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Supporting information

Designing Inhalable Metal Organic Framework Drug Aerosols for Pulmonary Tuberculosis Treatment and Theragnostics via Spray Drying

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Materials. All chemicals were used as received from MilliporeSigma (St. Louis, MO, USA) without further purification. $Cu(NO_3)_2 \cdot 2.5H_2O$, $Gd(NO)_3 \cdot 6H_2O$ and pyrazinoic acid (HPOA).

Synthesis of bulk Cu(POA).

0.5 g $Cu(NO_3)_2 \cdot 2.5H_2O$ and 0.533 g HPOA were dissolved in 360 mL of water. Light blue crystals attributed to $Cu(POA)_2 \cdot 2H_2O$ or hydrated phase are formed after approximately 1 hour at RT and in several minutes at 60 ˚C.

Spray drying $Cu(POA)_2$.

A Buchi B-290 spray dryer equipped with two-fluid nozzle (inner orifice = 0.7 mm, outer orifice = 1.5 mm), drying chamber with elbow outlet connection, and the standard cyclone was used to manufacture microparticulate dry powders. A dehumidifier was placed at the inlet of the aspirator. Aspiration rate (75%; ~30 m^3/h) and solution pump speed (15%; ~5 mL/min) were kept constant throughout all experiments. The reagents were added to 80%:20% DI H_2O:EtOH (by volume) in a 1:2 Cu:POA molar ratio (0.5 g $Cu(NO_3)_2 \cdot 2.5H_2O$ and 0.533 g POA). Immediately prior to spray drying, the POA solution was poured gently into the Cu solution. Concentration of this feed solution was labelled as high, medium, or low based on the total solution volume: 180 mL (3 mg/mL POA), 360 mL (1.5 mg/mL POA), or 540 mL (1 mg/mL POA) where the POA and Cu solutions were each 90 mL, 180 mL, and 270 mL respectively. The inlet temperature was either set to 180 °C (high) or 150 °C (low) resulting in outlet temperature of 88 – 90 °C and 72 °C (low N_2 flow) or 65 °C (high N_2 flow) respectively. Atomization gas (N_2) flow was either 283 L/hour (20 mm on B-290 rotameter, 15 kPA pressure drop) or 1052 L/hour (50 mm on B290 rotameter, 75 kPa pressure drop).

Spray drying $Gd_{0.1}Cu_{0.9}(POA)_2$

Gd-doped material was obtained adding 10% molar of $Gd(NO)_3 \cdot 6\ H_2O$ to the synthesis of $Cu(POA)_2$ under the optimal spray drying conditions: 0.1Gd: 0.9Cu: 2 POA, 150 ˚C inlet temperature, N_2 = 1052 L/h (75 kPa pressure drop), 1.5 mg POA/mL and 80%:20% H_2O:EtOH.

Characterization.

X-ray powder diffraction (XRPD; Bruker AXS Inc., Germany) was used to confirm the crystalline structure of MOF materials. XRPD patterns were recorded using a Panalytical Empyrean X-ray diffractometer with Cu Kα radiation (λ=1.54778 Å). **Attenuated Total Reflection** (ATR) infrared spectroscopy measurements were performed in the range of 4000–400 cm^{-1} with a Perkin Elmer Spectrum 100 FTIR spectrometer (manufacturer location). **Thermogravimetric analysis** (TGA) was measured on a Q50, TA Instruments (New Castle, DE, USA) under air at 3 ˚C/min.

Scanning electron microscopy (SEM) images were acquired on a FEI Quanta 200 FEG Analytical Scanning Electron Microscope (Hillsborough, OR, USA) using a beam energy of 15 keV. **Transmission electron microscopy** (TEM) images were acquired on a Hitachi H-7000 100 keV transmission electron microscope (Japan) equipped with AMT digital camera and Kevex energy dispersive x-ray detector (EDX) with 4pi software.

Laser diffraction was performed using a Malvern Mastersizer 2000 (Malvern Panalytical, Malvern, UK) with the Sirocco 2000 dry powder system. Approximately 100-150 mg of powder was loaded on to the sample tray and fed into the instrument at a feed ratio of 40% and dispersive air pressure of 2 bar.

Triplicate measurements were taken for 2.5 seconds after the obscuration limit was reached (0.5-10) and in between 5 second background checks.

Inertial impaction was performed using a Next Generation Impactor (NGI; MSI Corp., MN, USA). Stages of the impactor were precoated with 1% silicone oil in hexanes (w/w) and the pre-separator was filled with 15 mL DI H_2O. A nominal mass of 10 mg $Cu(POA)_2$ powder was loaded into a #3 hydroxypropylmehtylcellulose (HPMC) capsule. The capsule was placed in a RS01 inhaler (Plastiape, Italy), pierced, and the inhaler was inserted into the NGI inlet mouthpiece adapter. The solenoid-controlled vacuum in line with the NGI was set to 60 L/min for 4 seconds and then turned on to begin the experiment. NGI characterization was performed in triplicate. All stages of the NGI, the inlet, inhaler, and capsule were washed with 10 mL DI H_2O and assayed for POA content at 269 nm vis UV spectroscopy (SynergyMX, Biotech, Winooski, TX, USA). Cu content was quantified via inductively coupled plasma atomic emission spectroscopy (ICP-OES). An aliquot of the NGI collections were diluted 1:1 with 1% HNO_3 and vortex mixed. The samples were then loaded into an iCAP 7600 ICP-OES (Thermo Scientific, Waltham, MA, USA) instrument autosampler. The ICP-OES measures copper by detecting the characteristic wavelengths emitted by the copper in the argon plasma and comparing the response to a standard curve. Aerodynamic particle size distributions (APSD) were generated for both Cu and POA by plotting mass collected vs stage cutoff diameter. Mass median aerodynamic diameter (MMAD) was calculated by plotting the cumulative percentage of Cu or POA mass deposited in the NGI stages (y – axis), using a probability scale, against the corresponding cutoff diameter (x – axis) and applying a log-linear fit on either side of 50% cumulative mass. Geometric standard deviation (GSD) was calculated by the square root of the ratio of the particle size one standard deviation above and below the median particle size (84^{th} and 16^{th} percentile, respectively, or 1 and -1 on a probit scale). Fine particle fraction of the emitted (FPF_{ED}) dose was calculated as a ratio of the sum of drug mass collected below 4.46 µm (stage 3 to the micro-orifice filter) to the mass collected at the inlet of the NGI and below.

Dosator. All dosator supplies were purchased from McMaster Carr (Elmhurst, IL, USA). Manufacture and filling procedure was based on previous literature methods[1]). Briefly, the luer portion of a 21 G 2.54 cm blunt stainless steel needle was sanded down until ~ 2-3 mm of material remained (note a smaller needle was inserted into the 21 G to remove excess after sanding). This needle was tamped 1 – 5 times into a $Cu(POA)_2$ dry powder bed (~35 mg) held in the bottom of a 0.5 conical tube. Lastly, this powder filled steel needle was inserted horizontally into a 20 G 5.08 cm Teflon needle. The powder was delivered into a small vial (equipped with a large needle protruding through a rubber bung for dosator access) with a volume of HNO_3 (pH ~ 3.5) via 1 mL syringe (pre filled with 0.3 mL of air before attaching to the needle-in-needle setup). Cu and POA contents were quantified as above.

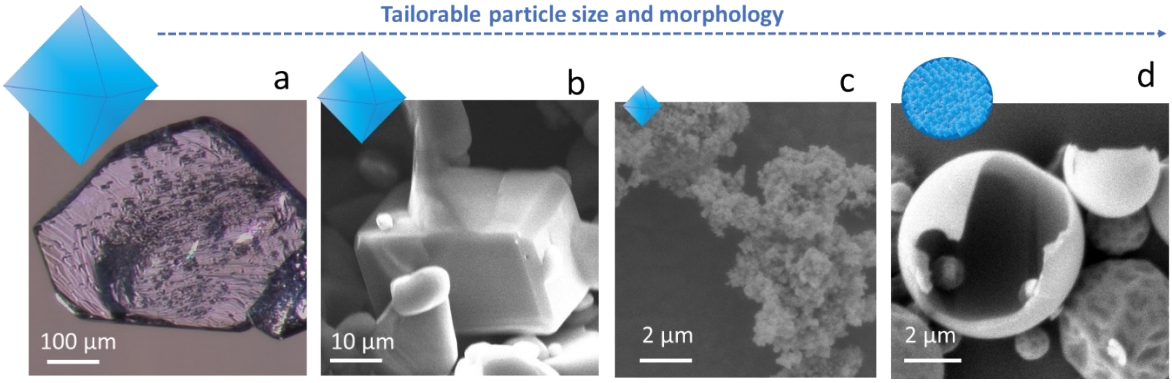

Figure S1. Optical microscope and SEM images for bulk $Cu(POA)_2$ MOF particles prepared at different temperatures: RT (a and b) and 60 ˚C (c); compared to hollow spherical particles manufactured via spray drying (in this case 150 ˚C inlet temperature) (d).

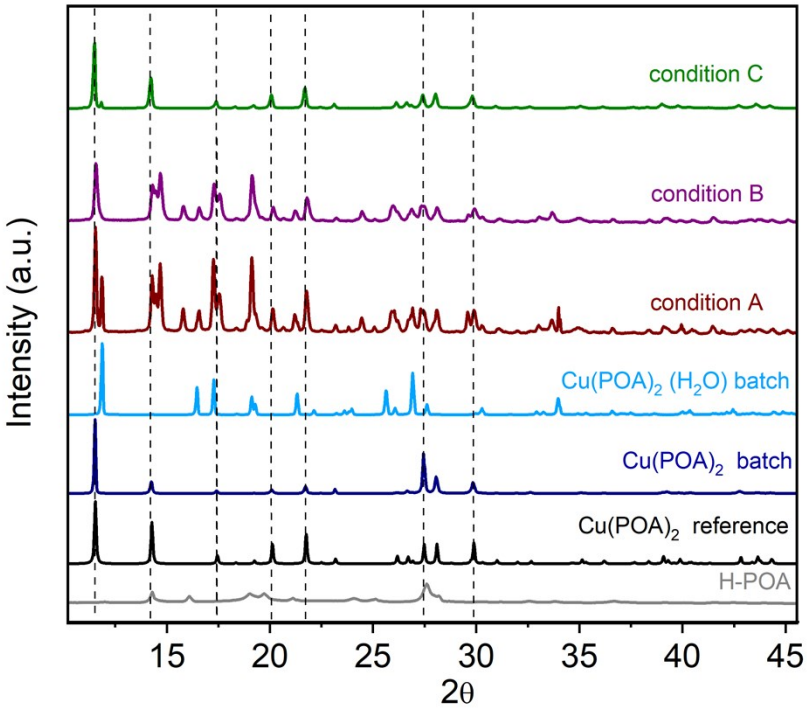

Figure S2. XRPD patterns for spray dried MOF materials prepared under the following conditions: A) 150 ˚C inlet temperature, 1 Cu: 1.6 POA, 1.5 mg POA/mL, 100 % H_2O and 293 L/h N_2 flow (15 kPa pressure drop) (green line); B) 150 ˚C inlet temperature, 1 Cu: 1.6 POA, 1.5 mg POA/mL, 80 %:20% H_2O:EtOH and 293 L/h N_2 flow (15 kPa pressure drop) (purple line); and C) 180 ˚C inlet temperature, 1 Cu: 2 POA, 1.5 mg POA/mL, 80 %:20% H_2O:EtOH and 293 L/h N_2 flow (15 kPa pressure drop) (wine red line). The patterns are compared to bulk hydrated (light blue line) and dehydrated (dark blue line) bulk $Cu(POA)_2$, simulated dehydrated $Cu(POA)_2$ (black line), and POA (grey line)

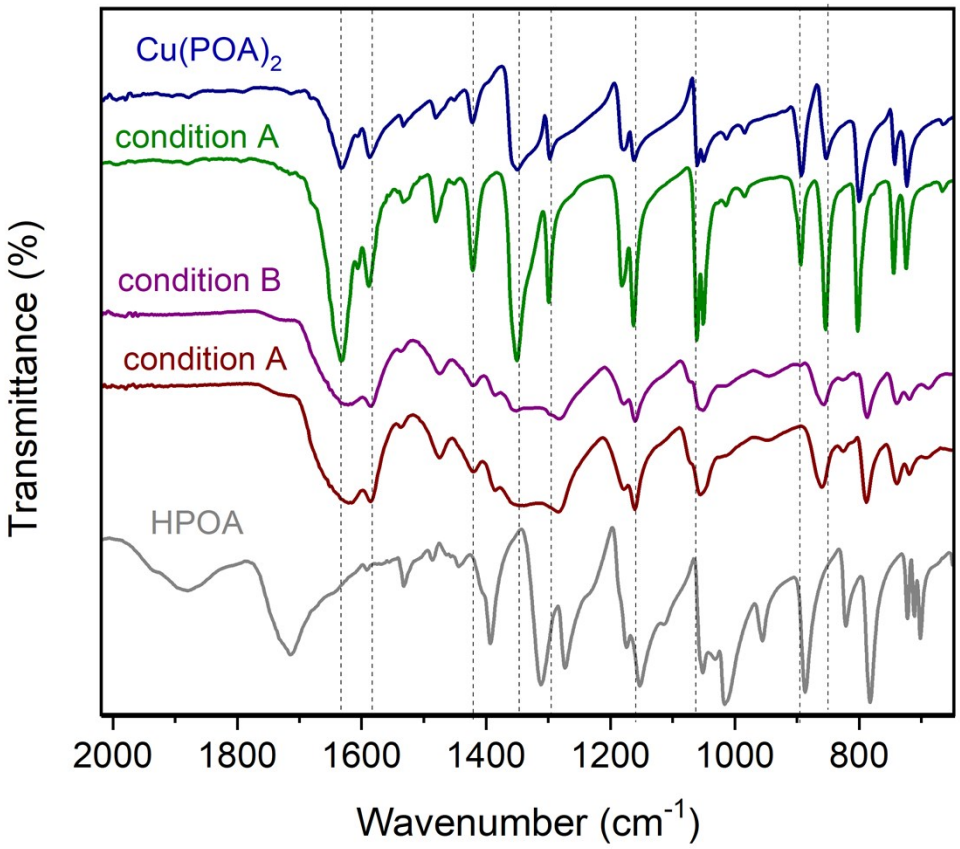

Figure S3. FTIR analysis for spray dried MOF materials prepared under the following conditions: A) 150 ˚C inlet temperature, 1 Cu: 1.6 POA, 1.5 mg POA/mL, 100 % H_2O and 293 L/h N_2 flow (15 kPa pressure drop) (green line); B) 150 ˚C inlet temperature, 1 Cu: 1.6 POA, 1.5 mg POA/mL, 80 %:20% H_2O:EtOH and 293 L/h N_2 flow (15 kPa pressure drop) (purple line); and C) A) 180˚C inlet temperature, 1 Cu: 2 POA, 1.5 mg POA/mL, 80 %:20% H_2O:EtOH and 293 L/h N_2 flow (15 kPa pressure drop) (wine red line). The spectra are compared to bulk dehydrated $Cu(POA)_2$ (dark blue line) and POA (grey line).

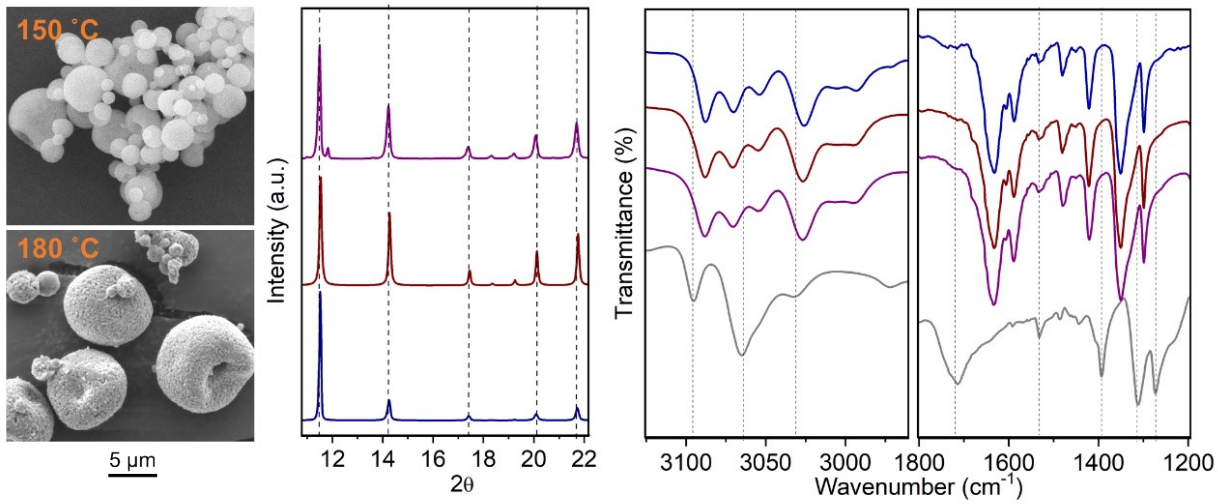

Figure S4. SEM images, XRPD patterns and FTIR analysis for evaluating the effect of inlet temperature on the spray dried MOF materials: 150 ˚C (wine red line) and 180 ˚C (purple line) compared to bulk dehydrated $Cu(POA)_2$ (dark blue line) and pyrazinoic acid (HPOA). The other spray drying input parameters that were held constant during this temperature variation were: 1 Cu: 2 POA, 1.5 mg POA/mL, 80 %:20% H_2O:EtOH and 293 L/h N_2 flow (15 kPa pressure drop).

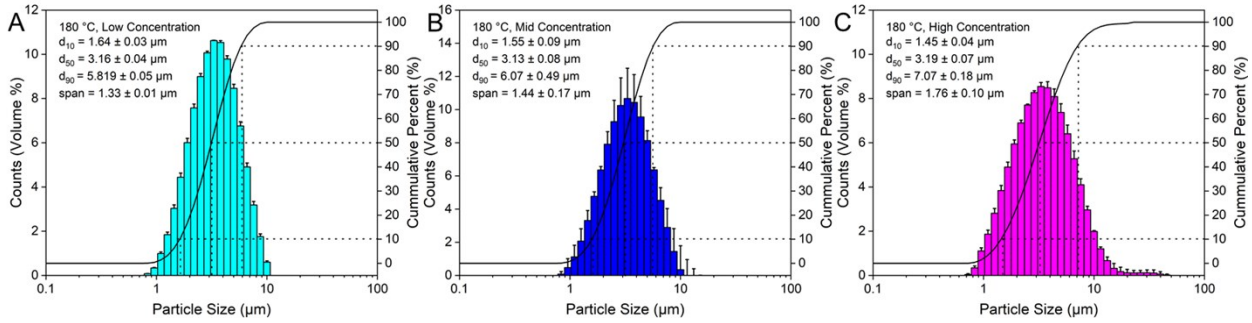

Figure S5. Laser diffraction data for $CuPOA_2$ MOF microparticles evaluating the effect of precursor concentration on volume particle size: A) 1 Cu: 2 POA ratio at 1.0 mg/mL POA, B) 1 Cu: 2 POA ratio at 1.5 mg/mL POA, and C) 1 Cu: 2 POA ratio at 3.0 mg/mL POA. The other spray drying input parameters that were held constant during this concentration variation were: 180 °C inlet temperature, 80 %:20% H_2O:EtOH, and 293 L/h N_2 flow (15 kPa pressure drop).

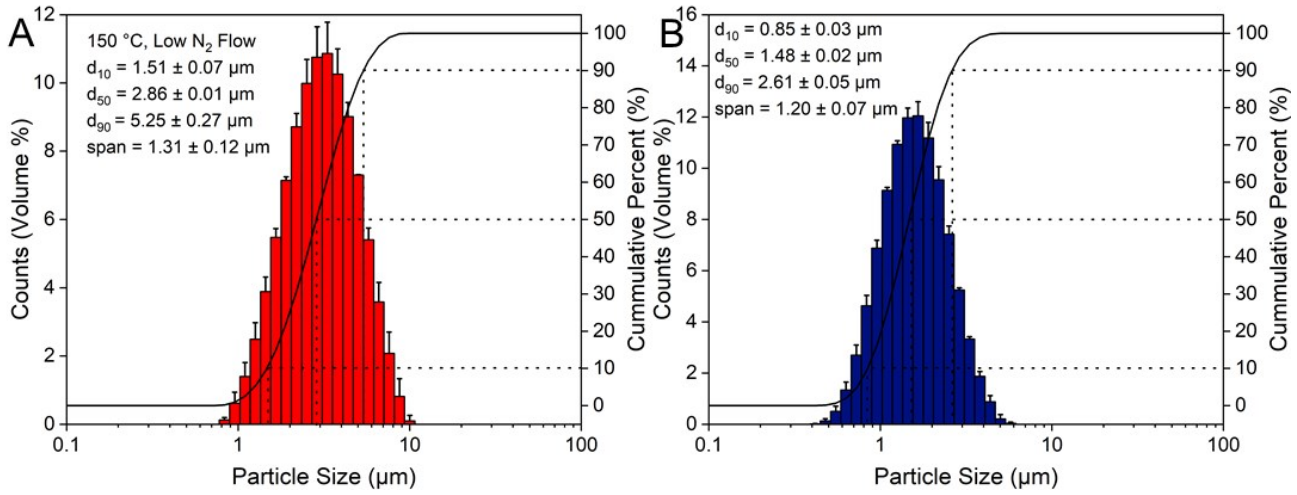

Figure S6. Laser diffraction data for $Cu(POA)_2$ MOF microparticles evaluating the effect of atomizing gas flow (N_2) on volume particle size: A) Low N_2 = 293 L/h (15 kPa pressure drop) and B) High N_2 = 1052 L/h (75 kPa pressure drop) The other spray drying input parameters that were held constant during this concentration variation were: 1:2 Cu:POA, 150 °C inlet temperature, 1.5 mg/mL POA, and 80%:20% H_2O:EtOH.

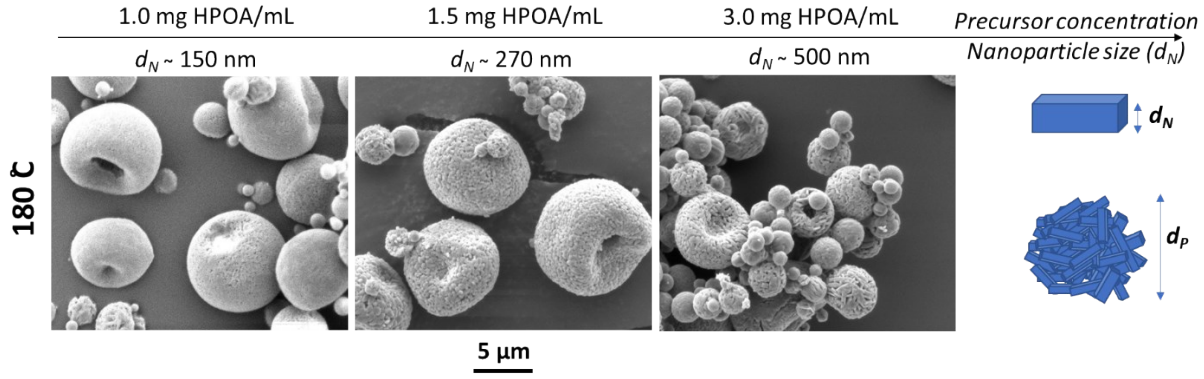

Figure S7. SEM images for evaluating the effect of precursor concentration on the spray dried MOF materials (d_N = nanoparticle diameter; d_P = total particle diameter). Spray drying input parameters that were held constant during this concentration variation were: 1 Cu: 2 POA, 180 ˚C inlet temperature, 80%:20% H_2O:EtOH and 293 L/h N_2 flow (15 kPa pressure drop).

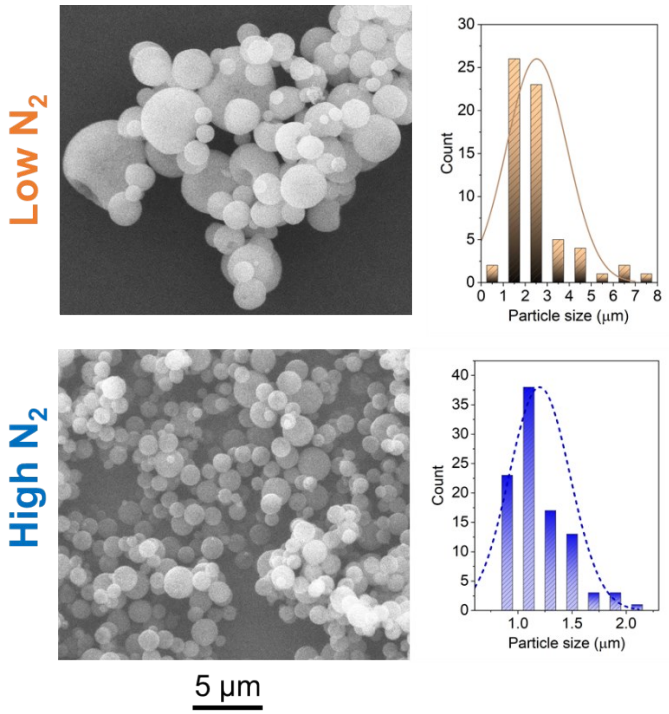

Figure S8. SEM images for evaluating the effect of the N_2 flow on the spray dried MOF materials. Low N_2 = 293 L/h flow rate (15 kPa pressure drop) and high N_2 = 1052 L/h (75 kPa pressure drop) Spray drying input parameters that were held constant during this atomizing gas variation were: 1 Cu: 2 POA, 150 ˚C inlet temperature, 1.5 mg POA/mL and 80%:20% H_2O:EtOH.

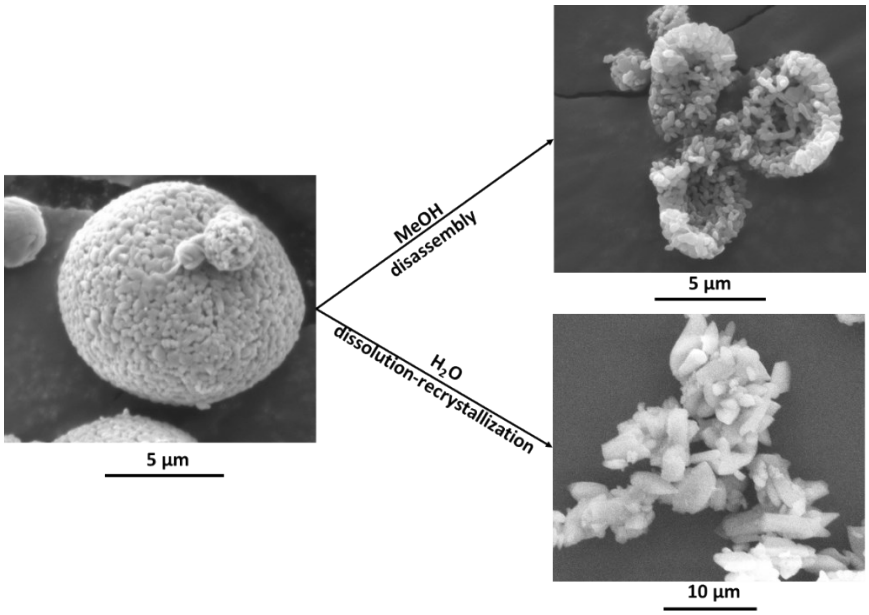

Figure S9. SEM images for $Cu(POA)_2$ obtained under the following conditions: 1 Cu: 2 POA, 15 mg POA/mL, 180 ˚C, 80%:20% H_2O:EtOH and 293 L/h N_2 flow (15 kPa pressure drop); and after soaking/sonicating this material in MeOH and H_2O.

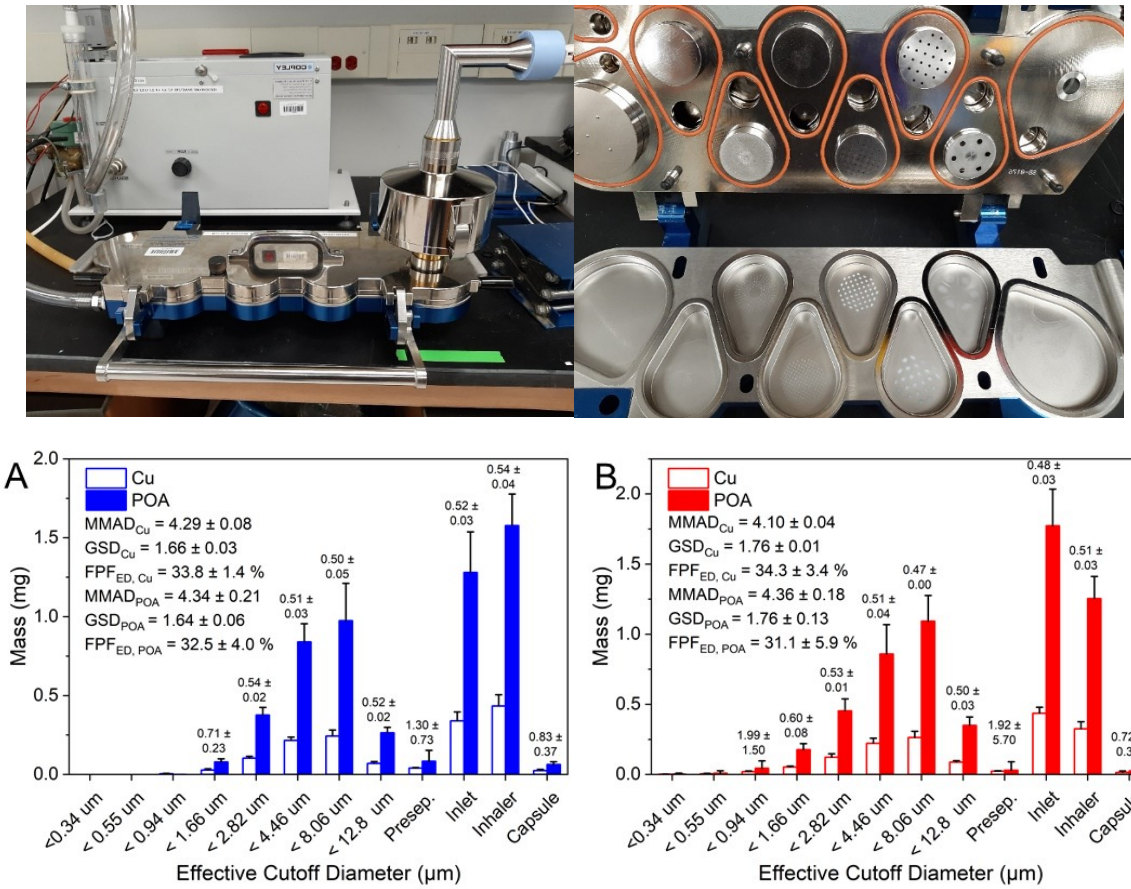

Figure S10. (above) Photograph of a closed (left and open (right) NGI. (below) NGI data evaluating impact of spray dryer inlet temperature on MMAD: A) 180 °C inlet temperature and low N_2 (293 L/h, 15 kPa pressure drop) and B) 150 °C inlet temperature and low N_2 (293 L/h, 15 kPa pressure drop). Note consistency of MMAD, GSD, and FPF_{ED} whether determined via Cu or POA content. A 1:2 Cu:POA molar ratio is maintained throughout characterization. NGI results are expressed as mean +/- standard deviation after three replicates.

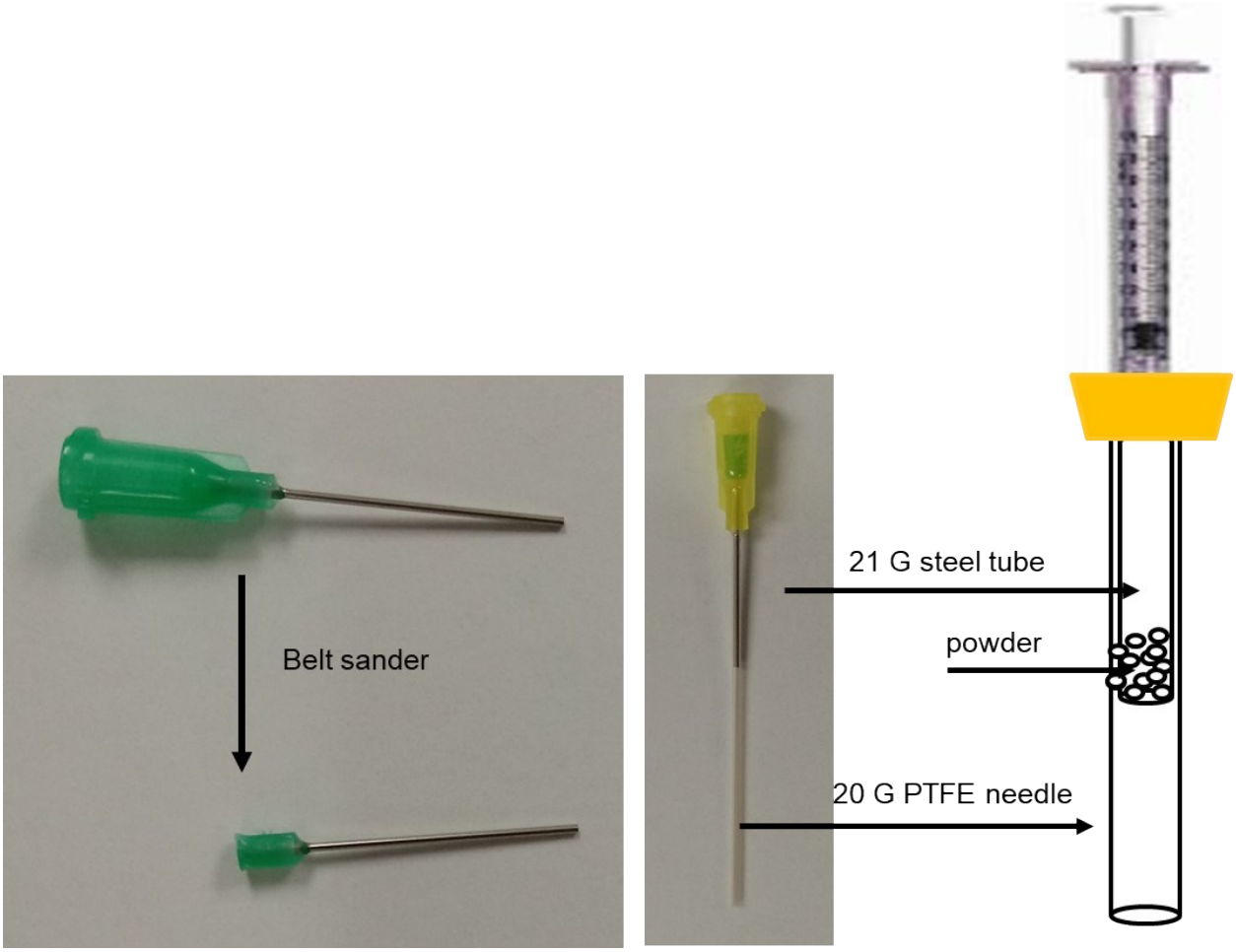

Figure S11. Assembly and loading of dosators intended to deliver < 1 mg spectinamide 1599 powder. (Left) Photograph showing the fabrication of the dosator and where the powder is intended to be held in the dosator and (Right) scheme of dosator construction[1].

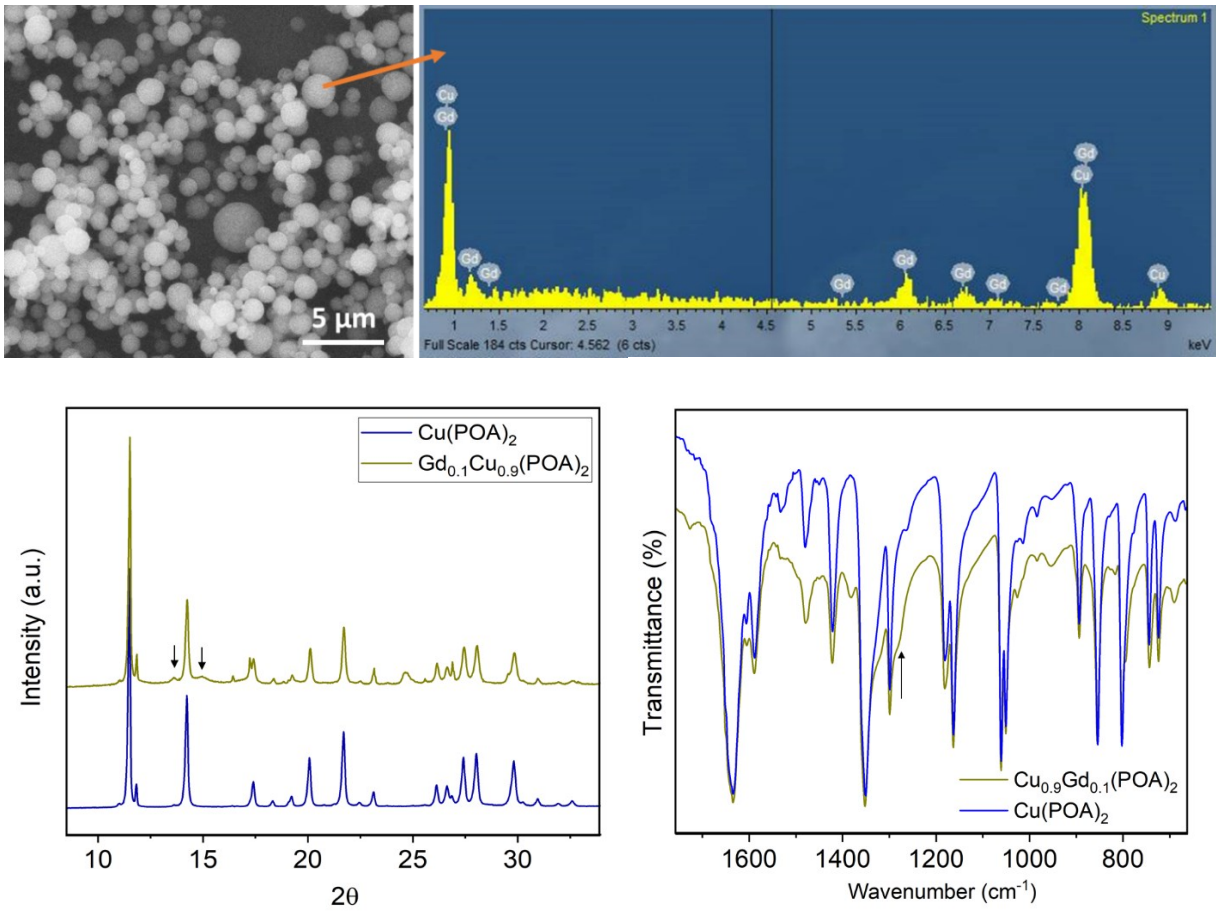

Figure S12. SEM image and EDS analysis (above), FTIR spectra (bottom left) and XRD pattern for Gd-doped material $Gd_{0.1}Cu_{0.9}(POA)_2$ compared to $Cu(POA)_2$ obtained under the optimal spray drying conditions: 0.1Gd: 0.9Cu: 2 POA, 150 ˚C inlet temperature, N_2 = 1052 L/h (75 kPa pressure drop), 1.5 mg POA/mL and 80%:20% H_2O:EtOH. Doping with 10 %molar of Gd results in very small and broad peaks on XRD pattern and a shoulder around 1260 cm^{-1} on FTIR spectra suggesting the possible formation of additional phases but difficult to determine due to their low concentrations. Nevertheless, EDS/SEM confirms the presence and homogeneity (*ca* 10 % Gd) of both Gd and Cu elements in several single spherical particles.

1. I. E. Stewart, P. B. Lukka, J. Liu, B. Meibohm, M. Gonzalez-Juarrero, M. S. Braunstein, R. E. Lee and A. J. Hickey, *Pharm. Res.*, 2019, **36**, 136.