 °C) and enthalpy (11.36 J/g) (curve b); freeze-dried nanoformulation demonstrated a very similar pattern as freeze-dried raw drug with a melting point of 193.3 °C and enthalpy of 11.10 J/g.

3.4. Dissolution

Dissolution profile of freeze-dried spironolactone nanoformulation and raw drug is illustrated in Fig. 5. The main difference between the freeze-dried raw drug and nanoformulation is the particle size. In 2.5 min, 20.1% of the drug was dissolved from the nanoformulation; while only 7.8% was dissolved from raw drug formulation. Nanoformulation is 2.6 times ($p < 0.05$) faster than raw drug in dissolution. In 5 min, dissolution of spironolactone from the nanoformulation was nearly complete. In the same period, only 72.0% of raw drug dissolved. The result demonstrates that the dissolution of spironolactone nanoformulation is faster than raw drug formulation, which could be ascribed to the enlarged surface area of the precipitated drug nanoparticles according to Noyes-Whitney equation (Kesisoglou et al., 2007). Supersaturation was also observed for nanoformulation from 5 to 15 min, which could be

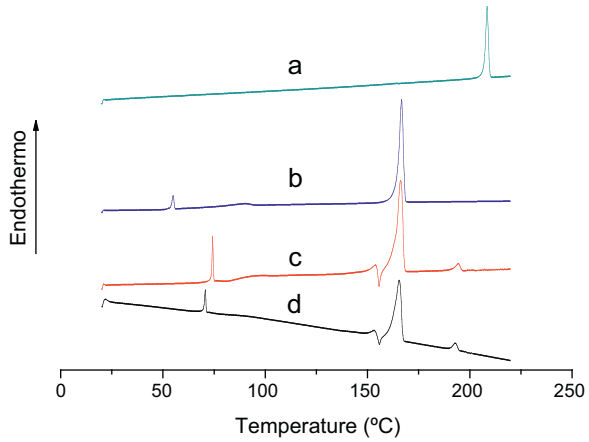

Fig. 4. DSC thermograms of (a) raw spironolactone, (b) mannitol, (c) freeze dried raw drug, and (d) freeze-dried nanoformulation.

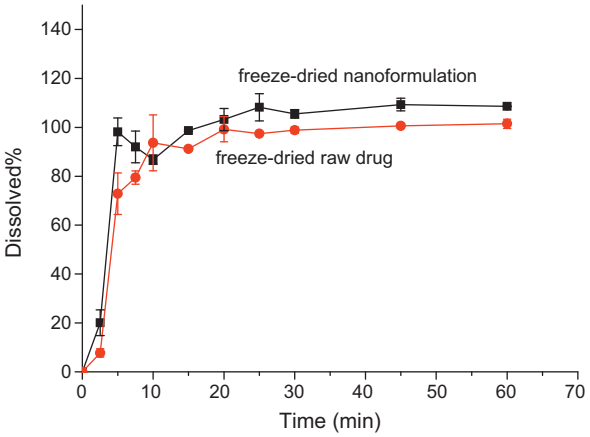

Fig. 5. Dissolution of the freeze dried raw drug formulation and nanoformulation.

ascribed to the enhanced solubility of nanoparticles in comparison with the micronized drug particles.

4. Conclusions

Impingement mixing technique was applied in this work to produce poorly water-soluble drug nanoparticles by antisolvent precipitation. It was found that 302–360 nm spironolactone nanoparticles were achieved in the range of Reynolds number 2108–6325 for the antisolvent water stream and 1771–5313 for the acetonic drug solution stream. Increasing drug concentration could result in significant increase in particle size and polydispersity index. The freshly precipitated nanoparticles were spherical cluster initially but would evolve to crystalline state. The lyophilized nanoformulation showed faster dissolution rate in comparison with bulk raw drug formulation. The results demonstrate that the size of the precipitated particles could be conveniently controlled by tuning the process parameters. Combining the impingement mixer with an industry scale spray dryer, a fully continuous process may be realized to produce dried hydrophobic drug nanoparticles at large scale. As a metastable system, drug nanoparticles may grow in size and/or change in crystallinity. Therefore, their long-term storage stability is of great concern, which will be done in our future work.

Acknowledgement

This work was supported by project grant ICES/07-122B01 from A*STAR (Agency for Science, Technology and Research) of Singapore.

References

Ali, H.S.M., York, P., Blagden, N., 2009. Preparation of hydrocortisone nanosuspension through a bottom-up nanoprecipitation technique using microfluidic reactors. Int. J. Pharm. 375, 107–113.

Beck, C., Dalvi, S.V., Dave, R.N., 2010. Controlled liquid antisolvent precipitation using a rapid mixing device. Chem. Eng. Sci. 65, 5669–5675.

Chen, J.-F., Zhang, J.-Y., Shen, Z.-G., Zhong, J., Yun, J., 2006. Preparation and characterization of amorphous cefuroxime axetil drug nanoparticles with novel technology: high-gravity antisolvent precipitation. Ind. Eng. Chem. Res. 45, 8723–8727.

China Pharmacopeia, 2005. Spironolactone, 886. ISBN 7-117-06982-1 (Volume I); ISBN 7-117-06983-X (Volume II); ISBN 7-117-06984-8 (Volume III).

Clarke, J.M., Ramsay, L.E., Shelton, J.R., Tidd, M.J., Murray, S., Palmer, R.F., 1977. Factors influencing comparative bioavailability of spironolactone tablet. J. Pharm. Sci. 66, 1429–1432.

Dong, Y., Ng, W.K., Hu, J., Shen, S., Tan, R.B.H., 2010. A continuous and highly effective static mixing process for antisolvent precipitation of nanoparticles of poorly water-soluble drugs. Int. J. Pharm. 386, 256–261.

Douroumis, D., Fahr, A., 2006. Nano- and micro-particulate formulations of poorly water-soluble drugs by using a novel optimized technique. Eur. J. Pharm. Biopharm. 63, 173–175.

El-Shabouri, M.H., 2002. Nanoparticles for improving the dissolution and oral bioavailability of spironolactone, a poorly-soluble drug. STP Pharm. Sci. 12, 97–101.

Erdemir, D., Lee, A.Y., Myerson, A.S., 2009. Nucleation of crystals from solution: classical and two-step models. Acc. Chem. Res. 42, 621–629.

Gassmann, P., List, M., Schweitzer, A., Sucker, H., 1994. Hydrosols-alternatives for the parenteral application of poorly water-soluble drugs. Eur. J. Pharm. Biopharm. 40, 64–72.

Gavi, E., Marchisio, D.L., Barresi, A.A., 2007a. CFD modelling and scale-up of confined impinging jet reactor. Chem. Eng. Sci. 62, 2228–2241.

Gavi, E., Rivautella, L., Marchisio, D.L., Vanni, M., Barresi, A.A., Baldi, G., 2007b. CFD modelling of nano-particle precipitation in confined impinging jet reactor. Chem. Eng. Res. Des. 85, 735–744.

Horn, D., Rieger, J., 2001. Organic nanoparticles in the aqueous phase-theory, experiment and use. Angew. Chem. Int. Ed. 40, 4330–4361.

Johnson, B.K., Prud'homme, R.K., 2003. Chemical processing and micromixing in confined impinging jets. AIChE J. 49, 2264–2282.

Junghanns, J.-U.A.H., Muller, R.H., 2008. Nanocrystal technology, drug delivery and clinical applications. Int. J. Nanomed. 3, 295–309.

Keck, C.M., Muller, R.H., 2006. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenization. Eur. J. Pharm. Biopharm. 62, 3–16.

Kesisoglou, F., Panmai, S., Wu, Y., 2007. Nanosizing-oral formulation development and biopharmaceutical evaluation. Adv. Drug Del. Rev. 59, 631–644.

Langguth, P., Hanafy, A., Frenzel, D., Grenier, P., Nhamias, A., Ohlig, T., Vergnault, G., Spahn-Langguth, H., 2005. Nanosuspension formulation for low-soluble drugs: pharmacokinetic evaluation using spironolactone as model compound. Drug Dev. Ind. Pharm. 31, 319–329.

Levy, G., 1962. Availability of spironolactone given by mouth. Lancet 2, 723–724.

Lince, F., Marchisio, D.L., Barresi, A.A., 2009. Smart mixers and reactors for the production of pharmaceutical nanoparticles: proof of concept. Chem. Eng. Res. Des. 87, 543–549.

Liu, Y., Fox, R.O., 2006. CFD predictions for chemical processing in a confined impinging-jets reactor. AIChE J. 52, 731–744.

Liu, Y., Kathan, K., Saad, W., Prud'homme, R.K., 2007. Ostwald ripening of β-Carotene nanoparticles. Phy. Rev. Lett. 98, 1–4, 036102.

Liu, Y., Chen, C., Prudhomme, R., Fox, R., 2008. Mixing in a multi-inlet vortex mixer (MIVM) for flash nano-precipitation. Chem. Eng. Sci. 63, 2829–2842.

Mahajan, A.J., Kirwan, D.J., 1996. Micromixing effects in a two-impinging-jets precipitator. AIChE J. 42, 1801–1814.

Marchisio, D.L., Rivautella, L., Barresi, A.A., 2006. Design and scale-up of chemical reactors for nanoparticle precipitation. AIChE J. 52, 1877–1887.

Matteucci, M.E., Hotze, M.A., Johnston, K.P., Williams III, R.O., 2006. Drug nanoparticles by antisolvent precipitation: mixing energy versus surfactant stabilization. Langmuir 22, 8951–8959.

Merisko-Liversidge, E., Liversidge, G.G., Cooper, E.R., 2003. Nanosizing: a formulation approach for poorly-water-soluble-compounds. Eur. J. Pharm. Sci. 18, 113–120.

McInnes, G.T., Asbury, M.J., Ramsay, L.E., Shelton, J.R., Harrison, I.I., 1982. Effect of micronization on the bioavailability and pharmacologic activity of spironolactone. J. Clin. Pharmacol. 22, 410–417.

Rabinow, B.E., 2004. Nanosuspensions in drug delivery. Nat. Rev. Drug Discov. 3, 785–796.

Zhao, H., Wang, J.-X., Wang, Q.-A., Chen, J.-F., Yun, J., 2007. Controlled liquid antisolvent precipitation of hydrophobic pharmaceutical nanoparticles in a microchannel reactor. Ind. Eng. Chem. Res. 46, 8229–8235.