Nexo Revista Científica
Vol. 34, No. 01, pp. 112-119/Marzo 2021

ISSN-E 1995-9516
Universidad Nacional de Ingeniería

http://revistas.uni.edu.ni/index.php/Nexo
https://doi.org/10.5377/nexo.v34i01.11290

Synthesis of an organic metal nanoporous structure for controlled azathioprine delivery

Síntesis de una estructura nanoporosa de metal orgánico para el suministro controlado de azatioprina

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(recibido/received: 03-January-2021; aceptado/accepted: 07-February-2021)

ABSTRACT

New drug delivery systems are highly efficient in diseases diagnosis and treatment and also controlled release of drugs. The use of this technology has given rise to the invention of new porous nanoparticles which are called metal organic frameworks (MOFs). In the present research, a kind of MOFs with formula $Cu_3(BTC)_2$ (HKUST-1, BTC ¼ benzene-1,3,5-tricarboxylate) with Fe_3O_4 magnetic nanoparticles (MNPs) as a core have been able to create three-dimensional magnetic porous structures. This magnetic and porous structure and the pores capability in being controlled have made these frameworks to be used as one of the best carriers in drug delivery. This system could magnetically be directed to the considered point inside body if it contains a drug that has side effects and may harm other body organs. Azathioprine is used in rheumatoid arthritis, granulomatosis with polyangiitis, Crohn's disease, ulcerative colitis, systemic lupus erythematosus, and in kidney transplants to prevent rejection. However, in the present work we consider the drug injection for kidney transplants to prevent rejection.
Keywords: MOF, Nanoporous structure, MNPs, Drug release, Azathioprine.

RESUMEN

Los nuevos sistemas de administración de fármacos son altamente eficientes en el diagnóstico y tratamiento de enfermedades y también en la liberación controlada de fármacos. El uso de esta tecnología ha dado lugar a la invención de nuevas nanopartículas porosas que se denominan estructuras organometálicas (MOF). En la presente investigación, una especie de MOF con fórmula Cu3 (BTC) 2 (HKUST-1, BTC ¼ benceno-1,3,5-tricarboxilato) con nanopartículas magnéticas (MNP) de Fe3O4 como núcleo han podido crear tres- estructuras porosas magnéticas dimensionales. Esta estructura magnética y porosa y la capacidad de control de los poros han hecho que estas estructuras se utilicen como uno de los mejores vehículos en la administración de fármacos. Este sistema podría dirigirse magnéticamente al punto considerado dentro del cuerpo si contiene un medicamento que tiene efectos secundarios y puede dañar otros órganos del cuerpo. La azatioprina se usa en artritis reumatoide, granulomatosis con poliangeítis, enfermedad de Crohn, colitis ulcerosa, lupus eritematoso sistémico y en trasplantes de riñón

para prevenir el rechazo. Sin embargo, en el presente trabajo consideramos la inyección de fármacos para trasplantes de riñón para prevenir el rechazo.

Palabras clave: MOF, estructura nanoporosa, MNP, liberación de fármaco, azatioprina.

1. INTRODUCTION

Magnetic nanoparticles (MNPs) are responsive to external magnetic field. The size, compositions and the synthesis procedure of these magnetic nanoparticles are different according to their application. Super paramagnetic nanoparticles have tremendous applications in drug delivery and recently have drawn significant attention among pharmaceutical chemists. These materials due to possessing high magnetic property could highly be affected by an external magnetic field and it has created a vast area in drug delivery as magnetic targeted carriers (Dobson, 2006; Goodwin, Peterson, Hoh, & Bittner, 1999; Huang, Lu, & Chen, 2017; Lübbe, Alexiou, & Bergemann, 2001; McBain, Yiu, & Dobson, 2008). Drug delivery systems based on nanotechnology because of the increase in the time of the drug presence in blood, the decrease in toxicity and the increase in the drug lifetime, have given rise to an underlying improvement in medicinal treatments (Hughes, 2017; Li & Mooney, 2016; Parveen, Misra, & Sahoo, 2012; Tibbitt, Dahlman, & Langer, 2016; Vader, Mol, Pasterkamp, & Schiffelers, 2016; Webber & Langer, 2017).

Although zeolite as a porous material has been very successful in industrial applications, they are not successful in drug delivery systems because their pores size is not enough small to accept tiny molecules (Colella, 2011). Accordingly, researchers are trying to overcome the problems and are challenging to modify it for medicinal applications (Ananthoji et al., 2011). Metal organic frameworks (MOF) could easily be designed to accept guest tiny molecules. Designing these materials generally seems to be an art than a science because in their construction process chemists are able to control the crystalline structure, pores size and chemical and physical properties. In addition, they alleviate the side effects of the active pharmaceutical ingredient (API) and can increase in the efficiency of drug. Accordingly, in recent years, they have been extensively investigated especially in the field of drug delivery. In addition to the above mentioned advantages, MOFs have very unified pores (Akhtar, Chen, AlDamen, & Tong, 2017; Deria, Yu, Balaraman, Mashni, & White, 2016; Janiak & Vieth, 2010; Lee, Kim, & Ahn, 2013; Reinsch et al., 2013; Shekhah, Liu, Fischer, & Wöll, 2011).

MOFs due to possessing organic and inorganic parts in their structure provide underlying advantages in drug delivery such as biocompatibility, loading high amounts of drug and controlled drug release. One of the other goals of developing these systems is to obtain a system which has the lowest toxicity in body (Gupta et al., 2019; Ibrahim, Sabouni, & A Husseini, 2017; Lazaro & Forgan, 2019; Orellana-Tavra, Köppen, Li, Stock, & Fairen-Jimenez, 2020; Park, Kim, Murray, Koo, & Kim, 2017; Xie et al., 2018; Xue et al., 2019). In the present work we synthesize $Cu_3(BTC)_2$ (HKUST-1, BTC ¼ benzene-1,3,5-tricarboxylate) encapsulated with Fe_3O_4 MNPs ($Fe_3O_4/Cu_3(BTC)_2$) and load azathioprine inside it in order to inject for kidney transplants to prevent rejection. To the best of our knowledge, this is the first application of a MOF for azathioprine delivery.

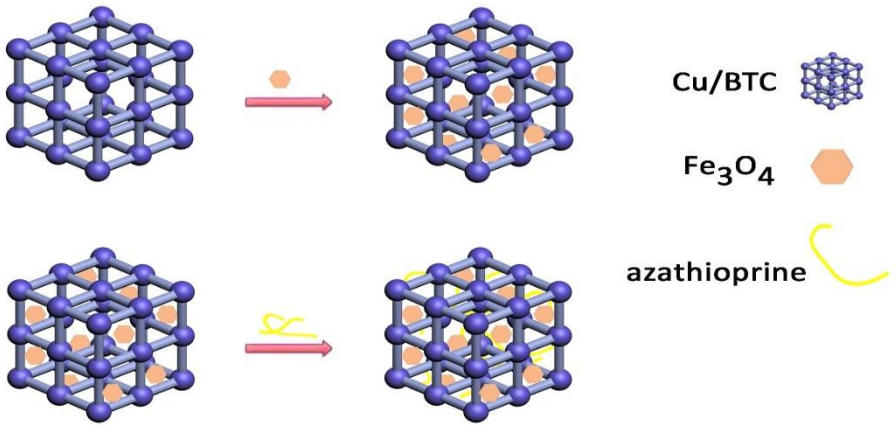

Figure 1. The synthesis of $Fe_3O_4/Cu_3(BTC)_2$ MOF and loading azathioprine inside it.

2. EXPERIMENTAL

General: $Cu(CO_2CH_3)_2$, $FeCl_2$, $Fe_2(SO_4)_3$, DMSO, dimethylformamide, $NaHCO_3$, ethylenediaminetetraacetic acid, sodium azide, Na_2HPO_4-$7H_2O$, ethanol, benzene-1,3,5-tricarboxylic acid and azathioprine were purchased from Merck, Fluka and Aldrich. FE-SEM images were obtained on a Sigma Zeiss. XRD measurements were carried out using a JEOL JDX–8030 (30 kV, 20 mA). UV/VIS Spectroscopy was obtained by PerkinElmer lambda 25 uv/vis spectrometer.

Preparation of phosphate buffer: 1000 mL of distilled water in a suitable container was prepared. 7.8 g NaCl, 1.82 g Na_2HPO_4-$7H_2O$ and 0.23 g $NaH_2PO_4H_2O$ were dissolved in the solution. The solution pH was adjusted by HCl in 7.4.

Preparation of dialysis container: Dialysis container was submerged into a solution containing 2% $NaHCO_3$ and 0.05% ethylenediaminetetraacetic acid and boiled for 10 minutes. After cooling it remained inside sodium azide for 4 hours.

Synthesis of Fe_3O_4 MNPs: $FeCl_3 \cdot 6H_2O$ and $FeCl_2 \cdot 4H_2O$ with molar ratio of 1:2 were dissolved in ethanol in a 25 cc suitable container. Then, NaOH solution (3 $mol \cdot L^{-1}$) was loaded into the solution by using of a peristaltic pump and stirred magnetically for 30 min. the final pH was 10. Next, the sodiumcitrate and oleic acid were respectively added into the suspensions to modify the obtained Fe_3O_4 MNPs for 12h.

Synthesis of $Fe_3O_4/Cu_3(BTC)_2$ MOF: 0.5 g H3BTC was dissolved in 80 mL ethanol and 0.1 g Fe_3O_4 MNPs were added inside the solution. In this stage the color of the solution changed into orange. Then, 0.68 g $Cu(OAc)_2$ was added to the solution and heated for 4 h on a heater. The obtained solution was washed by distilled water and ethanol. The solution was centrifuged and the product was separated. It was placed inside oven at 80 ^oC for half an hour.

Azathioprine loading inside the MOF: The loading process was carried out to insert drug particles inside the synthesized magnetic MOF pores. Because azathioprine in 14 ppm has UV absorption, this amount of the drug was dissolved in a solution containing distilled water and ethanol in a suitable 20 mL container. Then, 3 flasks with the drug to nanocarrier ratio of 2:1, 1:1 and 1:2 were prepared to obtain three different concentrations of drug and MOF in order to find out the best ratio for the loading. The three flasks were shaken on a shaker with 350 rpm at room temperature. After three days, the loading capability of the three

samples was investigated by UV spectroscopy. Next, the products were dried at 70 ^{o}C for 24 h. This temperature doesn't damage the drug because its decomposition temperature happens at more than 200 ^{o}C.

Drug release: A suitable amount of the three obtained samples were loaded into the prepared dialysis container and it was immersed inside a falcon 30 mL centrifuge containing phosphate buffer. The falcon tube was centrifuged with 350 rpm at 37 ^{o}C. The samples are cationic because of the drug presence. Hence, these cations could be replaced with phosphate buffer cations, which gives rise to the extraction of the drug from the samples structure. To avoid this problem, 3 mL of phosphate buffer was taken and a same amount of fresh buffer was replaced. The release profile was obtained by soaking the samples in 90 mL of a simulated body fluid, SBF (1 mg of azathioprine of the three prepared samples per mL of fluid), and measuring the drug concentration in the fluid by means of a UV-vis spectrophotometer.

3. RESULTS AND DISCUSSION

SEM images were used for showing nanostructured surface of both Fe_3O_4 MNPs and $Fe_3O_4/Cu_3(BTC)_2$ (Fig. 2). Because of $Cu_3(BTC)_2$ coating surrounded by Fe_3O_4 MNPs, MOFs are observed as cubic particles (Fig. 2, E and F). Furthermore, particle size distribution chart was obtained and it was realized that the particle size distribution is narrow and the size of most of the particles is about 15-20 nm.

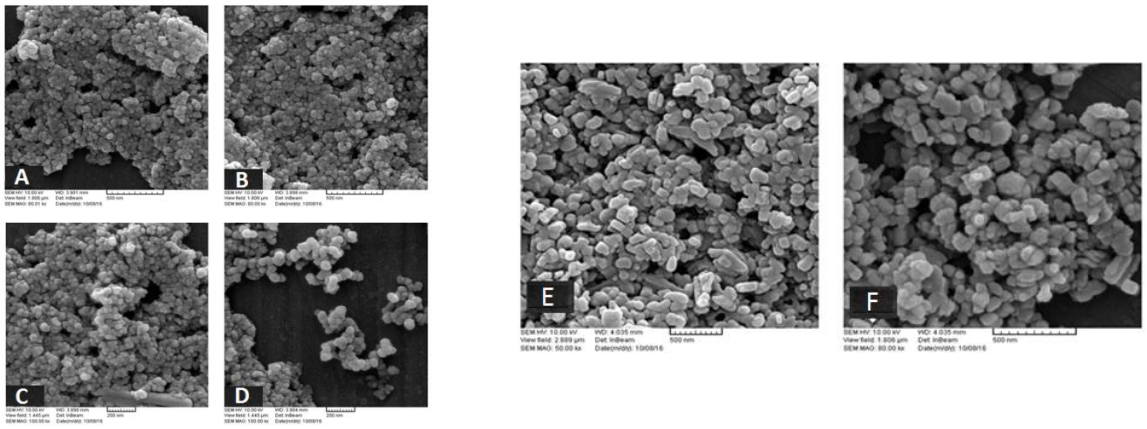

Figure 2. SEM images of Fe_3O_4 MNPs (A-D) and $Fe_3O_4/Cu_3(BTC)_2$ (E and F).

FT-IR spectroscopy for both $Fe_3O_4/Cu_3(BTC)_2$ and azathiaprine were obtained for charachterization (Fig. 3). An intense peak at 3400 to 3600 cm^{-1} is related to the water absorbed by the sample. The peak at 1500-1600 cm^{-1} is related to C-C stretching vibrations. The peak appeared at 1550-1610 cm^{-1} is related to CO-OH (carboxylic acid) indicating connecting factors in MOFs. The appeared peaks at 1395-1405 cm^{-1} are related to C-O stretching vibrations. The peaks at 1350-1450 cm^{-1} show S=O (sulphate) bond. The peaks at 1400-1500 cm^{-1} are related to aromatic rings. The peaks at 1615-1700 cm^{-1} are shown C=N bond. N=O peak could be seen at 1500-1600 cm^{-1}. Stretching C-H vibrations are appeared at 2850-3000 cm^{-1}.

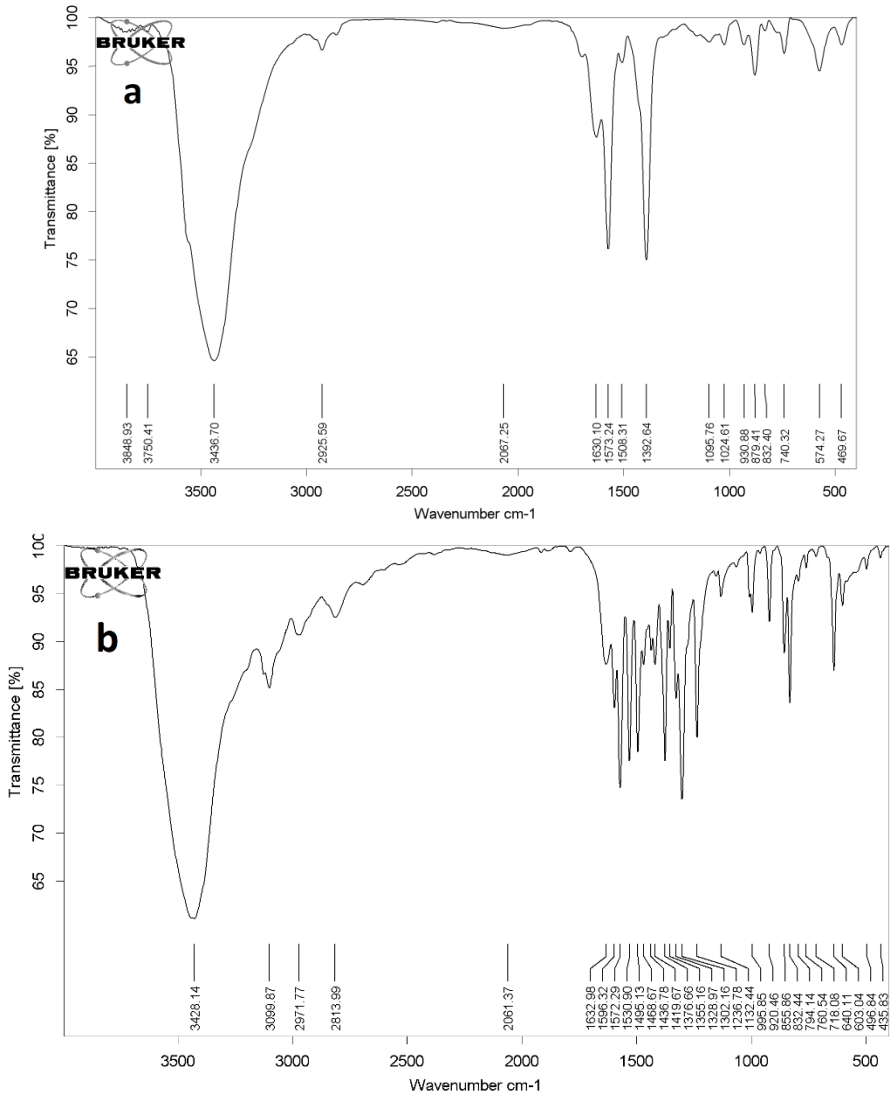

Figure 3. FT-IR spectrum of a) $Fe_3O_4/Cu_3(BTC)_2$ and b) azathioprine.

The effective loading of azathioprine by the MOF MNPs when immersed into the hexane solution of the drug can be monitored by TGA, which indicates a maximum of 37 wt % of azathioprine. Comparing a and b diagrams in Fig. 4, it can be said that after drug loading TGA diagram shows 37 wt % loss which is due to the decomposition of the drug in temperature ranging from 300 to 400 ^{o}C. It can be concluded that this weight loss in this range of temperature confirms the effective uptake of azathioprine in the synthesized nanocarrier.

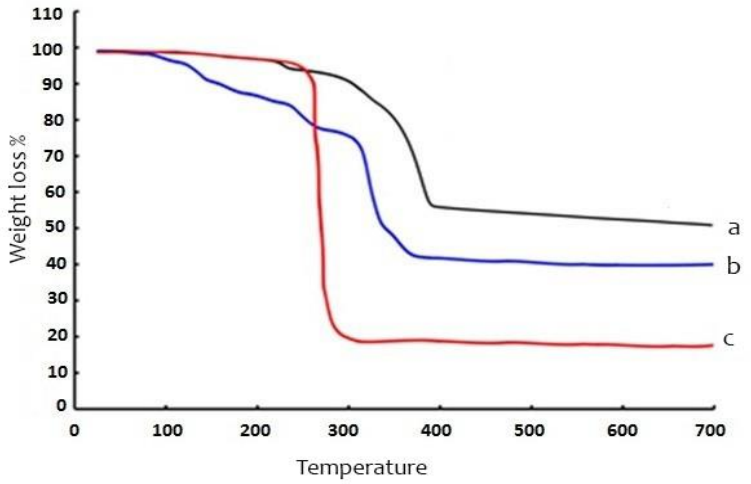

Figure 4. TGA diagram of a) before drug loading, b) after drug loading and c) azathioprine.

Fig. 5 shows the percentage of azathioprine release as a function of time for the $Fe_3O_4/Cu_3(BTC)_2$ with the three obtained samples with the drug to nanocarrier ratio of 2:1, 1:1 and 1:2 loaded with azathioprine. As it can be seen from the release diagrams, by increasing the nanocarrier ratio the drug release conditions improve. According diagram c, it can be said that it releases the loaded drug gently and with high percentage (85%). The drug to nanocarrier ratio of 1:1 (Fig. 5, b) has a medium release with medium percentage (71%) and for the ratio of 2:1 (Fig. 6, a) the highest release percentage happens after 72 h (49%). The azathioprine release in drug to nanocarrier ratio of 1:2 (Fig. 5, c) increases by passing the time and reaches to its highest point after 100 h. It is noteworthy that, after releasing azathioprine the XRD of $Fe_3O_4/Cu_3(BTC)_2$ was obtained and it showed no loss of structural ordering.

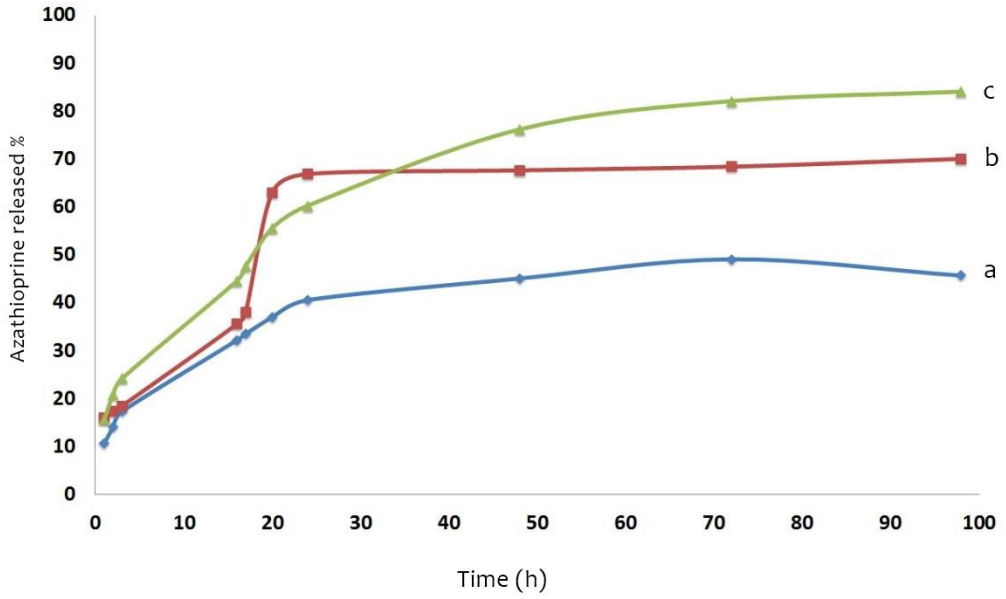

Figure 5. Azathioprine % release from $Fe_3O_4/Cu_3(BTC)_2$ in drug to MOF ratio of a) 2:1, b) 1:1 and c) 1:2.

4. CONCLUSION

We developed an interesting MOFs property which is their capability for accepting and delivering organic compounds. For the first time, we used MOF MNPs as nanocarrier for azathioprine delivery. The used nanocarrier was $Fe_3O_4/Cu_3(BTC)_2$ which was synthesized in a facial way. We showed that the synthesized MOF pore size was 2.1 nm and the size of the azathioprine molecules estimated to be ≈1.4 × 0.7 nm. Hence, the drug molecules could fit inside the MOF pores. The drug loading was monitored by TGA and it was estimated to be 37%. The percentage of azathioprine release was investigated by SBF and UV which in the optimized conditions after 100 h it reached to 85%. This MOF is magnetic and could be directed to the considered part of patient body then start to release the drug to avoid the drug side effects and possible harms to other parts of body. As the release process takes place gently during 4 days magnetically, this drug delivery system should be considered as one of the best candidates in azathioprine delivery.

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