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# Potential of the isolated lung technique for the examination of sildenafil absorption from lung-delivered poly(lactide-*co*-glycolide) microparticles☆

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ABSTRACT

Herein, we challenged the isolated lung (IL) technique to discriminate the performance of lung-delivered polymeric microparticles (MPs) having distinct drug release rates. For this purpose, sildenafil-loaded poly(lactide-*co*-glycolide) MPs were administered to the airspace of an IL model and the drug absorption profile was monitored.

MPs (particle size of ~5 μm) composed of PLGA of lower molecular weight (and glass transition temperature) manifested in the most rapid *in vitro* drug release (half-times ranging from <15 to ~200 min). Moreover, microencapsulation resulted in a delayed sildenafil transfer over the air/perfusate barrier (half-times ranging from <5 to ~230 min), where the actual *ex vivo* absorption profile depended on the release behavior of the utilized formulation. Finally, the obtained *in vitro* and *ex vivo* results were tested for level C, B and A correlations. The plotted data showed good agreement ($R^2 > 0.96$) and the slopes of the resulting lines of regression (*i.e.*, 0.80–0.85) indicated a slightly elevated *in vitro* drug release behavior.

Overall, the IL model was able to differentiate between distinct microparticulate formulations and is, therefore, a valuable technique for early testing of potential inhalable controlled release medications.



## 1. Introduction

Inhalation of aerosolized drug formulations is currently the *gold standard* for the treatment of numerous airway diseases (*e.g.*, asthma, chronic obstructive pulmonary disease and pulmonary hypertension), due to the advantageous therapeutic index of lung-delivered medications [1]. Unfortunately, many medications such as vasodilators provide only a short duration of action (minutes to hours) and thus, require frequent, time-consuming inhalations (up to 9 times per day) and cause inevitable night breaks [2].

In recent years, several strategies have been conceived with the potential to reduce the burden of inhalation therapy [3], among them promising delivery vehicles that enable control over the spatial release of the encapsulated drug at a therapeutically optimal rate [4]. The application of controlled release medications would not only improve the pharmacokinetic profile of a drug, but also enhance the *convenience* and *compliance* of patients [5]. Although these therapeutic benefits are well-recognized by academia and the pharmaceutical industry [4,5], no formulation with such properties has so far reached the market (liposomes containing amikacin (Arikayce®) and ciprofloxacin (Lipoquin®) are in the late clinical trials [6]). One possible explanation is the surprising lack of feasibility reports describing the potential of preclinical lung models for the analysis of such formulations [7–9]. The isolated lung (IL) technique has provided valuable insights into lung-specific pharmacokinetics of inhaled drugs [10,11], but it has rarely been challenged with prolonged release medications [12,13]. Such studies would clearly enhance our knowledge on the applicability and limitations of IL models for prolonged release medications, which in turn could further enhance formulation development.

*Abbreviations: ABC*, area between the curves; BALF, bronchoalveolar lavage fluid; BSA, bovine serum albumin; $d_n$, median geometric microparticle diameter (based on the volume distribution); HPLC, high pressure liquid chromatography; IL, isolated lung; *M*, amount of sildenafil released; *MDT*, mean dissolution time; $M_n$, number average molecular weight; *MRT*, mean residence time; MPs, microparticles; n.a., not applicable; n.d., not detected; PLGA, poly(lactide-*co*-glycolide); SD, standard deviation; SEM, scanning electron microscopy; $t_{1/2}$, half-time; $T_g$, glass transition temperature; TMR-PLGA, tetramethylrhodamine-labeled poly(lactide-*co*-glycolide); wt.%, weight percent.

☆ This article is dedicated to the memory of Thomas Schmehl, PhD.

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To close this gap, the current study aimed at challenging the IL technique with polymeric microparticles (MPs) showing distinct drug release rates. For this purpose, diverse sildenafil-loaded poly(lactide-*co*-glycolide) (PLGA) MPs were prepared by vibrational spray-drying (sildenafil is of relevance for the treatment of pulmonary hypertension [14]). Following a thorough *in vitro* characterization, MP formulations were delivered to the airspace of an isolated rabbit lung and the sildenafil transfer over the air/perfusate barrier was monitored. The drug release data were then correlated with the obtained drug absorption profiles.

## 2. Materials and methods

### 2.1. Materials

PLGA, Resomer® RG502H ($M_n$ = 12.3 kDa) and Resomer® Condensate RG 50:50 ($M_n$ = 2.3 kDa) were obtained from Boehringer Ingelheim (Ingelheim, Germany). Furthermore, fluorescently-labeled PLGA (TMR-PLGA) was prepared by reacting tetramethylrhodamine-5-carbonyl azide (Invitrogen, Karlsruhe, Germany) with Resomer® RG502H in dry N-methyl-2-pyrrolidone (Sigma-Aldrich, Steinheim, Germany) as previously described [15,16]. Sildenafil (free base) was acquired from bioKEMIX (Nienburg, Germany). Double-distilled water was purchased from B. Braun (Melsungen, Germany). All other chemicals and solvents were of analytical grade.

### 2.2. Preparation of MPs

Four distinct formulations composed of Resomer® RG502H and Resomer® Condensate RG 50:50 and blends thereof (*i.e.*, *a*) 0/100, *b*) 40/60, *c*) 60/40, and *d*) 100/0 (m/m)) were prepared on a B-90 spray-dryer (Büchi, Flawil, Switzerland) using 4.0 μm nozzles for feed solution (*i.e.*, polymer and sildenafil (10 wt.% per polymer mass) dissolved in acetone; solid concentration of 20 mg/ml) atomization [17–21]. The process parameters were as follows: inlet temperature = 45 °C, outlet temperature = 25–30 °C, drying gas flow rate = 100 l/min ($N_2/CO_2$), relative spray rate = 100%. The collected formulations were subjected to vacuum (72 h, ~0.1 mbar; Beta I, Christ, Osterode, Germany) and then stored in a sealed desiccator at 4 °C until further analysis. TMR-PLGA-MPs (*i.e.*, blend of TMR-Resomer® RG502H and Resomer® RG502H of 10/90 (m/m)) were prepared and treated as described above.

### 2.3. Characterization of MPs

The median geometric diameter of spray-dried formulations ($d_p$, based on the volume distribution) was determined by laser diffraction (Mastersizer X, Malvern Instruments, Herrenberg, Germany). Samples were dispersed in double-distilled water, containing 0.1 wt.% of polysorbate 80 (Sigma-Aldrich, Steinheim, Germany), using ultrasound (SONOREX DIGITEC, BANDELIN, Berlin, Germany). Aliquots were then placed in a stirred cuvette until a laser obscuration of >10% was achieved. The obtained diffraction patterns were analyzed in Mie mode (real part of the refractive index of water and polymeric MPs was set to 1.33 and 1.59, respectively). The size distribution (*i.e.*, *span* = $(d_{90\%} - d_{10\%}) / d_{50\%}$, with $d_n$ as the diameter at the percentile *n* of the cumulative distribution) was calculated from the obtained laser diffraction values.

The morphology of spray-dried MPs (deposited on silica wafers) was visualized by scanning electron microscopy (SEM). Before imaging, all samples were sputter-coated with a platinum layer (Gatan Alto 2500, Gatan, München, Germany) and then observed under a scanning electron microscope (JSM-7500F, JEOL, Eching, Germany).

To determine the drug content of the spray-dried formulations, sildenafil-loaded MPs were dissolved in acetonitrile (a common solvent for the employed polymers and sildenafil). Sildenafil concentrations were quantified by UV/Vis spectroscopy (λ = 300 nm; Ultrospec® 3000, Pharmacia Biotech, Freiburg, Germany). The drug incorporation efficiency is reported in terms of drug loading (wt.%).

Glass transition temperatures ($T_g$) of formulations were determined using a differential scanning calorimeter (Discovery DSC, TA Instruments, Hedehusene, Denmark). Samples (~3–4 mg) were scanned at a rate of 10 °C/min from −20 °C to 100 °C under a nitrogen atmosphere. $T_g$ was calculated from the second heating cycle.

The *in vitro* drug release studies were carried out in Krebs–Henseleit buffer (Serag-Wiessner, Germany) supplemented with 4 wt.% of bovine serum albumin (BSA; Carl Roth, Germany). The solubility of sildenafil in the release medium at 37 °C amounted to >1 mg/ml. Spray-dried sildenafil-loaded PLGA-MPs (10 mg, theoretical sildenafil loading of 10 wt.%) were dispersed in the drug release medium (600 ml) by brief sonication. Incubation occurred at 37 °C with stirring. Samples were taken at predetermined time points, centrifuged (Centrifuge 5418, Eppendorf, Hamburg, Germany) and analyzed for the sildenafil content by high pressure liquid chromatography (HPLC). The cumulative amount of drug released was then calculated by the following equation

$$\text{cumulative released sildenafil } [\%] = \mathrm{Mt}/M_\infty \cdot 100,$$

with $M_t$ and $M_\infty$ as the amount of sildenafil released at time *t* and the sildenafil loading in the MPs. In parallel, no loss or degradation was observed for sildenafil when incubated under the same conditions.

For mass loss studies, spray-dried sildenafil-loaded PLGA-MPs (10 mg, theoretical sildenafil loading of 10 wt.%) were dispersed in the drug release medium (10 ml) by brief sonication. Incubation occurred at 37 °C with shaking (Rotatherm®, Gebr. Liebisch, Bielefeld, Germany). After 300 min the samples were centrifuged (Sorvall centrifuge, DuPont, Bad Homburg, Germany) and the supernatant was carefully removed. The pellet was washed three-times with double-distilled water and then freeze-dried. The remaining MP mass was determined gravimetrically (BP 211 D, Sartorius, Göttingen, Germany).

### 2.4. IL experimentation

The IL setup has been previously described [12,22–26]. All experiments were performed in accordance with the German Law on the Use and Protection of Laboratory Animals (*TierSchG*). The Federal Authorities for Animal Research of the "Regierungspräsidium Giessen" (Giessen, Germany) approved the study protocol (17/02/2012, GI 20/10 Nr. A 1/2012).

Briefly, following isolation, rabbit lungs were placed in a temperature-equilibrated housing chamber, perfused with 600 ml of Krebs–Henseleit buffer supplemented with 4 wt.% of BSA kept at 37 ± 1 °C and ventilated with a gas mixture of 21% $O_2$, 5.3% $CO_2$, and 73.7% $N_2$ (tidal volume: 30 ml, frequency: 30 strokes/min).

Spray-dried MPs (10 mg, theoretical sildenafil loading of 10 wt.%) were delivered to the airspace of the IL using a Dry Powder Insufflator® (Model DP-4, Penn-Century, Wyndmoor, USA) [27]. Sildenafil solution (1 ml, 1 mg/ml) was applied using a MicroSprayer® (Model IA-1B, Penn-Century, Wyndmoor, USA) [25] as a control.

In order to determine the sildenafil perfusate concentration, samples were taken from the perfusion system at predetermined time points. Additionally, the sildenafil concentration in the bronchoalveolar lavage fluid (BALF) was determined after the drug absorption experiment. Therefore, lavages of the lungs were first performed with ice-cold isotonic saline and then with methanol. The sildenafil concentration in the samples was determined as previously described [12,23–25] by HPLC [28].

For qualitative analysis of drug carrier distribution within the isolated organ, TMR-PLGA-MPs were delivered to the airspace of the IL model as described above. The isolated organ was then removed from the system, perfused with a gelatin solution, and immediately frozen over liquid nitrogen [29]. The distribution of fluorescently-labeled MPs in the