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Zn-based porous coordination solid as diclofenac sodium carrier

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ABSTRACT

Drug delivery systems produced with biocompatible components can be used to reduce adverse effects and improve therapy efficacy. Most of the carrier materials reported in the literature show poor drug loading and rapid release. However, porous hybrid solids, such as metal-organic frameworks, are well suited to serve as carriers for delivery and imaging applications. In this work, a luminescent and nontoxic porous Zn(II) coordination polymer with 4,4′-biphenyl-dicarboxylic acid (BPDC) and adenine linkers (**BioMOF-Zn**) was synthesized by a solvothermal process and characterized by PXRD, TGA, SEM-FEG, and FTIR. Nitrogen adsorption measurements revealed the presence of micropores as well as mesopores in the framework after activation of the material. The blue-emitting **BioMOF-Zn** exhibited an outstanding loading capacity (1.72 g g^{-1}) and satisfactory release capability (56% after two days) for diclofenac sodium.

1. Introduction

Metal-organic frameworks (MOFs) are a class of crystalline porous coordination solids constructed from the self-assembly of metal ions and organic spacer ligands [1]. Interest in the study of MOFs has emerged rapidly, due to their exceptionally high surface area (up to 10,000 m^2 g^{-1}), permanent porosity, low density, and additional unique features such as the possibility of functionalization of their internal surfaces [2]. For this reason, they are potential candidates for numerous applications including gas storage [3], selective gas separation [4], heterogeneous catalysis [5], sensors [6], and photonics [7], among others [8–10]. In the biomedical area, MOFs have recently attracted attention as nanoencapsulation and controlled-release agents in drug delivery [11–13]. Several kinds of therapeutic agents have been efficiently delivered from the pores of different MOFs [14–20]. Another approach involves the direct introduction of the therapeutically active compound into the MOF structure, in the form of a ligand [21,22]. In this case, no pores are required and the degradation of the MOF under biological conditions is essential to release the drug molecule. More recently, a number of computational studies have also been undertaken to predict the potential capacities of MOFs for drug storage, in order to identify those materials with the highest performances [23]. The main requirements for effective drug delivery in the body include high capacity of the solid for drug loading, control of the release of the therapeutic compound in order to avoid the burst effect [24], high cell uptake, intrinsic biodegradability (due to relatively labile metal-ligand bonds), biocompatibility, and negligible cytotoxicity [25]. Biomolecules such as amino acids, peptides, and nucleobases, as well as biocompatible metal cations (Fe, Zn, and Cu), have been used as ligands in the construction of this sub-class of MOFs [26]. The first compound of this family was **bio-MOF-1** ($[(Zn_8(adenine)_4(bpdc)_6O)]\cdot 2Me_2NH_2\cdot 8DMF\cdot 11H_2O$), synthesized by An and co-workers [27]. This material, based on zinc-adeninate columnar SBUs, is composed of apex-sharing zinc-adeninate octahedral cages, with the zinc-adeninate columns being interconnected by multiple bpdc (4,4′-biphenyl-dicarboxylic acid) linkers. The **bio-MOF-1** microporous material has a BET surface area of around 1700 m^2 g^{-1} and exhibits an intrinsic anionic porous nature. For this reason, it has been used as a host for adsorption of cationic drug molecules and other cationic guests [28,29]. Here, we report the synthesis of a luminescent biocompatible zinc-adeninate MOF, denoted **BioMOF-Zn**, and evaluate its drug delivery potential. The model drug selected was diclofenac sodium (DS), a common non-steroidal anti-inflammatory agent that exhibits a short half-life *in vivo* (1–2 h), requiring many daily doses [30]. Diclofenac sodium is therefore an ideal candidate for controlled release administration, since the inorganic matrix can protect the drug from rapid biodegradation, minimize

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toxicity, and increase the therapeutic effect, hence ensuring suitable concentration levels at active sites.

2. Experimental section

2.1. Materials and measurements

For the synthesis, adenine ($C_5H_5N_5$) and 4,4′-biphenyl-dicarboxylic acid (BPDC) were purchased from Sigma-Aldrich, and zinc acetate dihydrate ($Zn(CH_3COO)_2{\cdot}2H_2O$) and nitric acid ($HNO_3$) were acquired from Vetec. For the cytotoxicity assays, trypsin was purchased from Vitro Cell and resazurin ($C_{12}H_7NO_4$) and doxorubicin ($C_{27}H_{29}NO_{11}$) were obtained from Sigma-Aldrich. All the starting materials and solvents were used without further purification. Elemental analyses (C, H, and N) were performed with a Perkin Elmer 2400 series II analyzer. Fourier transform infrared (FTIR) spectra were acquired in the range 4000–400 cm^{-1}, using KBr pellets and a Nicolet IS5 spectrophotometer (Thermo Scientific). The absorption bands were described as follows: strong (s), medium (m), weak (w), and broad (br). Powder X-ray diffraction (PXRD) patterns were obtained at room temperature using a Difrac Plus XRD Commander diffractometer, in the 2θ range 5–30°, with Cu Kα radiation (λ = 1.5418 Å), scan speed of 1 s/step, and step size of 0.05°. Scanning electron microscopy (SEM) images were acquired with a Topcon SM-300 field emission gun (FEG) instrument, using carbon as a sample support. Thermogravimetric analyses (TGA) were performed using a TA Instruments SDT Q600 system, with heating from room temperature to 750 °C at a rate of 10 °C min^{-1}, under an air atmosphere (150 mL min^{-1}). Nitrogen adsorption measurements were performed at liquid nitrogen temperature using a Micrometrics ASAP 2010 system. Solid-state luminescence spectra were obtained at room temperature using a Jobin Yvon-Spex Fluorolog FL3-22 spectrofluorimeter equipped with a 450 W xenon lamp.

2.2. Preparation of BioMOF-Zn

A mixture of adenine (0.125 mmol), BPDC (0.25 mmol), zinc acetate dihydrate (0.375 mmol), HNO_3 (1 mmol), and 14 mL of DMF/MetOH (1:1) was added to a 35 mL vial and stirred for 30 min. The vial was then capped and the resulting mixture was heated at 130 °C for 24 h. After cooling to room temperature, the white microcrystalline **bio-MOF-1** powder obtained was washed with DMF/MetOH (1:1) and dried under vacuum for 8 h. The as-synthesized **bio-MOF-1** was activated by applying vacuum (50 mbar) for 24 h, at 150 °C, and the material obtained was denoted **BioMOF-Zn** (see next section). The FTIR spectrum (KBr, 4000–400 cm^{-1}) of this material was as follows: 3347 (br), 3176 (br), 2925 (w), 1665 (s), 1604 (s), 1543 (m), 1474 (m), 1383 (s), 1213 (m), 1178 (m), 1154 (m), 1101 (m), 849 (m), 772 (s), 703 (m).

2.3. Calibration curve

The calibration curve for diclofenac sodium was obtained using a Thermo Scientific Evolution Array UV–Vis spectrophotometer at λ = 275 nm. Calibration standard solutions of diclofenac sodium were prepared in the concentration range from 10 to 50 μg mL^{-1}.

2.4. Drug loading efficiency

The **BioMOF-Zn** drug loading was performed by impregnation, using diclofenac sodium (DS) as a model drug. The loading tests were performed by suspending 60 mg of MOF in an aqueous solution (42 mL of Milli-Q water) containing 200 mg of the drug (4.76 mg mL^{-1}), under stirring at room temperature for four days. The loaded drug was then separated by centrifugation. The drug in the supernatant was determined by UV–Vis spectroscopy, at 275 nm, in triplicate. The drug loading percentage (DL%) was calculated according to Eq. (1):

$$DL(\%) = \frac{(DS)_i - (DS)_s}{(DS)_i} x \quad 100\% \tag{1}$$

where, DS_i is the initial concentration of DS (4.76 mg mL^{-1}) and DS_s is the concentration of DS in the supernatant. The powder obtained after centrifugation, denoted **DS@BioMOF-Zn**, was submitted to FTIR, PXRD, and TGA analyses.

2.5. In vitro drug released experiments

In vitro release profiles of diclofenac sodium were obtained using a medium that simulated physiological conditions (1 mol L^{-1} phosphate buffered saline (PBS), at pH 7.4). The **DS@BioMOF-Zn** (5 mg) was immersed in 10 mL of PBS and incubated at room temperature, under constant stirring, in triplicate.

Determination of the amount of diclofenac sodium released into the medium was determined by UV spectroscopy, at 275 nm. Aliquots were removed after 0.5, 1, 2, 3, 5, 9, 22, 36, and 48 h. For each aliquot removed, an identical volume of fresh medium was immediately added, in order to maintain sink conditions.

2.6. Cytotoxicity assays

Resazurin reduction assays were used to investigate the cytotoxicities of the **BioMOF-Zn** and **DS@BioMOF-Zn** samples towards MRC-5 cells [31]. This assay is based on reduction of the indicator dye, resazurin, to highly fluorescent resorufin by viable cells. Nonviable cells rapidly lose the metabolic capacity to reduce resazurin and therefore do not produce a fluorescence signal. Briefly, the cells were detached by treatment with 0.25% trypsin/EDTA and 2.5×10^4 cells were placed in each well of a 96-well cell culture plate, in a total volume of 100 μL. The cells were allowed to adhere overnight and were then treated with different concentrations of the drug. After 24 h incubation in the presence of the compounds, the medium was removed and a 50 μL volume of resazurin (0.01% w/v) in DMEM (Dulbeccos' Modified Eagle Medium) was added to each well. The plates were incubated at 37 °C for 3 h, followed by measurement of fluorescence using a Biotek Synergy H1 plate reader at excitation and emission wavelengths of 530 and 590 nm, respectively. The negative control consisted of untreated (viable) cells, and the positive control was cells treated with doxorubicin at 100 nmol (dead cells). All the tests were performed using three independent assays. The IC_{50} values, representing the sample concentrations required to inhibit 50% of cell proliferation, were calculated from a calibration curve by regression, using GraphPad Prism v. 5.01 software.

3. Results and discussion

3.1. Preparation of BioMOF-Zn

The reaction of zinc acetate dihydrate, adenine, and BPDC in a mixed solvent (DMF and methanol) resulted in a white microcrystalline powder of **bio-MOF-1**. The literature describes two MOFs based on the same zinc-adenine-BPDC system, namely **bio-MOF-1** (synthesized in a DMF/water mixture at 130 °C for 24 h) [27], and **bio-MOF-100** (synthesized in a DMF/MetOH/water mixture at 85 °C for 24 h) [32]. Fig. 1 shows the powder XRD diffractograms of the as-synthesized **bio-MOF-1** (black line) and simulated crystals of **bio-MOF-1** (blue line) and **bio-MOF-100** (green line). The diffraction peaks obtained for the simulated **bio-MOF-100** model differed considerably from those for the as-synthesized **bio-MOF-1**. However, matching curves were obtained between the PXRD profiles of **bio-MOF-1** and the as-synthesized material in this work, indicating formation of the same phase.

The SEM-FEG image shown in Fig. 2a reveals the formation of rod-like particles of **bio-MOF-1**, ~5–7 μm in size, which organized into sheaf-like structures. It can also be seen in Fig. 2b that the surface of