International Journal of Pharmaceutics 382 (2009) 61–66

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Preparation of rifampicin-loaded PLGA microspheres for lung delivery as aerosol by premix membrane homogenization

T.V.P. Doan[a,b], J.C. Olivier[a,b,*]

[a] INSERM, ERI-23, 40 avenue du Recteur Pineau, 86000 Poitiers, France
[b] Université de Poitiers, Faculté de Médecine et de Pharmacie, 6 rue de la Milétrie, 86000 Poitiers, France

ARTICLE INFO

Article history:
Received 26 June 2009
Received in revised form 30 July 2009
Accepted 4 August 2009
Available online 12 August 2009

Keywords:
PLGA
Microspheres
SPG membrane
Premix membrane emulsification
Rifampicin

ABSTRACT

The water-in-oil solvent evaporation method with premix membrane homogenization was investigated to improve productivity of the preparation of narrowly size-distributed poly(lactide-co-glycolide) (PLGA) microspheres for rifampicin lung delivery as dry aerosols. Using ethyl acetate as organic solvent, a coarse oil-in-water emulsion (or premix) was prepared under magnetic stirring and homogenized by extrusion through a Shirasu porous glass (SPG) membrane (5.9 μm porosity). Microspheres were obtained after dilution and solvent evaporation. Formulation parameters investigated were: PLGA concentration, transmembrane pressure and oil:water volume ratio. The optimal formulation parameters were then applied to prepare rifampicin-loaded microspheres. Loaded microspheres were 1.72 ± 0.16 μm in diameter with a span of 0.86 ± 0.04 and a rifampicin content of 52 ± 6 μg/mg microspheres. Release studies in phosphate-buffered saline showed a linear release profile with 40% rifampicin release over 4.5 days. The MMAD of 2.63 μm of freeze-dried microspheres should be suitable for aerosol administration and delivery into the rat lungs by intratracheal insufflation.

1. Introduction

Over the past decade aerosol antibiotics have gained clinicians' interest for the treatment of pulmonary infections by providing high drug concentrations at the lung target site while reducing systemic exposure and toxicity. Aerosol antibiotic dosage forms that are used in clinical practice or that are currently under investigations are aqueous solutions or dry powders (Dudley et al., 2008). As they provide transient effect, these dosage forms require multiple daily dosing. In addition, the direct contact of the drug particles or of concentrated drug solutions with the lung mucosa may lead to adverse effects such as cough, bronchostriction, irritation, etc. (Westerman et al., 2004; Le Brun et al., 2002). Innovative microsphere aerosols may improve treatment efficiency and tolerance by providing sustained release and mucosal protection. In addition they may facilitate the dispersion of therapeutic agents in the airways, reduce loss by deposition in the oropharynx, and target specific stages of the respiratory tract (Sakagami and Byron, 2005). For lower airway deposition microspheres need to be in the 1–5 μm aerodynamic diameter range. Furthermore for a well-controlled lung deposition and predictable release rates of entrapped drug, microsphere diameter should be narrowly distributed (Telko and Hickey, 2005). The conventional emulsification/solvent evaporation methods commonly used to prepare biodegradable polylactide (PLA) microspheres generally result in polydisperse microspheres, due to heterogeneity of emulsion droplet sizes inherent to the homogenization processes. The direct (or conventional) membrane emulsification (ME) which consists of the permeation of a dispersed phase through a microporous membrane into a stirred or cross-flowing continuous phase permitted to produce monodisperse emulsions and in turn to generate narrowly size-distributed microspheres (Vladisavljević and Williams, 2005; Ma et al., 1999). This method was successfully applied to the preparation of highly monodisperse rifampicin-loaded PLA microspheres (Ito et al., 2009; Makino et al., 2004). However, due to a low dispersed phase transmembrane flux (typically 0.01–0.1 $m^3\ m^{-2}\ h^{-1}$) the direct ME is a low-productivity process (Vladisavljević and Williams, 2005). The premix ME, or membrane homogenization, method which consists of the size reduction and homogenization of a coarse emulsion, the so-called premix, by extruding it through a porous membrane allowed for higher transmembrane flux (generally above 1 $m^3\ m^{-2}\ h^{-1}$). However, higher productivity was obtained at the expense of droplet monodispersity, which was corrected by repeating homogenization cycles (Vladisavljević et al., 2004). This method was recently used for the preparation of relatively narrowly size-distributed PLA microspheres by applying 1 (Wei et al., 2008) or 11 (Sawalha et al., 2008) homogenization cycles. The aim of the present work was to produce rapidly, using the premix ME

* Corresponding author at: Université de Poitiers, Faculté de Médecine et de Pharmacie, 6 rue de la Milétrie, 86000 Poitiers, France. Tel.: +33 5 49 45 43 79; fax: +33 5 49 45 43 78.
E-mail address: jean.christophe.olivier@univ-poitiers.fr (J.C. Olivier).

0378-5173/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2009.08.008

method, monodisperse rifampicin-loaded microspheres to be used ultimately as dry aerosol for the treatment of lung infection.

2. Materials and methods

2.1. Materials

Resomer® RG 502 H (poly(D,L-lactide-co-glycolide) 50:50 (PLGA), inherent viscosity of 0.19 dl/g (25 °C, 0.1% in chloroform)) was supplied by Boehringer Ingelheim (Ingelheim, Germany). Ethyl acetate (EA) and dimethyl sulfoxide (DMSO) were obtained from Sigma. Polyvinylalcohol (PVA) 72000 (degree of hydrolysis ≥98%) was provided by Merck Schuchardt. Purified water was produced using a MilliQ gradient® Plus Millipore system.

2.2. Methods

2.2.1. SPG porous membrane cleaning and conditioning

SPG membranes were cleaned under sonication as follows: 30 min in detergent solution, 2 h in ethyl acetate, then 2 h in 2 M hydrochloric acid. They were finally rinsed with purified water. For conditioning before emulsion homogenization SPG membranes were water-bath sonicated for 30 min in the continuous phase used to prepare microspheres.

2.2.2. PLGA microsphere preparation

A coarse emulsion or premix (total volume = 10 mL) was first prepared by mixing under magnetic stirring (200 rpm for 5 min) the dispersed phase, a PLGA solution in EA (3–30 wt.%, 1.5–5 mL), with the continuous phase and a 3% (m/v) PVA solution (8.5–5 mL) saturated with EA (8.5 vol.%). Then the premix was subjected to homogenization cycles (up to 6) in an open system under positive transmembrane pressure ΔP_{tm} (10–500 kPa) using an external pressure-type micro-kit emulsification device (SPG Technology, Sadowara, Japan) equipped with a Shirasu porous glass (SPG) membrane (5.9 μm pore size, MCTech, Korea). The obtained emulsion was poured quickly into 100 mL 0.4% (w/v) PVA solution and left under magnetic stirring (500 rpm) for 4 h to allow EA evaporation. Microspheres were collected by centrifugation (280 × *g* for 15 min), washed three times with purified water, and freeze-dried.

Formulation parameters that were investigated as their effects on microsphere size and span were: PLGA concentration, volume percent φ of dispersed phase, homogenization cycle number, and transmembrane pressure ΔP_{tm}.

2.2.3. Rifampicin-loaded PLGA microsphere preparation

To prepare rifampicin-loaded PLGA microspheres the procedure was similar as in Section 2.2.2, except that rifampicin was dissolved in the organic phase at the concentration of 1 wt.% and that the pH of the continuous aqueous phase was adjusted to 4 using 2 M hydrochloric acid. For rifampicin content determination, freeze-dried rifampicin-loaded PLGA microspheres were dissolved in DMSO. The rifampicin content was determined at 485 nm using a Varian Cary 50 UV-Visible spectrophotometer, with a rifampicin calibration curve (0.004–0.050 mg/mL concentration range in DMSO). Entrapment efficiency (%) was calculated as the percent ratio of the determined content to the theoretical content calculated considering a 100% entrapment efficiency. Yield (%) was calculated from the recovered mass of freeze-dried microspheres versus the initial weight of rifampicin plus PLGA.

2.2.4. Microsphere sizing

Microspheres were dispersed in purified water and analyzed using laser light diffraction (Microtrac® X100 particle size analyzer). Particle size was expressed as the mean diameter of the volume distribution (D_v) calculated by the PSA software (version 9.0g) according to Eq. (1):

$$D_v = \frac{\sum V_i d_i}{\sum V_i} \quad (1)$$

where V_i is the volume percent in channel size and d_i the channel diameter.

The width of microsphere size distribution, or span, was calculated according to Eq. (2) (Vladisavljević and Schubert, 2003).

$$\text{span} = \frac{d_{90} - d_{10}}{d_{50}} \quad (2)$$

where d_{10}, d_{50} and d_{90} are the diameters corresponding to 10, 50 and 90 vol.% on a relative cumulative microsphere size distribution curve.

Microsphere size distribution was considered as satisfactory (i.e., narrow size distribution) for span values below or equal to 1.

2.2.5. Aerodynamic property evaluation

Freeze-dried microsphere powder was aerosolized using a Model DP-4 dry powder insufflator™ for rat (PennCentury Inc., USA) with 2.5 mL of air contained in a syringe connected to the device. The resulting aerosol was analyzed at room temperature using a 7-stage NGI cascade impactor (Copley Scientific) fitted with a preseparator and connected to a Copley HCPS pump. Collection cups were coated with silicon oil and airflow rate was set at 30 L/min. Several insufflations were carried out in order to deliver approximately 20 mg freeze-dried microspheres into the NGI. Microspheres collected on each cup were dissolved with 1 mL DMSO for rifampicin determination as in Section 2.2.3. The cumulative mass fractions versus log aerodynamic diameters plot allowed the calculation of the mass median aerodynamic diameter (MMAD) and of the respirable fraction (i.e., 1–5 μm aerodynamic diameter cumulated fractions in percentile).

2.2.6. Microscopy

Microspheres were observed with an optical microscope (Zeiss Axioscope A1). The microsphere surface was examined by scanning electron microscopy (SEM) using a JSM-840A JEOL electron microscope at 15 kV, after gold-sputtering the microspheres in argon atmosphere.

2.2.7. In vitro drug release study

Rifampicin-loaded microspheres (amount corresponding to 100 μg/mL rifampicin concentration) were incubated at 37 °C and under magnetic stirring in 8 mL phosphate buffer saline (PBS), pH 7.4, containing 1% (m/v) ascorbic acid as antioxidant. For rifampicin release determination the microsphere suspension was centrifuged (10 min, 160 × *g*) and 100 μL of supernatant was collected for rifampicin quantification by HPLC as previously described (Tewes et al., 2008).

3. Results

3.1. PLGA microsphere preparation and effects of formulation parameters on microsphere size distribution

3.1.1. Effect of homogenization cycle number

Fig. 1 is a representative observation of emulsions and resulting microspheres. The premix coarse emulsion was heterogeneous in size (Fig. 1A) resulting in large polydisperse microspheres (Fig. 1D). From homogenization cycle number 1 to cycle number 6 emulsions and microspheres were gradually homogenized and reduced in size (Fig. 1B–F). The sizing of microspheres derived from these emulsion (Fig. 1G–I) confirmed the microscope observations, with broad size

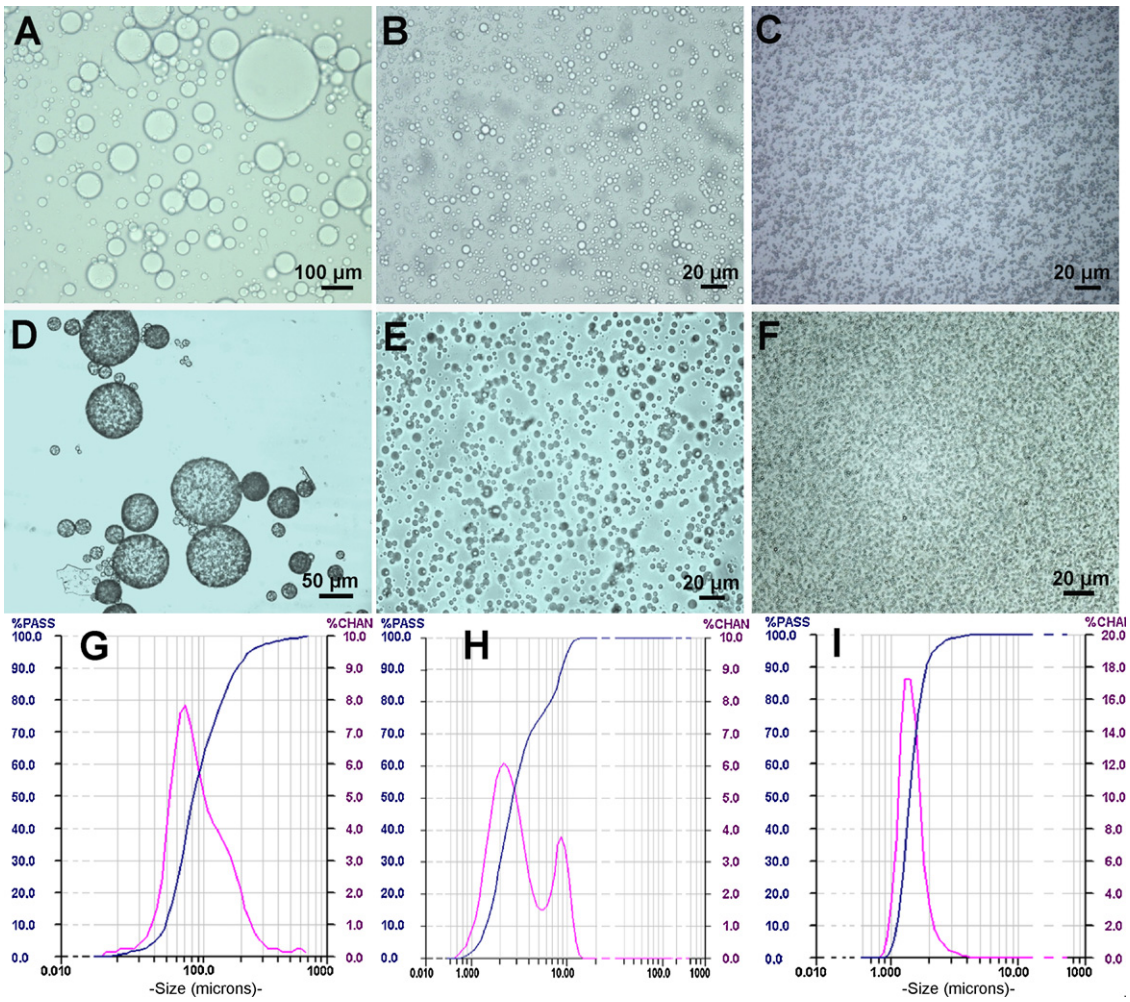

Fig. 1. Optical micrographs of a representative premix emulsion (A), of membrane-homogenized emulsions after 1 homogenization cycle (B) and after 6 homogenization cycles (C), and of the deriving microspheres (D–F) with their respective size distributions (G–I). Experimental conditions: 3% wt.% PLGA concentration in EA, φ = 30 vol.%, 200 rpm magnetic stirring for 5 min for premix preparation, and homogenization cycles performed at critical transmembrane pressure P_c. For further details see text.

distribution centred on D_v = 104 μm (span = 1.60) for microspheres derived from premix emulsion, multimodal size distributions for microspheres derived from emulsion obtained after 1 homogenization cycle (D_v = 4.54 μm, span = 1.88) and narrowly distributed diameters for microspheres derived from emulsions obtained after 6 homogenization cycles (D_v = 1.20, span = 0.50).

3.1.2. *Effect of PLGA concentration and of the cycle number on critical pressure P_c*

Except at the 1st homogenization cycle, higher PLGA concentration resulted in higher transmembrane pressures P_c (Fig. 2). P_c increased almost linearly with homogenization cycle number. At a 30 wt.% PLGA concentration, a 6th cycle could not be performed due to PLGA precipitation within the SPG membrane. Microsphere diameter decreased significantly after the 1st cycle and apparently stabilized from the 3rd homogenization cycle (Fig. 3). The span values also stabilized from the 3rd cycle, with a targeted span value of 1 or below obtained only with a 3 wt.% PLGA concentration.

3.1.3. *Effect of constant transmembrane pressure*

At a 3 wt.% PLGA concentration, transmembrane pressure of 50 or 75 kPa resulted in similar particle sizes (final D_v around 2 μm) and span values (around 1 from the 4th cycle) (Fig. 4). However, a transmembrane pressure of 150 kPa led to higher microsphere

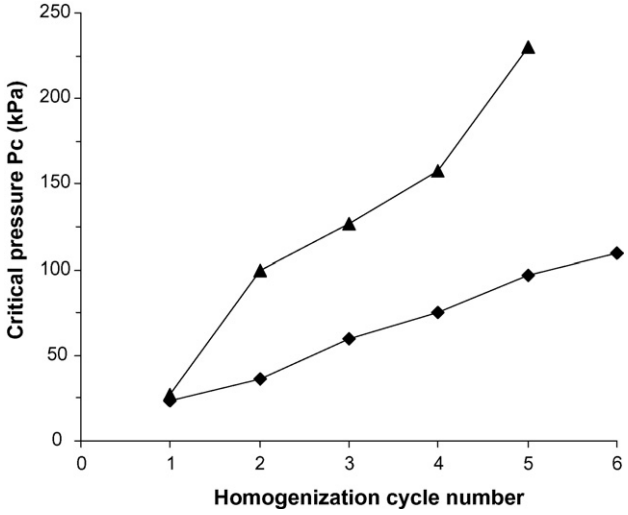

Fig. 2. Effect of PLGA concentration and of the cycle number on critical transmembrane pressure P_c. Experimental conditions: 3 (♦) or 30 (▲) wt.% PLGA concentration in EA and φ = 30 vol.%. For further details see text.

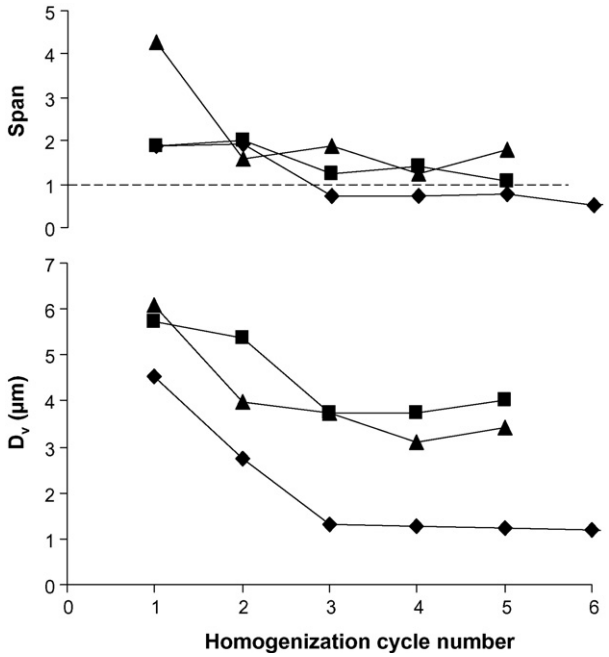

Fig. 3. Effect of PLGA concentration on mean volume diameter (D_v) and span of microspheres. Experimental conditions: 3 (♦), 15 (■) or 30 (▲) wt.% PLGA concentration in EA and φ = 30 vol.%. For further details see text.

diameters (final D_v around 4 µm) and unacceptable span values (around 2).

3.1.4. Effect of volume percent φ of dispersed phase

At a constant pressure of 75 kPa and a 3 wt.% PLGA concentration, increasing φ from 15 to 50 vol.% resulted in an increasing D_v and span values (Fig. 5). With φ of 15 or 30 vol.% D_v were 1.73 ± 0.31 and 2.18 ± 0.22 µm, respectively, with satisfactory span values (0.76 ± 0.10 and 0.96 ± 0.05).

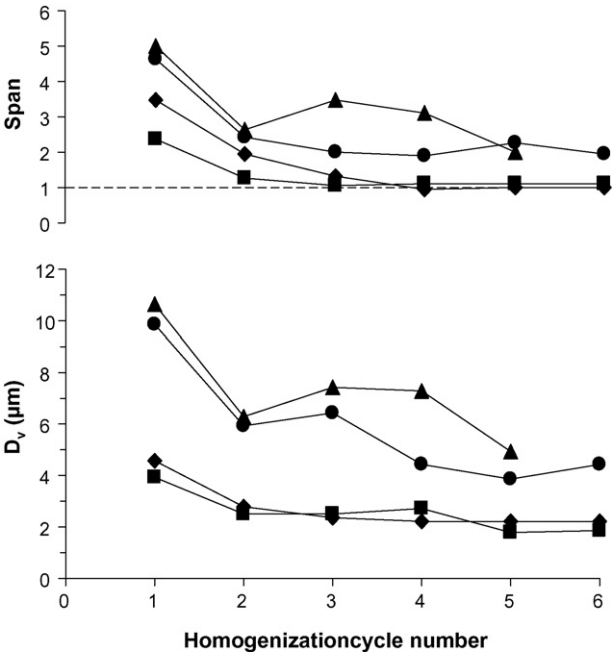

Fig. 4. Effect of constant transmembrane pressure ΔP_{tm} on mean volume diameter (D_v) and span of microspheres. Experimental conditions: ΔP_{tm} = 50 (♦), 75 (■), 100 (▲) or 150 (●) kPa, 3 wt.% PLGA concentration in EA and φ = 30 vol.%. For further details see text.

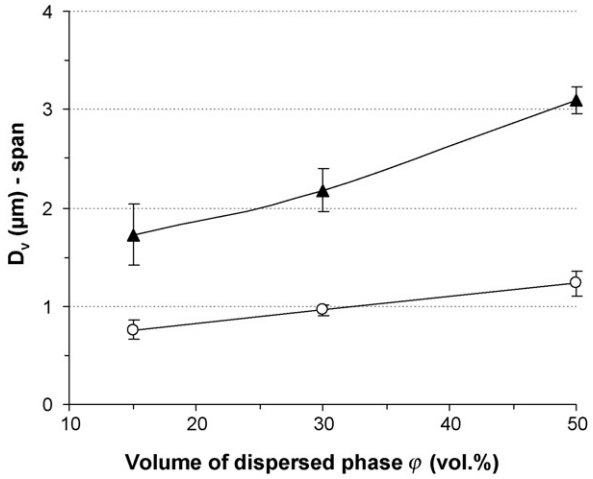

Fig. 5. Effect of φ on mean volume diameter D_v (▲) and span (○) of microspheres (means ± SD, n = 3). Experimental conditions: 3 wt.% PLGA concentration in EA, ΔP_{tm} = 75 kPa and 6 homogenization cycles. For further details see text.

3.2. Rifampicin-loaded PLGA microspheres

Rifampicin-loaded PLGA microspheres were prepared under a transmembrane pressure of 75 kPa, with φ = 15 or 30 vol.% and with 3 and 1 wt.% PLGA and rifampicin respective concentrations in EA. With φ = 15 vol.% rifampicin-loaded PLGA microspheres had size distribution profiles similar to those of unloaded PLGA microspheres (1.72 ± 0.16 µm D_v, 0.86 ± 0.04 span value). However with φ = 30 vol.% larger microspheres (4.29 ± 0.76 µm D_v) and broader size distribution (2.34 ± 0.69 span value) were obtained. Furthermore, nanospheres of around 300 nm, cumulating a volume fraction of around 5%, were found in the preparations. Electron micrograph (Fig. 6) of rifampicin-loaded microspheres prepared at φ = 15 vol.% confirmed the presence of narrowly size-distributed microspheres and of a small fraction of nanospheres. Drug loading and entrapment efficiency were 52 ± 6 µg/mg microsphere and 20.8 ± 2.4%, respectively, with a yield of 48.8 ± 3.6%.

Aerodynamic assessment with NGI gave a MMAD of 2.63 µm and a respirable fraction of 54% (i.e., aerodynamic diameter between 1 and 5 µm). Release profile was roughly linear over 4.5 days with about 40% of the rifampicin content released (Fig. 7).

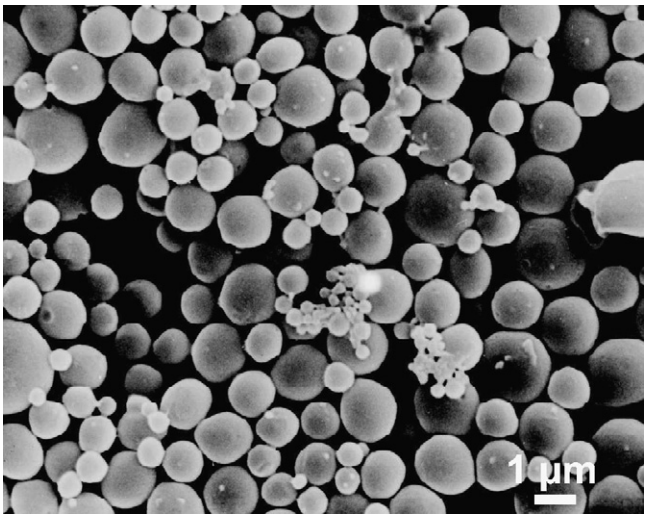

Fig. 6. Scanning electron micrograph of rifampicin-loaded PLGA microspheres. Experimental conditions: 3 wt.% PLGA and 1 wt.% rifampicin concentrations in EA, φ = 30 vol.%, ΔP_{tm} = 75 kPa and 6 homogenization cycles. For further details see text.

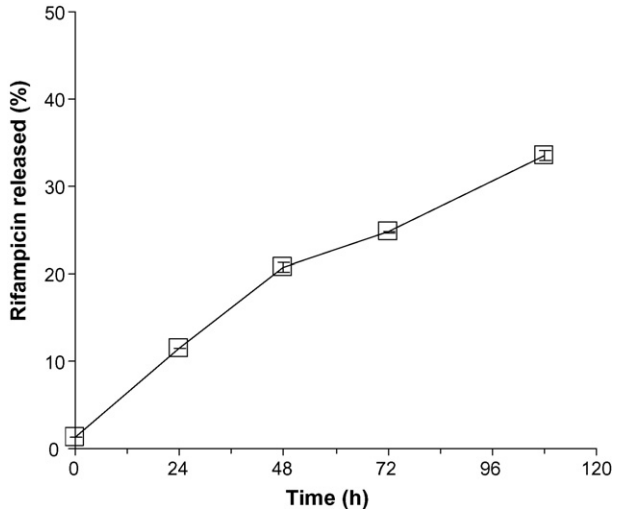

Fig. 7. In vitro release of rifampicin in PBS, pH 7.4, from 2.2 mg rifampicin-loaded microspheres/mL corresponding to 100 μg/mL rifampicin (means ± SD, $n = 3$). Experimental conditions for microsphere preparation: 3 wt.% PLGA and 1 wt.% rifampicin concentrations in EA, $\varphi = 30$ vol.%, $\Delta P_{tm} = 75$ kPa and 6 homogenization cycles. For further details see text.

4. Discussion

Formulation parameters were first investigated preparing unloaded PLGA microspheres. The premix membrane homogenization process was studied at critical pressure P_c, i.e., the minimal transmembrane pressure ΔP_{tm} at which the inner phase droplets flow through the membrane into the continuous aqueous phase, irrespective of their size (Vladisavljević et al., 2004). For toxicity and environmental concerns, the class-3 ethyl acetate (EA) solvent was used as organic solvent in place of the more toxic class-2 dichloromethane (Sah, 1997). As shown in Fig. 1 emulsion was gradually reduced in size and homogenized with an SPG membrane of 5.9 μm pore size, resulting in narrowly size-distributed microspheres. Six homogenization cycles and 10 mL emulsion (containing 3–5 mL dispersed phase) could be prepared within 1 h and the resulting microspheres were about 1–2 μm diameter. Except at the 1st homogenization cycle, critical transmembrane pressures P_c were shown to be higher with a higher PLGA concentration (Fig. 2). In addition P_c gradually increased as cycle number increased. Vladisavljević et al. (2004) hypothesized that transmembrane pressure ΔP_{tm} has to overcome the resistance to flow within the membrane pores and the droplet interfacial tension forces, which can be described by Eq. (3):

$$\Delta P_{tm} = \Delta P_{flow} + \Delta P_{disruption} \quad (3)$$

These authors showed that, at constant ΔP_{tm}, as the homogenization cycle number increased and the emulsion droplet size decreased, the energy needed for disrupting droplets, i.e., $\Delta P_{disruption}$, decreased down to zero (i.e., when droplet size reached a steady-state minimum), whereas ΔP_{flow} increased up to reaching the whole pressure drop ΔP_{tm}, resulting in a parallel increase in the transmembrane flux. In the present work however, a gradual increase in critical pressure P_c was observed over the 6 homogenization cycles (Fig. 2). The evaporation of ethyl acetate during the process carried out in an open system, resulting in an increase in PLGA concentration, may account for an increase in viscosity of the inner phase which impacted (increased) both ΔP_{flow} and $\Delta P_{disruption}$. The impossibility of performing 6 homogenization cycles with a PLGA concentration of 30 wt.% due to polymer precipitation within the SPG membrane corroborated this hypothesis. Whatever the PLGA concentrations, 3 or 4 homogenization cycles resulted in minimal span values (Fig. 3), in agreement with previous studies on emulsion preparation (Vladisavljević et al., 2004, 2006). Satisfactory span values (i.e., equal to or below 1) were only obtained for a 3 wt.% PLGA concentration.

As operating at P_c required a tedious setting of pressure (to be repeated at each homogenization cycle) and resulted in a slow transmembrane flux permitting ethyl acetate evaporation, the homogenization process was studied at constant ΔP_{tm} over a 50–150 kPa range (i.e., beyond the critical pressure determined at the 1st cycle, see Fig. 2), and was evaluated regarding microsphere D_v and spans. In all pressure conditions 6 homogenization cycles could be performed within 30 min. Higher ΔP_{tm} resulted in higher D_v and span. Span values were satisfactory at ΔP_{tm} up to 75 kPa (Fig. 4). Further studies showed that at a ΔP_{tm} of 75 kPa, D_v and span increased as the volume percent φ of dispersed phase increased (Fig. 5) and that satisfactory span values were obtained with φ values up to 30 vol.%. Beyond ΔP_{tm} of 75 kPa or φ of 30 vol.%, an increase of shear stress forces may induce droplet collision and fusion (Jafari et al., 2008), resulting in larger microspheres with unsatisfactory span.

Based on these studies, rifampicin-loaded microspheres were prepared applying the optimal conditions found for unloaded microspheres. Microspheres of a D_v of 1.72 ± 0.16 μm with a satisfactory span of 0.86 ± 0.04 were obtained with φ of 15 vol.%. Size analysis indicated the presence of a small fraction (less than 5% in volume) of 300 nm-diameter nanospheres, which was confirmed by SEM observation of freeze-dried preparation (Fig. 6). SEM further showed that microspheres were spherical and non-aggregated. The rifampicin content was 52 ± 6 μg rifampicin/mg microspheres which corresponded to an entrapment efficiency of 20.8 ± 2.4%. It was in agreement with previous works carried out applying the conventional solvent evaporation method (i.e., emulsification by high shear homogenizer) and dichloromethane as organic solvent (O'Hara and Hickey, 2000) though using a higher rifampicin-to-PLGA initial weight ratio (0.50) than in the present work (0.33). Preparing microspheres with the conventional ME method and dichloromethane as organic solvent, Ito et al. (2009) obtained a similar rifampicin content with a rifampicin-to-PLGA initial weight ratio of 0.10 (therefore with a higher entrapment efficiency) and Makino et al. (2004) obtained up to 150 μg/mg rifampicin loading with a ratio of 0.20. Therefore, in addition to a lower rifampicin ethyl acetate/water partition coefficient ($\log P_{EA/w} = 0.9$) compared to dichloromethane/water partition ($\log P_{DCM/w}$ 1.9) (Ito et al., 2009), the premix emulsification process itself, by prolonging mixing before solvent extraction and microsphere solidification may have facilitated drug diffusion into the aqueous phase. The rifampicin content should be adequate for lung administration as microsphere aerosol. Considering a dosing of 1–2 mg rifampicin/kg for therapeutic activity (Suarez et al., 2001), the amount of microspheres to be administered into the lungs of a 250-g rat would be 4.8–9.6 mg, which is acceptable. Using a PennCentury™ insufflator as aerosolizer, the MMAD was determined to be 2.63 μm, a value suitable for delivery in the rat lungs by intratracheal insufflation (Bosquillon et al., 2004). Release profiles in phosphate-buffered saline showed a steady release over 4.5 days. with a 40% release of entrapped rifampicin which should permit a sustained release of rifampicin after delivery into the lungs (Fig. 7). Release profile optimization will be conducted, based on in vivo pharmacokinetic and pharmacodynamic evaluations.

5. Conclusion

Premix membrane homogenization permitted to prepare monodisperse rifampicin-loaded microspheres with sustained-release profiles and adequate aerodynamic properties for lung

delivery by intratracheal insufflation in rats. The homogenization process (with up to 6 cycles) could be carried out within less than 30 min, which greatly improved productivity compared to conventional membrane emulsification.

Acknowledgements

The authors wish to thank Mr Emile Bere from the Service Interdisciplinaire de Microscopie et d'Imagerie Scientifique (SIMIS) for his technical assistance in photonic and electron microscopy. The Ph.D. grant of T.V.P.D. was funded by the Regional Council of the Région Poitou-Charentes, France.

References

Bosquillon, C., Préat, V., Vanbever, R., 2004. Pulmonary delivery of growth hormone using dry powders and visualization of its local fate in rats. J. Control. Release 96, 233–244.

Dudley, M.N., Loutit, J., Griffith, D.C., 2008. Aerosol antibiotics: considerations in pharmacological and clinical evaluation. Curr. Opin. Biotechnol. 19, 637–643.

Ito, F., Fujimori, H., Honnami, H., Kawakami, H., Kanamura, K., Makino, K., 2009. Study of types and mixture ratio of organic solvent used to dissolve polymers for preparation of drug-containing PLGA microspheres. Eur. Polym. J. 45, 658–667.

Jafari, S.M., Assadpoor, E., He, Y., Bhandari, B., 2008. Re-coalescence of emulsion droplets during high-energy emulsification. Food Hydrocolloids 22, 1191–1202.

Le Brun, P.P.H., de Boer, A.H., Mannes, G.P.M., de Fraîture, D.M.I., Brimicombe, R.W., Touw, D.J., Vinks, A.A., Frijlink, H.W., Heijerman, H.G.M., 2002. Dry powder inhalation of antibiotics in cystic fibrosis therapy. Part 2. Inhalation of a novel colistin dry powder formulation: a feasibility study in healthy volunteers and patients. Eur. J. Pharm. Biopharm. 54, 25–32.

Ma, G., Nagai, M., Omi, S., 1999. Preparation of uniform poly(lactide) microspheres by employing the Shirasu porous glass (SPG) emulsification technique. Colloid Surf. A 153, 383–394.

Makino, K., Nakajima, T., Shikamura, M., Ito, F., Ando, S., Kochi, C., Inagawa, H., Soma, G.I., Terada, H., 2004. Efficient intracellular delivery of rifampicin to alveolar macrophages using rifampicin-loaded PLGA microspheres: effects of molecular weight and composition of PLGA on release of rifampicin. Colloid Surf. B 36, 35–42.

O'Hara, P., Hickey, A.J., 2000. Respirable PLGA microspheres containing rifampicin for the treatment of tuberculosis: manufacture and characterization. Pharm. Res. 17, 955–961.

Sakagami, M., Byron, P.R., 2005. Respirable microspheres for inhalation: the potential of manipulating pulmonary disposition for improved therapeutic efficacy. Clin. Pharmacokinet. 44, 263–277.

Sah, H., 1997. Microencapsulation techniques using ethyl acetate as a dispersed solvent: effects of its extraction rate on the characteristics of PLGA microspheres. J. Control. Release 47, 233–245.

Sawalha, H., Purwanti, N., Rinzema, A., Schroën, K., Boom, R., 2008. Polylactide microspheres prepared by premix membrane, emulsification-Effects of solvent removal rate. J. Membr. Sci. 310, 484–493.

Suarez, S., O'Hara, P., Kazantseva, M., Newcomer, C.E., Hopfer, R., McMurray, D.N., Hickey, A.J., 2001. Respirable PLGA microspheres containing rifampicin for the treatment of tuberculosis: screening in an infectious disease model. Pharm. Res. 18, 1315–1319.

Telko, M.J., Hickey, A.J., 2005. Dry powder inhaler formulation. Respir. Care 50, 1209–1227.

Tewes, F., Brillault, J., Couet, W., Olivier, J.-C., 2008. Formulation of rifampicin–cyclodextrin complexes for lung nebulisation. J. Control. Release 129, 93–99.

Vladisavljević, G.T., Schubert, H., 2003. Influence of process parameters on droplet size distribution in SPG membrane emulsification and stability of prepared emulsion droplets. J. Membr. Sci. 225, 15–23.

Vladisavljević, G.T., Shimizu, M., Nakashima, T., 2006. Production of multiple emulsions for drug delivery systems by repeated SPG membrane homogenization: influence of mean pore size, interfacial tension and continuous phase viscosity. J. Membr. Sci. 284, 373–383.

Vladisavljević, G.T., Shimizu, M., Nakashima, T., 2004. Preparation of monodisperse multiple emulsions at high production rates by multi-stage premix membrane emulsification. J. Membr. Sci. 244, 97–106.

Vladisavljević, G.T., Williams, R.A., 2005. Recent developments in manufacturing emulsions and particulate products using membranes. Adv. Colloid Interface Sci. 113, 1–20.

Wei, Q., Wei, W., Tian, R., Wang, L.-y., Su, Z.-G., Ma, G.-H., 2008. Preparation of uniform-sized PELA microspheres with high encapsulation efficiency of antigen by premix membrane emulsification. J. Colloid Interface Sci. 323, 267–273.

Westerman, E.M., Le Brun, P.P.H., Touw, D.J., Frijlink, H.W., Heijerman, H.G.M., 2004. Effect of nebulized colistin sulphate and colistin sulphomethate on lung function in patients with cystic fibrosis: a pilot study. J. Cyst. Fibros. 3, 23–28.