Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supporting Information

One-pot Preparation of a Novel CO-Releasing Material based on a CO-Releasing Molecule@Metal-Organic Framework System

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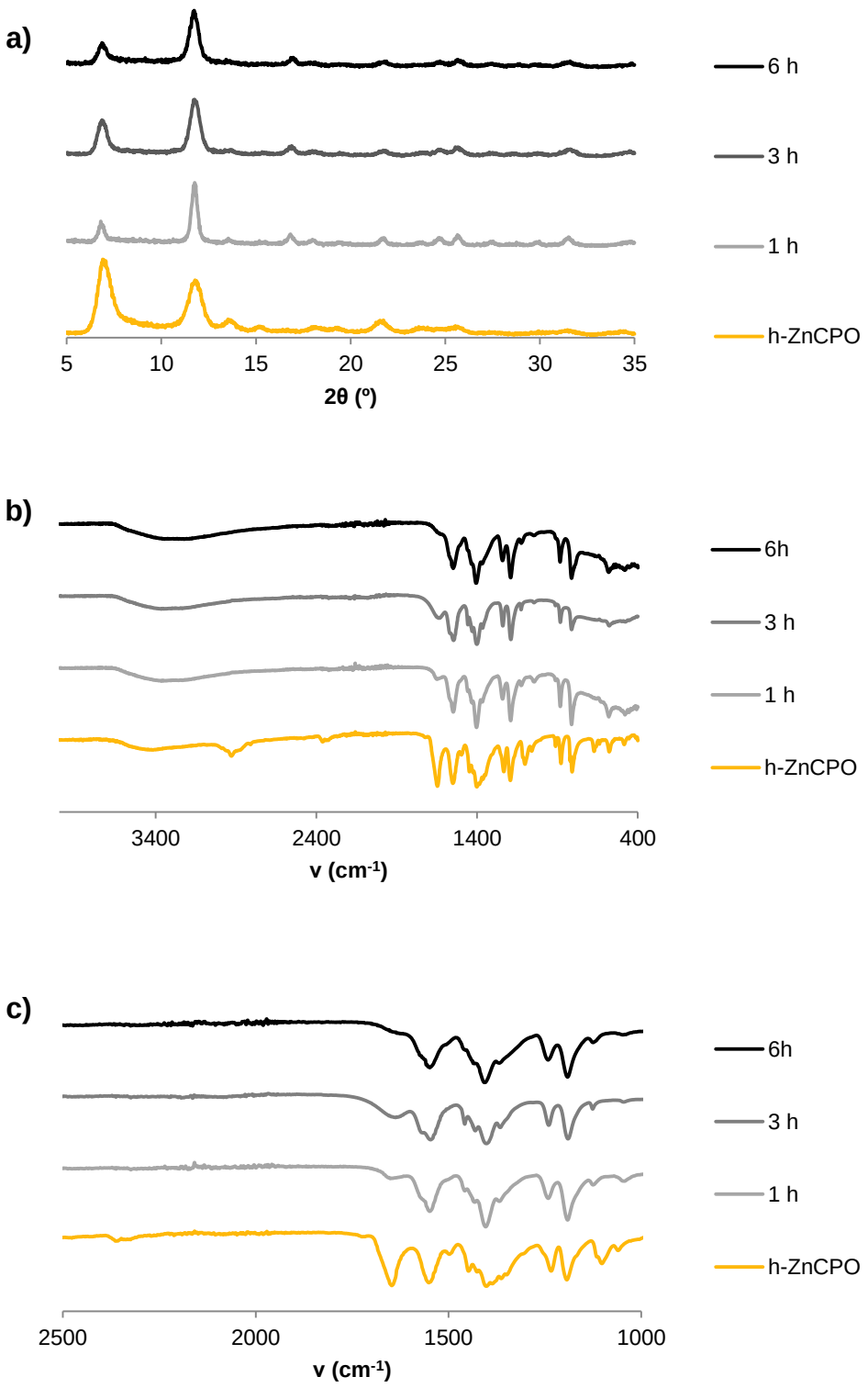

Fig. S1 Stability studies of h-ZnCPO material after being suspended in HEPES (10 mM, pH 7.4) for different periods of time (1, 3, 6 h). a) XRPD patterns. b) IR spectra. c) Focus on IR spectra in the 1000-2500 cm^{-1} region. The loss of a peak at 1650 cm^{-1} due to the exchange of hosted DMF molecules can be appreciated.

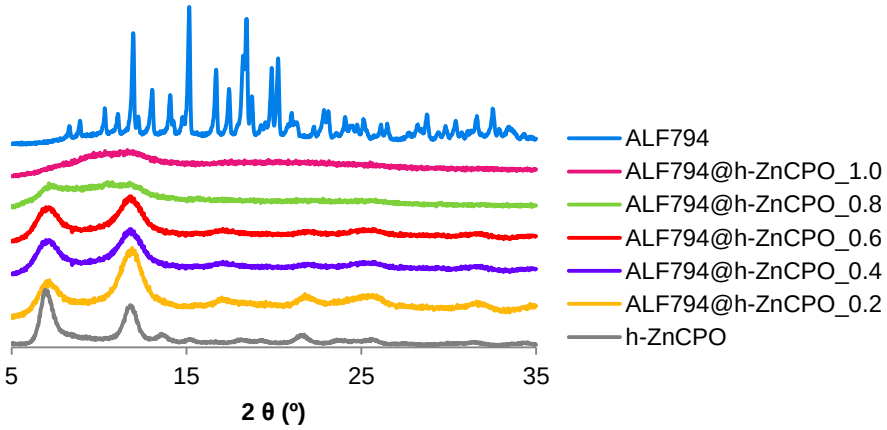

Fig. S2 X-ray powder diffractograms of ALF794, h-ZnCPO and the corresponding hybrid materials ALF794@h-ZnCPO_x (x = CORM/Zn^{2+} molar ratio used during the synthesis).

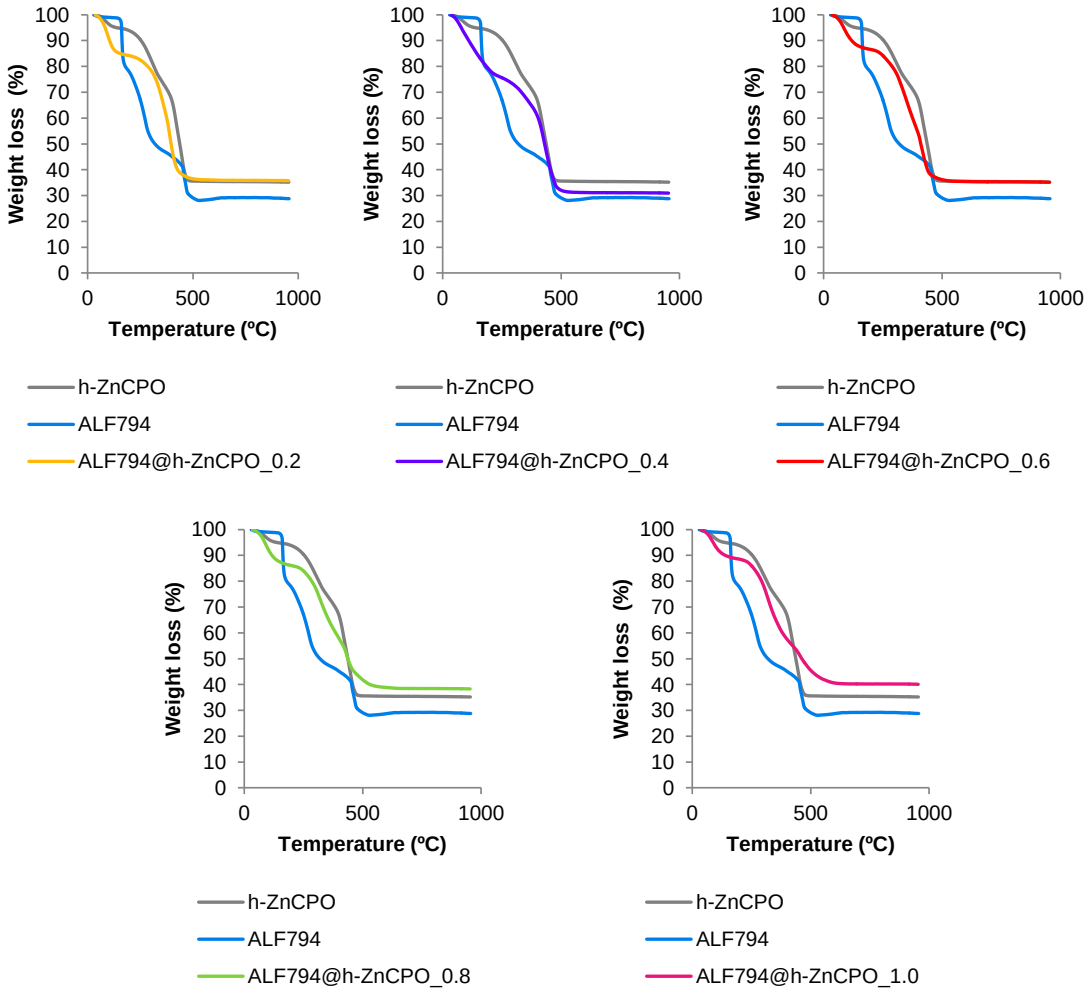

Fig. S3 Thermogravimetric analysis of ALF794, h-ZnCPO and the corresponding hybrid materials ALF794@h-ZnCPO_x (x = CORM/Zn^{2+} molar ratio used during the synthesis).

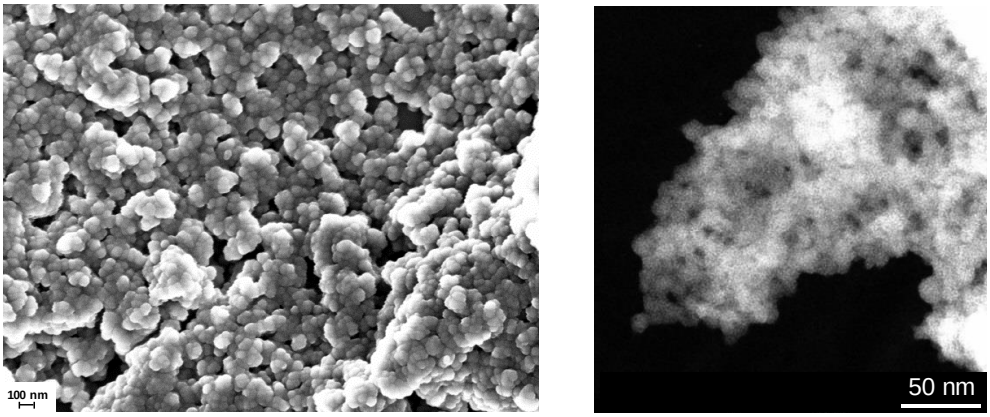

Fig. S4 SEM (left) and HR-TEM (right) images of ALF794@h-ZnCPO_0.2 material.

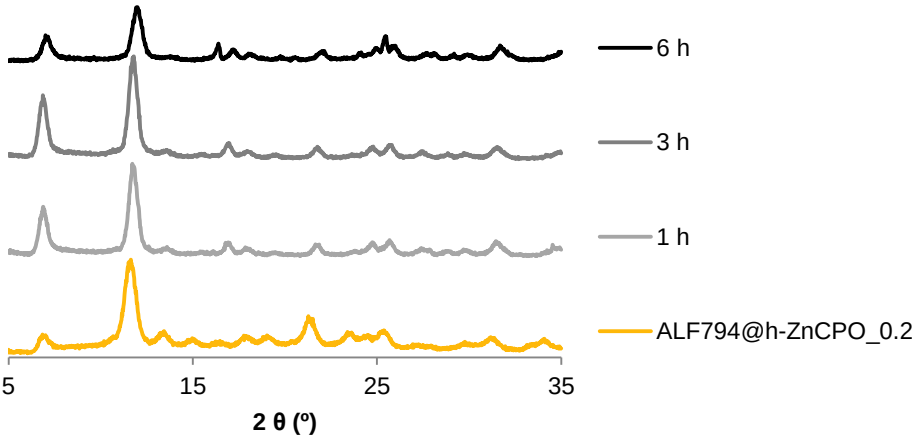

Fig. S5 XRPD patterns of ALF794@h-ZnCPO_0.2 material after being suspended in HEPES (10 mM, pH 7.4) for different periods of time (1, 3, 6 h).

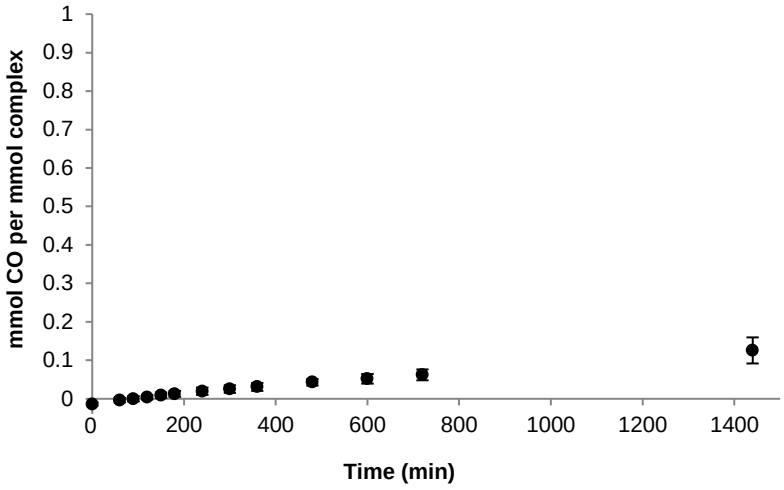

Fig. S6 24 h CO kinetic profile of ALF794 in darkness. Experimental conditions: Ar atmosphere, HEPES, 37 °C, 10 µM of ALF794 and 10 µM of myoglobin.

Table S1 Quantification of ALF794 loadings (mmol g^{-1}) in the hybrid materials and encapsulation efficiency (EE, %) of the process ($\mathrm{EE}(\%) = \frac{\mathrm{Dl}}{\mathrm{Dt}} \cdot 100$; D_t is the total amount of drug employed and D_l is the amount of loaded drug).

CORM@MOF material	ALF794 (mmol g^{-1})	EE (%)
ALF794@h-ZnCPO_0.2	0.28	97
ALF794@h-ZnCPO_0.4	0.30	75
ALF794@h-ZnCPO_0.6	0.34	53
ALF794@h-ZnCPO_0.8	0.26	33
ALF794@h-ZnCPO_1.0	0.40	38

Table S2 Metal leaching studies of ALF794@h-ZnCPO_0.2 in HEPES under UV irradiation and in darkness.

	Mo (%)		Zn (%)	
Time (h)	UV	darkness	UV	darkness
1	11.2	12.5	7.9	8.3
3	14.2	14.9	8.9	8.1
6	16.9	17.5	8.9	9.0

EXPERIMENTAL SECTION

1. General methods

XRPD data were collected on a Bruker D2-PHASER diffractometer (2θ) using CuKα radiation (λ = 1.5418 Å) and LYNXEYE detector, from 5 to 35º (2θ) and a step size of 0.02º. Elemental (C, H, N) analyses were obtained in a THERMO SCIENTIFIC Flash 2000 instrument (Centre of Scientific Instrumentation, University of Granada). Thermogravimetric analyses were performed using a Mettler Toledo TGA/DSC STAR system under oxygen flow (10 mL min^{-1}) running from RT to 950 ºC with a heating rate of 20 ºC min^{-1} (Centre of Scientific Instrumentation, University of Granada). The infrared spectra data were collected with a Fourier transform infrared spectrophotometer Bruker Tensor 27. Inductively Coupled Plasma Optic Emission Spectrometry analyses (ICP-OES) were carried out in an OPTIMA 7300 DV (Central Services Research Support, University of Malaga). UV−vis spectra were collected on a Shimadzu UV spectrophotometer. HR-TEM images and TEM-EDX elemental mapping were performed using a JEOL JEM-1400 (Central Services Research Support, University of Malaga) while SEM images were recorded using a Zeiss SUPRA 40VP microscope (Centre of Scientific Instrumentation of the University of Granada). Samples were prepared by dispersing a small amount of the material (3 mg) in absolute ethanol (1 mL) followed by sonication for 10 min and deposition on a copper grid (TEM) or on a piece of glass (SEM). In the latter case, the sample was coated with carbon before acquisition.

2. Synthesis and characterization of materials

All chemical and solvents were commercially available and used without further purification. ALF794 [$Mo(CO)_3(CNCMe_2CO_2H)_3$] was provided by Alfama Ltd.

2.1. Preparation of the hierarchical matrix [Zn_2(dhtp)] (dhtp = 2,5-dihydroxyterephtalate) (h-ZnCPO)

The synthesis of h-ZnCPO was performed as previously described.[1] 10 mL of N,N-dimethylformamide (DMF) containing 2,5-dihydroxyterephthalic acid (49.5 mg, 0.25 mmol) were added to 10 mL of DMF containing $Zn(OAc)_2{\cdot}2H_2O$ (171.5 mg, 0.78 mmol) under stirring. The mixture was stirred (300 rpm) for 1 h at room temperature, and the resulting yellow precipitate was separated by centrifugation (4000 rpm, 5 min), washed with DMF and methanol (3 x 10 mL), and subsequently dried under N_2 stream (ca. 2 h). Yield: 67%.

Anal. calcd for $(Zn_2C_8H_2O_6)(H_2O)_2(C_3ONH_7)_{1.5}$ (**h-ZnCPO**): C 31.91, H 3.53, N 4.47; found: C 32.04, H 3.55, N 4.40. M.W: 470.55 g/mol. Calculated residue after thermal treatment of h-ZnCPO: $(ZnO)_2$ 34.59%; found: 33.15%.

2.2. One-pot synthesis of ALF794@h-ZnCPO_x hybrid materials

In a typical synthesis, 3.33 mL of DMF containing 2,5-dihydroxyterephthalic acid (16.5 mg, 0.083 mmol) and different amounts of ALF974 (ALF794/$Zn(OAc)_2$ ratio: 0.2, 0.4, 0.6, 0.8 and 1.0) were immediately added to a 3.33 mL DMF solution of $Zn(OAc)_2{\cdot}2H_2O$ (57.17 mg, 0.3 mmol) under stirring. The mixture was stirred (300 rpm) for 1 hour at room temperature. The resulting precipitate was separated by centrifugation (4000 rpm, 5 min), washed with DMF and methanol (3 x 10 mL) and dried under N_2 stream (ca. 2 h). Yield: 66%, 45%, 44%, 28%, and 37% (for 0.2, 0.4, 0.6, 0.8 and 1.0, respectively). All the experiments were performed in darkness.

Anal. calcd for $(Zn_2C_8H_2O_6)(H_2O)_{4.6}(C_{18}H_{21}O_9N_3Mo)_{0.14}(C_3ONH_7)_{0.23}$ (**ALF794@h-ZnCPO_0.2**): C 27.07, H 3.19, N 1.83; found C 26.86, H 2.95, N 1.89. M.W: 497.26 g mol^{-1}. Calculated residue after thermal treatment of ALF794@h-ZnCPO_0.2: $(ZnO)_2(MoO_3)_{0.14}$ 35.81%; found 36.79%. Zn/Mo ratio determined by ICP-OES: 2:0.14. EE: 97%.

Anal. calcd for $(Zn_2C_8H_2O_6)(H_2O)_{4.5}(C_{18}H_{21}O_9N_3Mo)_{0.15}(C_3ONH_7)_{0.1}$ (**ALF794@h-ZnCPO_0.4**): C 26.89, H 3.04, N 1.56; found C 26.86, H 2.97, N 1.51. M.W: 491.15 g mol^{-1}. Calculated residue after thermal treatment of ALF794@h-ZnCPO_0.4: $(ZnO)_2(MoO_3)_{0.15}$ 31.03%; found 37.54%. Zn/Mo ratio determined by ICP-OES: 2:0.15. EE: 75%.

Anal. calcd for $(Zn_2C_8H_2O_6)(H_2O)_3(C_{18}H_{21}O_9N_3Mo)_{0.16}(C_3ONH_7)_{0.15}$ (**ALF794@h-ZnCPO_0.6**): C 28.77, H 2.64, N 1.87; found C 28.49, H 2.41, N 1.85. M.W: 472.98 g mol^{-1}. Calculated residue after thermal treatment of ALF794@h-ZnCPO_0.6: $(ZnO)_2(MoO_3)_{0.16}$ 35.34%; found 39.28%. Zn/Mo ratio determined by ICP-OES: 2:0.16. EE: 53%.

Anal. calcd for $(Zn_2C_8H_2O_6)(H_2O)_{3.4}(C_{18}H_{21}O_9N_3Mo)_{0.13}(Mo)_{0.19}(C_3ONH_7)_{0.3}$ (**ALF794@h-ZnCPO_0.8**): C 27.33, H 2.78, N 1.96; found C 26.87, H 2.40, N 2.16. M.W: 493.8 g mol^{-1}. Calculated residue after thermal treatment of ALF794@h-ZnCPO_0.8: $(ZnO)_2(MoO_3)_{0.32}$ 41.49%; found: 38.4%. Zn/Mo ratio determined by ICP-OES: 2:0.32. EE: 33%.

Anal. calcd for $(Zn_2C_8H_2O_6)(H_2O)_{4.2}(C_{18}H_{21}O_9N_3Mo)_{0.23}(Mo)_{0.31}(C_3ONH_7)_{0.3}$ (**ALF794@h-ZnCPO_1.0**): C 27.39, H 3.05, N 2.42; found: C 27.04, H 2.50, N 2.80. M.W: 571.65 g mol^{-1}. Calculated residue after thermal treatment of ALF794@h-ZnCPO_1.0: $(ZnO)_2(MoO_3)_{0.54}$ 41.80%; found: 40.18%. Zn/Mo ratio determined by ICP-OES: 2:0.0.53. EE: 38%.

3. Stability studies of the hierarchical h-ZnCPO matrix in HEPES

The structural stability of the hierarchical h-ZnCPO material was studied by suspending 10 mg of solid in 2 mL of HEPES (10 mM, pH 7.4). The resulting suspensions were kept on an orbital incubator shaker at 37 ºC for different periods of time (1, 3, and 6 h). At each time, the

corresponding suspension was centrifuged and X-ray powder diffractograms and IR spectrum recorded.

4. Determination of CO-release by Myoglobin Assay.

The amount of released CO was monitored as previously reported.[2,3] The CO release from ALF794 and the loaded material ALF794@h-ZnCPO_0.2 was studied spectrophotometrically by measuring the conversion of deoxy-myoglobin (deoxy-Mb) to carbonmonoxy-myoglobin (Mb-CO). The amount of Mb-CO formed was quantified by measuring the absorbance at 423 nm at different times. Stock solutions of myoglobin from equine heart (100 μM, adjusted by UV−visible spectroscopy), sodium dithionite (40 mg mL^{-1}), ALF794 (1 mM), and stock suspension of ALF794@h-ZnCPO_0.2 (1 mM, based on ALF794) were freshly prepared in degassed HEPES (10 mM, pH = 7.4). A 100 μL portion of myoglobin stock solution, 100 μL of sodium dithionite stock solution, 790 μL of degassed HEPES and 10 μL of ALF794 stock solution or CO-releasing material stock suspension (ALF794@h-ZnCPO_0.2) were placed in a sealed quartz cuvette under inert atmosphere (Ar) and kept at 37 °C under UV light for 5 h (using a UV lamp, λ = 365 nm, distance between lamp and cuvette 5 cm). The solutions were analyzed by means of UV−vis at different times (0, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, and 285 min) in order to determine the released amount of CO and follow the kinetics of the process. After 5 h, the solutions were bubbled with CO and the concentration of Mb-CO was calculated as previously reported.[3] Additional CO-releasing assays of free ALF794 (10 μM) in the dark were performed in order to confirm that the CO release only takes place after activation with UV light. All experiments were performed by triplicate.

5. Metal leaching assays

The leaching assays of the loaded ALF794@h-ZnCPO_0.2 material was studied, both under UV-light and in darkness, by suspending 10 mg of solid in 2 mL of HEPES (10 mM, pH 7.4). The resulting suspensions were kept on an orbital incubator shaker at 37 ºC for different times (1, 3, and 6 h). At each time, the corresponding suspension was centrifuged and the supernatant was collected to determine the total amount of leached Mo and Zn by ICP-OES.