

From Prediction to Performance: ML-Guided Co-Amorphous Rifampicin Formulations for Inhalation Therapy in Tuberculosis

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Summary

Machine learning (ML) offers a promising strategy for accelerating pharmaceutical development by enabling data-driven prediction of viable formulations. In this study, we expand on prior work by applying ML to predict co-amorphous systems (COAMS) suitable for inhalation-based treatment of tuberculosis (TB). COAMS, consisting of two active pharmaceutical ingredients (APIs) in a single amorphous phase, can enhance solubility, stability, and dose consistency—key factors in pulmonary drug delivery. Rifampicin-Ethambutol (RIF-ETH) and Rifampicin-Moxifloxacin (RIF-MOX) were selected based on ML predictions. Both combinations were prepared via co-milling and spray-drying at 1:1 molar ratios and adjusted to therapeutically relevant ratios (1:6 for RIF-ETH; 1:1.25 for RIF-MOX) to reflect inhalation dosing requirements. X-ray diffraction confirmed amorphous character for RIF-MOX across all conditions, while RIF-ETH showed incomplete amorphization at 1:6. Particle size analysis and SEM imaging indicated spray-drying produced smaller, more spherical particles, though RIF-ETH (1:6) spray-dried particles exhibited poor separation. Aerodynamic assessment of RIF-MOX (1:1.25) formulations demonstrated superior performance for the spray-dried system, with an emitted fraction (Ef) of 85.2% for MOX and 77.5% for RIF and a fine particle fraction (FPF) of 78.2% for MOX and 90.0% for RIF, outperforming both co-milled formulation and physical mixture. Future work will focus on evaluating dissolution behaviour for the COAMS.

Key Message

Machine learning successfully predicted COAMS for TB therapy. However, compared to RIF-ETH, only RIF-MOX formed a stable amorphous system at a therapeutically relevant ratio. The spray-dried RIF-MOX showed superior aerodynamic performance over the ball-milled formulation and crystalline mixture, confirming the model's predictive value and potential for effective lung delivery.

Introduction

The integration of machine learning (ML) in pharmaceutical development is transforming drug formulation by enabling rapid, data-driven predictions that reduce experimental effort and accelerate innovation. Co-amorphous systems (COAMS), which combine active pharmaceutical ingredients (APIs) into a stable, single-phase amorphous form, hold promise for improving solubility, stability, and patient compliance - particularly for combination therapies. Pulmonary delivery of COAMS for respiratory diseases such as tuberculosis (TB) is suggested to minimize variability in drug doses when administering combination products. Further, in TB treatment inhalation therapy could enhance local drug concentrations, reduce systemic side effects, and improve treatment outcomes [1].

At DDL 2021, we presented a ML approach to predict the successful formation of COAMS composed of two APIs, specifically targeting compounds used in the treatment of lung diseases such as asthma, COPD, and TB [2,3]. This model aimed to reduce the time and cost of empirical screening by narrowing down viable API combinations. In the present study, we build upon that work by experimentally evaluating COAMS formed from predicted API pairs for TB therapy. The positively predicted and selected combinations are Rifampicin-Ethambutol and Rifampicin-Moxifloxacin. These systems are prepared as dry powders for inhalation therapy via co-milling and spray-drying characterized with regards to solid-state, morphology and size and assessed for their potential suitability in inhalation-based delivery.

Material and Methods

Preparation of COAMS

For simplicity, the ML model predicts for molar ratios of 1:1. However, this ratio might not correspond to the therapeutic relevant doses of the 2 APIs. For effective inhalation therapy, a dose 10 times lower than the oral dose can be considered [4]. Taking this and common oral doses of the APIs (600 mg of RIF, 1200 mg of ETH and 400 mg of MOX were considered in this study) into account, the doses effective for inhalation treatment were calculated and translated into new molar ratios. This resulted in therapeutic relevant molar ratios of 1:6 for RIF-ETH and 1:1.25 for RIF-MOX. The formation of COAMS using the predicted molar ratio of 1:1 and the therapeutic relevant molar ratios was tested experimentally with co-milling and spray-drying and XRD analysis.

For co-milling, the two APIs (total amount 1 g) were placed in a 50 mL mixing cylinder together with 10 stainless-steel balls (10 mm diameter) and then milled for 200 min (RIF-ETH) and 150 min (RIF-MOX) at 650 rpm in a planetary ball mill (Pulverisette 6, Fritsch GmbH Austria). Spray-drying was done using a BUCHI Mini Spray-Dryer B-290 (Buchi Labortechnik AG, Switzerland) in closed loop configuration. Therefore, RIF-ETH combinations were dissolved in ethanol/water (80/20) with a solid concentration of 4% and RIF-MOX combinations in ethanol/water (60/40) with a solid concentration of 2%. The following spray-drying conditions were used: inlet temperature 90°C, airflow rate 1052 L/h, pump rate 10% and nozzle cap 1.4 mm.

COAMS characterization

The individual starting materials (rifampicin (RIF), ethambutol (ETH) and moxifloxacin HCl (MOX)) and the API-API pairs (molar ratio of 1:1 and therapeutic relevant molar ratio) were immediately analysed by X-ray diffraction (XRD) to assess amorphization. Measurements were performed using a Siemens D5005 diffractometer in Bragg-Brentano geometry, equipped with a copper anode ($\lambda = 1.54186 \text{ \AA}$), operating at 40 kV and 40 mA. Particle size distribution was analysed via laser diffraction (HELOS, Symantec, Germany) using wet dispersion in silicon oil. Additionally, scanning electron microscopy (SEM) was used to investigate particle morphology, using a Zeiss Ultra 55 microscope (Zeiss, Germany).

Inhalation performance of COAMS

For selected systems (RIF-MOX 1:1.25 spray-dried (SD) and ball-milled (BM)), the aerodynamic performance was evaluated using a fast-screening impactor (FSI, Copley Scientific, UK) in combination with a Cyclohaler® device (PB Pharma GmbH, Germany). Size 3 HPMC capsules were manually filled ($25 \pm 2 \text{ mg}$) with each formulation and 1 capsule used per experiment. A flowrate of 60 L/min was applied. The fine particle fraction (FPF) and the emitted fraction (EF) were determined and compared across formulations. API (RIF and MOX) contents were quantified with reverse-phase ultra-performance liquid chromatography (RP-UPLC) on an Acquity UPLC H-Class® system (Waters Corp., Milford, MA, USA) equipped with a photodiode array (PDA) detector. Chromatographic separation was performed on a Waters Acquity UPLC HSS Cyano column ($2.1 \times 100 \text{ mm}$, $1.8 \mu\text{m}$) at 25 °C using a gradient of 10 mM ammonium formate buffer (pH 4.0) and 0.1% formic acid in methanol at 0.35 mL/min. The 12 min run included detection at 295 nm for Moxifloxacin and 335 nm for Rifampicin.

As a reference, a physical mixtures (PM) of micronized RIF and MOX was prepared in a molar ratio of 1:1.25, in a tumble blender (T2F Turbula®, Willy A. Bachofen AG Maschinenfabrik, Switzerland) at 32 rpm for 30 min and used for FSI experiments as well.

Results and Discussion

Results in Figure 1 (right image) show the distinct peaks of crystalline RIF, MOX and ETH, while the RIF-ETH and RIF-MOX combinations in a molar ratio of 1:1 only revealed an amorphous halo, independent of the preparation technique ((ball-milled or spray-dried) Figure 1 left and middle image)). This matches with the prediction from the ML model. RIF-MOX spray-dried, and ball-milled in a therapeutic relevant molar ratio of 1:1.25 also only revealed an amorphous halo, confirming the formation of a COAMS (Figure 1 left image). By contrast ball-milling and spray-drying of RIF-ETH in a therapeutic relevant molar ratio of 1:6 still showed crystalline bragg peaks (Figure 1 middle image). Comparing the two XRD pattern with the XRD pattern of the starting materials reveals that the crystalline peaks present remain from ETH, indicating amorphization of RIF but not ETH. Further, the intensity of the counts is reduced compared to the full crystalline starting material, indicating reduced crystallinity of ETH in the formulations.

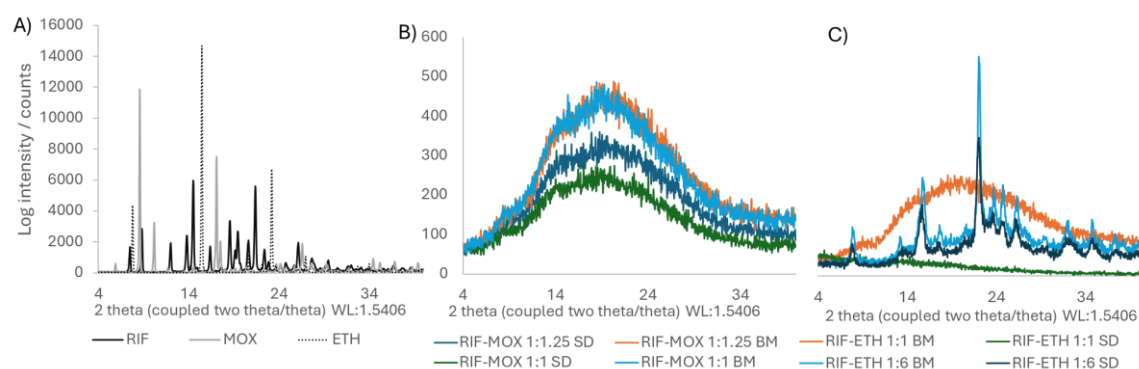


Figure 1 - XRD pattern of A) starting materials (RIF, MOX and ETH), B) RIF-MOX spray-dried, and ball-milled and C) RIF-ETH spray-dried and ball-milled particles in 2 molar ratios (1:1 and therapeutically relevant).

SEM images (Figure 2) and size measurements (Table 1) confirmed particle sizes suitable for inhalation ($< 5 \mu\text{m}$) for most formulations. RIF-MOX 1:1 BM, and RIF-ETH 1:6 BM and SD showed bigger particles not suitable for inhalation. Overall spray-drying yielded smaller particles, around $3 \mu\text{m}$ for RIF-MOX and $2 \mu\text{m}$ for RIF-ETH (1:1). Generally, milled particles showed bigger mean particle sizes.

Table 1 Mean particle size x_{50} of co-milled and spray-dried particles (molar ratios 1:1 and therapeutically relevant molar ratios) of RIF-MOX and RIF-ETH, (mean $\pm$ standard deviation, $n=3$).

	RIF-MOX 1:1 BM	RIF-MOX 1:1 SD	RIF-MOX 1:1.25 BM	RIF-MOX 1:1.25 SD	RIF-ETH 1:1 BM	RIF-ETH 1:1 SD	RIF-ETH 1:6 BM	RIF-ETH 1:6 SD
x_{50}	4.31 ± 0.18	2.82 ± 0.07	3.84 ± 0.25	3.00 ± 0.18	2.87 ± 0.95	2.01 ± 0.65	$25,72 \pm 4.31$	$6,68 \pm 0.22$

SEM images show that the co-milled particles (RIF-MOX and RIF-ETH in both molar ratios) retained an irregular appearance similar to that of the starting materials (data not shown), whereas the SD particles of RIF-MOX (1:1 and 1:1.25) and RIF-ETH (1:1) resemble shrivelled spheres. RIF-ETH spray-dried in a therapeutically relevant molar ratio of 1:6 did not result in single separated particles. This is also reflected in a comparably large mean particle size. However, as the molar ratio of 1:6 does not seem to be promising in terms of COAMS formation, no further trials were done to optimize the particles by changing spray-drying conditions or milling parameters. RIF-ETH combinations were excluded for further aerosolization tests as well.

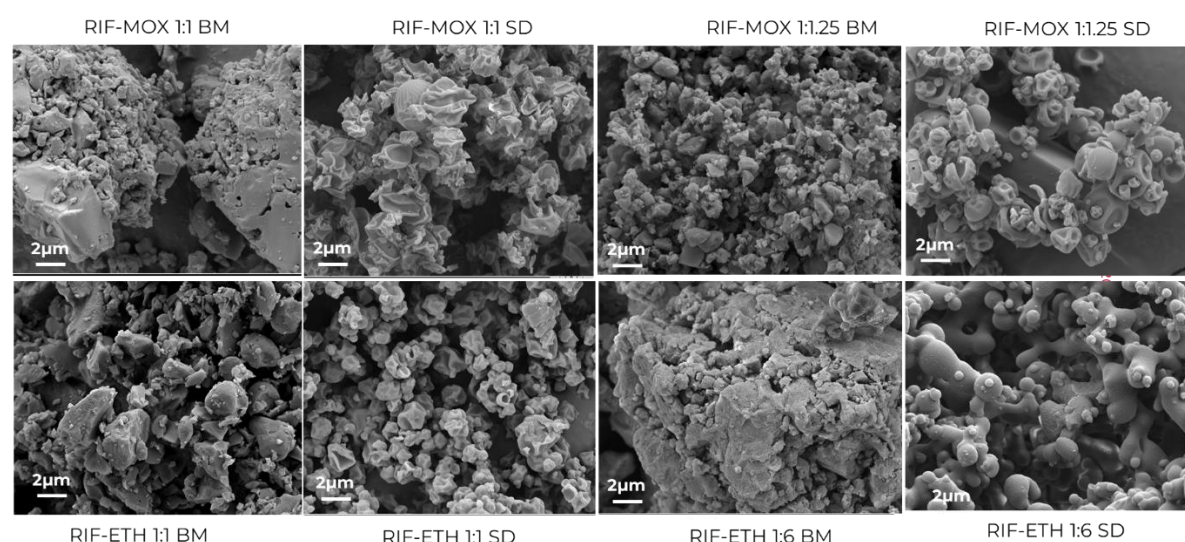


Figure 2 - SEM images of co-milled and spray-dried particles in molar ratios 1:1 and therapeutically relevant molar ratios for RIF-MOX (upper row) and for RIF-ETH (lower row).

Aerodynamic performance was tested for RIF-MOX particles in a molar ratio of 1:1.25. Since the effective inhalable dose is considered 10 times lower than the oral dose, inhalable doses still remain relatively high. Therefore, a carrier-free drug only formulation was used in our case, which is preferred for high-dose delivery to the lung. We compared RIF-MOX SD and RIF-MOX BM with a physical mixture of crystalline RIF and MOX (RIF-MOX PM 1:1.25).

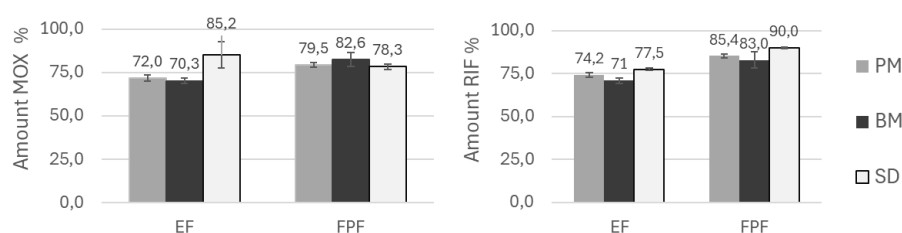


Figure 3 - Emitted fraction (EF) and Fine particle fraction (FPF) for the 3 RIF-MOX 1:1.25 formulations (PM-physical mixture, BM-ball-milled, and SD-spray-dried), (mean $\pm$ standard deviation, n=3).

Initial aerodynamic evaluation results in Figure 3 show a superior behaviour of the SD formulation in terms of EF (for MOX 85% and RIF 77%) and FPF of RIF (90%) and lower amounts of powder remaining in the capsule and inhaler (Figure 4). The EF of RIF and MOX for the PM and BM formulation is around 70-74% and the FPF around 80-85%. Figure 4 shows that for all formulations a significant amount of powder remains in the capsule and inhaler, resulting in comparable low EF values. This is to be optimized in further spray-drying tests (e.g. spray-drying conditions or addition of excipients).

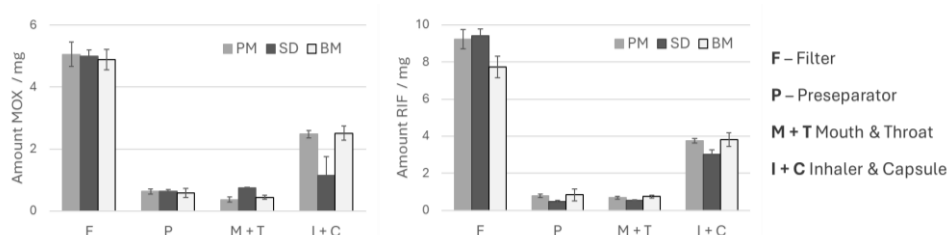


Figure 3 - Stagewise deposition from FSI experiments for the 3 RIF-MOX 1:1.25 formulations (PM-physical mixture, BM-ball milled and SD-spray-dried), (mean $\pm$ standard deviation, n=3).

Conclusion and Outlook

A data-driven approach was used to develop a predictive ML tool for rapid screening of COAMS formation, reducing experimental time and costs. Using the model, co-amorphous RIF-ETH and RIF-MOX combination for TB treatment were identified and successfully prepared. However, in therapeutic relevant molar ratios only RIF-MOX succeeded in co-amorphous formulations. Here, both SD and BM produced co-amorphous particles sized for inhalation. Initial aerodynamic tests showed promising lung delivery, especially for the spray-dried RIF-MOX 1:1.25 COAMS with higher EF and FPF. Next, dissolution experiments will assess the benefits of COAMS in this context. Overall, it is not possible to convert oral to inhalation doses directly for all systems, both experimentally (deviations from molar ratio 1:1, which is the most commonly used stoichiometric ratio in COAMS [5]) and in terms of metabolism.

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