Research Article

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Hydrogels loaded with atenolol drug metal–organic framework showing biological activity

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Abstract: Using the starting reactants of Zn(OAc)₂ and 2-mercaptonicotinic acid (H₂L), we successfully synthesized a fresh Zn(II) compound, that is, [Zn₃(L)₃]ₙ (1), under hydrothermal conditions and then successfully loaded with atenolol. Hualuronic acid/carboxymethyl chitosan hydrogels loaded with atenolol drug metal–organic framework were prepared based on a chemical synthesis method. The microscopic morphology of the hydrogels was investigated, and scanning electron microscopic results showed that the hydrogels had a highly porous morphology with good penetration between the pores. For the new hydrogels, the enzyme-linked immunosorbent assay detection was conducted, and the production of sVCAM-1 and TNF-α in vascular endothelial cells was determined. Besides, the levels of inflammatory response in the vascular endothelial cells were also determined with the real-time reverse transcriptase polymerase chain reaction assay.

Keywords: Zn(II) compound, hydrogels, atenolol

1 Introduction

Metal–organic frameworks (MOFs) with novel compositions and topological frameworks have evoked ever-increasing attention owing to their multiple properties that can be used in different fields, such as luminescence sensing, magnetism, gas storage, adsorption and separation, heterogeneous catalysis, molecular recognition, non-linear optics, and so on [1–5]. As far as we all know, MOFs that is complexed by organic ligands and metal ions combined the characteristics of inorganic compound and organic ligand. Hence, the major factor for controllable generation is the careful choice of organic ligands with suitable coordination sites and metal ions with appropriate coordination geometries. Isonicotinic acid is a bifunctional organic ligand and has been widely applied for the creation of MOFs having multifunctional properties [6–9]. To our knowledge, hitherto, thiol-modified isonicotinic acid ligand is rarely used for the synthesis of MOFs [10,11]. In consideration of that, in our article, we chose 2-mercaptop nicotinic acid, which has four potential coordination sites, as the organic ligand to construct novel MOFs in that the thiol group of 2-mercaptonicotinic acid can be in situ removed or can be in situ substituted by hydroxyl group, or can transform into a disulfi de ligand via the in situ formation of disulfi de bond under appropriate hydro(solvo)thermal conditions [12,13]. On the other hand, although zinc is not strictly a transition metal, it shares many bioinorganic properties with the transition metals due to the particular properties of its coordination compounds. As a borderline Lewis acid, zinc is a component of so many metalloenzymes and has a specific role in the bioinorganic processes of these enzymes. Therefore, it is the second most abundant element in the 3D transition metals after iron in the human body. Unlike iron and copper that have different oxidation states, Zn(II) ion plays only a structural role in forming appropriate enzymes by means of coordination with amino acids such as cysteine and histidine in an approximately tetrahedral coordination geometry. The coordination geometry around the Zn(II) in the active site of the enzymes is usually tetrahedral. Because in this structure, the bond distances and repulsion between the ligands are minimum and the zinc atom has available binding sites to interact with a substrate. In order to design and synthesized model compounds of the zinc enzymes, many coordination chemistry researchers have focused their work on the synthesis of Zn(II) complexes with nitrogen, oxygen, and sulfur donor ligands which can mimic the active site of these enzymes. Zinc is the only metal ion that can facilitate the rewinding of DNA. Additionally, many zinc

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complexes have shown anticonvulsant, antidiabetic, anti-inflammatory, antimicrobial, antioxidant, and anticancer properties, and some of them have also been tested for the treatment of Alzheimer’s disease [14–18]. Under hydrothermal conditions, the self-assembly reaction of 2-mercaptotronic acid and the Zn(II) ions triumphantly produced a fresh three-dimensional compound, i.e., [Zn3(L)3]n (1, H2L = 2-mercaptotronic acid). Compound 1 shows intense blue-green luminescence at room temperature.

Atenolol is a broad-spectrum antiepileptic drug that is effective against a variety of seizure types. Atenolol enhances the effect of Temozolomide on proliferation and apoptosis of glioblastoma stem cells. However, atenolol has a poor water and lipid solubility, low oral bioavailability (10–20%), and a short drug plasma half-life. Therefore, in order to improve the therapeutic efficacy of atenolol, domestic and international researchers have used various forms of administration to enhance its efficacy. The inside of hydrogel is a three-dimensional network structure with water absorption and swelling properties, which has a certain slow-release effect on drugs. Combining atenolol with hydrogel will develop a new drug delivery mode, which is conducive to provide research ideas for clinical application.

In this study, Hualuronic acid (HA)/carboxymethyl chitosan (CMCS) hydrogels loaded with atenolol drug meta-and lipid solubility, low oral bioavailability (10–20%), and could be directly applied. In order to investigate the therapeutic efficacy of atenolol, domestic and international researchers have used various forms of administration to enhance its efficacy. The inside of hydrogel is a three-dimensional network structure with water absorption and swelling properties, which has a certain slow-release effect on drugs. Combining atenolol with hydrogel will develop a new drug delivery mode, which is conducive to provide research ideas for clinical application.

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2 Experimental

2.1 Materials and instrumentation

In this article, all of the reagents together with chemicals were acquired from a market source, which was AR grade and could be directly applied. In order to investigate the elements of carbon, nitrogen together with hydrogen, Vario EL III was employed. Through applying PANalytical X’Pert Pro, the Powder X-ray diffraction pattern (PXRD) could be analyzed at 0.05° step size by applying the Cu/Kα radiation (in which λ is 1.54056 Å). In the temperature of 30–800°C, thermogravimetric analysis (TGA) was accomplished with the NETSCHZ STA-449C at 10°C per min heating rate under a flow of nitrogen. FLS980 was utilized to test the compound’s luminescent performance in the solid state.

2.2 Synthesis of compound [Zn3(L)3]n (1)

The mixture generated via 0.1 mmol H2L, 0.1 mmol of Zn (OAc)2, 12.0 mL of H2O, and 0.2 mmol NaOH was sealed into the stainless steel container (23 mL) lined by Teflon, and this container was heated under 180°C temperature for three days. With 2°C per min cooling rate, the mixture was cooled to environmental temperature, and after that, the colorless massive crystals for this compound were separated with 36% of yield in accordance with the H2L. Elemental analysis calculated for C18H9N3O6S3Zn3 (622.57): N, 6.78%; C, 34.72; and H, 1.45% and found were N, 6.78%; C, 34.72; and H, 1.42.

2.3 X-Ray crystallography

For the compound, its data of single crystal were recorded with the Rigaku Dmax 2500 using Cu-Kα radiation (where λ is 1.54056 Å). ShelXS with Direct Methods and SHELXL with Least Squares minimization were respectively employed to solve and refine the compound’s architectures [19,20]. The complex’s data of crystallography are displayed in Table 1.

| Table 1: The complex’s data of crystallography |
|-------------------|---|
| **Formula** | C18H9N3O6S3Zn3 |
| Fw | 622.57 |
| Crystal system | Triclinic |
| Space group | P-1 |
| a (Å) | 10.939(7) |
| b (Å) | 10.966(7) |
| c (Å) | 11.903(8) |
| α (°) | 106.758(8) |
| β (°) | 104.945(2) |
| γ (°) | 110.648(3) |
| Volume (Å3) | 1171.9(13) |
| Crystal size (mm3) | 0.22 × 0.21 × 0.16 |
| Z | 2 |
| Density (calculated) | 1.858 |
| Abs. coeff. (mm-1) | 3.352 |
| Total reflections | 9,207 |
| Unique reflections | 5,061 |
| Goodness of fit on F2 | 1.013 |
| Final R indices | 0.0381, 0.0972 |
| R (all data) | 0.0470, 0.0998 |

\[
\text{aR} = \frac{\sum |I_f| - |F_o|/\sqrt{\sum |F_o|^2}}{\sum |F_o|^2}/(\sum |F_f|^2)^{1/2},
\]

where w = 1/[\sum (F_f^2)], \( aP = \sum |F_f|^2/\sum |F_o|^2 \), and \( bP = \sum |F_f|^2/\sum |F_o|^2 \).
2.4 Preparation for hydrogels loaded with atenolol drug metal-organic framework

First, the MOF was immersed in a 20 mg/mL atenolol solution to prepare the atenolol-loaded complex. Then, a 1 wt% solution of HA and a 3, 5, 7 wt% solution of CMCS were configured. The 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride/N-Hydroxysuccinimide solution and the drug complex were added to the HA solution with rapid stirring for 30 min. Subsequently, the HA solution and the CMCS solution were added to the mold with stirring in a 1:1 volume ratio. The hydrogels were allowed to form at room temperature and then removed and washed with deionized water to prepare HA/CMCS hydrogels loaded with an atenolol drug metal–organic framework. The hydrogel samples were freeze-dried and gold-sprayed prior to testing. The microscopic morphology of the samples was observed using scanning electron microscopy (SEM).

2.5 Enzyme-linked immunosorbent assay (ELISA)

The ELISA detection was conducted in this present research to determine the inhibitory effect of the hydrogels on the releasing of sVCAM-1 and TNF-a into the serum. This preformation was finished totally under the guidance of the instructions with only a little change. Then, the bilateral uterine artery stenosis operation was conducted on the animal to induce the hypertension animal model during pregnancy. Then, the hydrogels were given for treatment at the concentration of 1, 2, and 5 mg/kg. The sVCAM-1 and TNF-a released into the serum were measured with ELISA.

2.6 Real-time reverse transcriptase-polymerase chain reaction (RT-PCR)

To determine the levels of inflammatory response in the vascular endothelial cells after hydrogels treatment, the real-time RT-PCR was conducted in this present research. This experiment was performed strictly in accordance with the protocols with some modifications. Then, the bilateral uterine artery stenosis operation was conducted on the animal to induce the hypertension animal model during pregnancy. Then, the hydrogels were given for treatment at the concentration of 1, 2, and 5 mg/kg. The vascular endothelial cells were collected, and the total RNA in the cells was extracted with TRIZOL reagent. After measuring the concentration of the total RNA, it was then reverse transcribed into cDNA. Finally, the real-time RT-PCR was conducted, and the relative expression of the nf-kb and p53 was measured.

3 Results and discussion

3.1 Crystal structure of compound 1

The diffraction study of X-ray displayed that the 1 reveals a three-dimensional framework which belongs to the triclinic space group of P-1. The fundamental unit of complex 1 is constituted by three independent L2− and Zn(II) ions. As displayed in Figure 1a, all Zn(II) ions are four-coordinated and located in a tetrahedral coordination sphere. For Zn1, the tetrahedron is defined through a sulfur atom, a N atom, as well as 2 carboxylate O atoms from three diverse L2−. For the Zn2 ion, the coordination tetrahedron is defined via a sulfur atom and 3 carboxylate O atoms offered by 3 distinct L2−. The Zn3 ion is coordinated through a sulfur atom, a carboxylate O atom together with 2 N atoms belonging to 3 diverse L2−. The angles and lengths of bond around the Zn(II) ions are all comparable with those of the reported related Zn(II) compound [21]. Under the hydrothermal conditions, the H2L ligand completely deprotonated two protons into the anion form of L2−, and the L2− ligand uses its four coordination sites linking three Zn(II) ions displaying a μ3-κ1O,κ2O,S,κ1N coordination mode (Figure 1b). Such bridging mode between Zn(II) ions and L2− ligands finally leads to the formation of a three-dimensional extended skeleton (Figure 1c). In the above-mentioned three-dimensional framework, each of the Zn(n) ions is surrounded via three distinct L2−, and each ligand of L2− connects with three diverse Zn(n). In topology, the complex’s entire framework can be specified into a 3-linked etb-type topological net with {83} point symbol through regarding L2− and Zn(n) ions as the three-linked nodes (Figure 1d).

3.2 PXRD and TGA

There presents excellent accordance between the PXRD pattern of experiment and simulation which was produced from the diffraction research of a single crystal, proving that the compound’s bulk products are single phase (Figure 2a).
The compound’s thermal behavior was also studied via the TGA experiment performed in a nitrogen atmosphere. The result shown in Figure 2b suggests that the framework of 1 can be stable up to 280°C. After that, rapid weightlessness appeared implying the organic ligand decomposition, eventually resulting in the creation of
ZnO (with 39.12 and 39.21% observed and calculated values)

### 3.3 Luminescent property of 1

The luminescent spectra of the free ligand of H$_2$L and complex 1 in the solid state were harvested with ambient temperature. As shown in Figure 3a, the emission band of free H$_2$L ligand can be observed with the maximum peak at 470 nm under 330 nm excitation. After the complexation of L$_2$ ligand and Zn(II) ions, the luminescent spectra of 1 are obviously red-shifted and show a maximum emission peak at 494 nm excited by 330 nm. Considering the d$^{10}$ electronic configuration of Zn(II) ion, the complex’s emission band can be owing to the L$_2^-$ centered transition of $\pi^*\rightarrow\pi$ [22]. The CIE chromaticity coordinate of 1 is calculated at (0.1616, 0.3264) in the CIE chromaticity diagram (Figure 3b), demonstrating that compound 1 may be a good candidate as blue-green fluorescent materials.

### 3.4 Micromorphology of the hydrogels

Natural polysaccharide hydrogels have good biocompatibility and degradability. In recent years, a series of achievements have been made in the fields of biomedicine and materials science. Many hydrogels have further expanded their application fields due to the characteristics of three-dimensional network structure and controllable shape. Figure 4 shows the internal microstructure of hydrogels prepared with different concentrations of CMCS. As a whole, the hydrogel has a typical porous morphology, and the perforations between the holes are good. When the concentration of CMCS was small, the cross-linking was not complete and showed typical macropore characteristics. As the concentration of CMCS increased to 7 wt%, the cross-linking network in the hydrogel was increased, which further increased the degree of cross-linking, resulting in a reduction of the pore size to 370 μm, which plays an important role in controlling the slow release of drugs.

### 3.5 Hydrogels significantly reduce the releasing of sVCAM-1 and TNF-a into the serum

After the synthesis of the new hydrogels, the related prevention activity was assessed. Thus, ELISA detection was first used to determine the content of sVCAM-1 and TNF-a released into the serum. As the results shown in Figure 5,
we can see that there was a higher level of the sVCAM-1 and TNF-a in the serum of the model group, which is much higher than the control group. After the treatment of the new hydrogels, the levels of sVCAM-1 and TNF-a released into the serum were reduced significantly. This inhibition showed a dose-dependent manner.

3.6 Hydrogels obviously inhibited the levels of inflammatory response in the vascular endothelial cells

In the above results, we can see that the hydrogels have an excellent inhibitory effect on the sVCAM-1 and TNF-a
released into the serum. As the activation of the inflammatory signaling pathway regulates the releasing of inflammatory cytokines; thus, the real-time RT-PCR was further conducted, and the relative expression of the nf-xb and p53 was measured. The results in Figure 6 showed that the relative expression of the nf-xb and p53 in the model group was higher than that of the control group, with P < 0.005. The hydrogels could obviously inhibit the relative expression of the nf-xb and p53 in a dose-dependent manner.

4 Conclusion
To sum up, a fresh three-dimensional Zn(n) compound has been triumphantly created via reactions of hydrothermal self-assembly between Zn(OAc)_{2} and 2-mercapto nicotinic acid. By reducing L^{2−} and Zn(n) ions into 3-linked nodes, such three-dimensional framework can be reduced into a three-linked etb-type topological net with (8^{3}) point symbol. Its intense blue-green luminescence indicates that the complex may act as a good photoactive material. In order to better control drug release, HA/CMC hydrogels loaded with atenolol drug metal-organic framework were prepared by the chemical synthesis method. SEM showed that the hydrogel had a highly porous morphology, and the pore size decreased with the increase in CMCS concentration. The results of the ELISA detection showed that the hydrogels could significantly reduce the release of sVCAM-1 and TNF-a into the serum. In addition to this, the levels of inflammatory response in the vascular endothelial cells were also inhibited by the new hydrogels in a dose-dependent manner. In the end, we draw this conclusion, and the hydrogels could be an excellent candidate for the treatment of hypertension during pregnancy by reducing the release of sVCAM-1 and TNF-a into the serum.

Conflict of interest: Authors state no conflict of interest.

References


