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Zirconium-Based Metal-Organic Framework Impregnated With Silver Nanoparticles (AgNPs@MOF-808) As The Anticancer Drug Delivery System

A. Zulys[1], N. Hanifah[1], A. Umar[1], A.D.M. Satya[1], A. Adawiah[2]*

[a]*Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Indonesia*
Jl. Lingkar Kamus Raya, Podok Cina, Beji, Depok, Jawa Barat 16242, Indonesia
[b]*Integrated Laboratory Centre, Faculty of Science and Technology, UIN Syarif Hidayatullah Jakarta*
Jl. Ir. H. Juanda No. 95 Ciputat Tangerang Selatan, Banten 15412, Indonesia

Abstract

Tamoxifen citrate (TMC) as a breast cancer drug has side effects at certain doses; hence a drug delivery system is needed. Zirconium-based metal-organic framework with benzene-1,3,5-tricarboxylic acid linkers (MOF-808) impregnated with silver nanoparticles (AgNPs) can form AgNPs@MOF-808 nanocomposites which is a potential candidate to be the drug delivery system. By carried out the synthesis using the solvothermal method, MOF-808 impregnated with AgNPs is having various topolog rather than MOF-808 without the impregnation. MOF-808 (72h) generated the highest crystallinity with the particle size of nano-MOF scale, ranging from ±77-277 nm. MOF-808 was then used as a template for the impregnation of Ag+ into AgNPs with various concentrations of AgNO3 (0.01; 0.05; 0.2; and 0.4 mmol) using DMF as a mild reductant. AgNPs@MOF-808 (0.4) produced several AgNPs at most which were scattered in the MOF pores, with ±5 nm AgNPs nanospherical size and ±130nm of octahedral MOF. The synthesized products were characterized using FT-IR, XRD, SEM-EDX, UV-Vis, and TEM. The anticancer drug delivery system was investigated through in vitro. MOF-808 and AgNPs@MOF-808 had a drug loading efficacy of 55.25% and 44.94% after 72 hours, respectively. Drug release efficacy of MOF-808-TMC reached 73.5% while AgNPs@MOF-808-TMC reached 77.1% on dialysis for 36 hours.

Keywords: AgNPs; MOF-808; tamoxifen citrate; Zr-BTC, drug delivery system

1. Introduction

The Global Cancer Observatory stated that in 2018 there were 18.1 million new cases of cancer in the world with a figure of deaths up to 9.6 million deaths, of which there is that 1 in 6 women in the world suffers from cancer and 1 in 11 women die from cancer. According to World Health Organization, WHO, breast cancer in 2.1 million women in the world every year and causes a high mortality rate with a 15% of breast cancer deaths among women.

Breast cancer treatment has been developed by giving drugs, irradiation therapy, targeted therapy, and chemotherapy. One of the main drugs in breast cancer treatment from early stage to long-term treatment is tamoxifen citrate since it provided an antiestrogenic effect. However, the use of tamoxifen citrate at certain doses will give side effects that are quite harmful to the body and damage healthy cells. Targeted therapy in cancer using drug delivery systems can minimize side effects and allows drug release controlled, prolonged, and targeted dose treatment, hence effectively kills cancer cell tissue without damaging healthy cells.

The metal-organic framework (MOF) is being developed as a drug delivery system [1]. MOF is a crystalline solid metal ion coordinated with organic linkers form a porous structure with high porosity. One of the potential MOFs as a drug delivery system is a zirconium-based MOF because it has a chemical stability in water and acidic conditions, also biocompatible [2]. Zr (IV) terephthalate has been introduced as an encapsulation system of caffeine and ibuprofen through their structural activity [3]. Zr(IV) 1,4-benzene dicarboxylate (Zr-BDC) also has been investigated which exhibited size of 50-600 nm as a carrier for anticancer drugs. A drug loading test has been conducted for ibuprofen on Zr-BDC [4].

In this study, a zirconium-based MOF synthesis has been carried out. Zr with 1,3,5-benzene tricarboxylate (MOF-808) impregnated with silver nanoparticles

*Corresponding author e-mail: adawiah@uinjkt.ac.id.
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(AgNPs) used as an anticancer drug delivery system (nanocarrier) for tamoxifen citrate. The synthesis result and, AgNPs@MOF-808, and its drug loading activity were confirmed using FT-IR, XRD, SEM-EDX, TEM, and UV-Vis spectroscopy.

2. Experimenital Works

2.1. Materials

Zirconium (IV) chloride ($ZrCl_4$), acetic acid (CH_3CHOOH), trimesic acid (H_3BTC), silver nitrate ($AgNO_3$), N,N-dimethylformamide (DMF), hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium acetate (CH_3COONa), sodium chloride (NaCl), sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), tamoxifen citrate, distilled water, dichloromethane (DCM), methanol (MeOH), ethanol (EtOH), and acetone have been used in this study.

2.2. Preparation of MOF-808

MOF-808 is prepared using a development method of Xu and co-workers [5]. $ZrCl_4$ was dissolved in the acetic acid and sonicated for 30 minutes. H3BTC as the linker then dissolved in the DMF and sonicated for 30 minutes. Two mixtures with mole ratio between $ZrCl_4$:H_3BTC were set for 3:1 and reacted in the Pyrex glass with a screw cap and sonicated for 30 minutes. The synthesis has been carried out with solvothermal method at 120 °C. An oven was used with varying reaction times of 24, 48, and 72 hours. After the heating process, the sample allowed to cool to room temperature for 24 hours. The white precipitate was separated and collected by centrifugation and further washed with methanol twice to remove excess reactants. Samples are filtered and dried at room temperature. The activation was conducted in chloroform for 24 hours toward the sample for 24 hours at a temperature of 50 °C thrice and heated at 120 °C for 24 hours in the oven.

2.3. Preparation of AgNPs@MOF-808

AgNPs@MOF-808 is prepared using a development method of Han and co-workers [6]. The synthesized MOF0-808 and $AgNO_3$ with various concentrations of $AgNO_3$ were conducted by dispersing them in 20 mL of DMF solvent at room temperature and stirred for 8 hours. After that, the samples were washed with DMF twice to remove the excess precursors. The resulting solid product was then dispersed in 20 mL DMF as Ag^+ reducing agent to form AgNPs and heated in an oven at 140 °C for 4 hours. The sample then allowed to cool to room temperature. AgNPs@MOF-808 powder was obtained by centrifugation and washed with DMF twice. DMF was removed by decantation and the product was dried at 80 °C under vacuum for one night.

2.4. Drug Loading Experiment

The stock solution of tamoxifen citrate 1000 ppm was prepared by dissolving 100 mg of tamoxifen citrate in 100 mL of DCM. The stock solution was diluted to 5, 10, 15, 20, and 25 ppm as standard series. This standard series solution was characterized by UV-Vis spectrophotometer to see the absorbance equation at max and concentration as well as linear regression of the standard curve.

Drug loading process of tamoxifen citrate (TMC) by MOF-808 and AgNPs@MOF-808 was carried out by immersion for 3 x 24 hours at a room temperature in a dark and closed system of each 25 mg of MOF-808 and AgNPs@MOF-808, respectively in 5 mL stock solution of 1000 ppm tamoxifen citrate and added 5 mL ethanol (2.5 mg/mL). The MOF-808-TMC and AgNPs@MOF-808-TMC products were washed with DCM and the suspension was collected by centrifugation at 6,000 rpm. Samples were stored in the dark system at 4 °C. The solid sample was then characterized by FT-IR to observe the chemical interactions that exist in the product compounds. The supernatant sample was diluted with a dilution factor of 10 and characterized by a UV-Vis spectrophotometer to analyze the concentration of drug that was not absorbed by the material.

2.5. Drug Release Experiment

The drug release process for tamoxifen citrate was carried out by dialysis technique [7]. Dialysis tube containing 5 mL of acetate buffer solution pH 5 as a reservoir for the pH conditions of cancer cells was added as much as 10 mg of test material (2 mg/mL). Dialysis was carried out in a glass beaker containing simulated body fluid using phosphate buffered saline (PBS) with a pH of 7.4 as the cell environment medium and heated at 37°C then stirred at low rpm. Sampling was carried out at 36 hours intervals and 5 mL of PBS solution was taken every 6th, 12th, 18th, 24th, 30th, and 36th hour. The samples were characterized by UV-Vis spectrophotometer at a wavelength of 200-400 nm [8].

3. Results and Discussion

The synthesis of MOF-808 was carried out by the solvothermal method. The solvothermal method is the option among other methods since it generated MOF particles with uniform size and shape, high crystallinity and purity, small and wide distribution of crystal nuclei [9] and provides relatively fast crystal growth and product with high surface area [10].

Impregnation of silver nanoparticles (AgNPs) on MOF-808 was carried out with variations in $AgNO_3$ concentration of 0.4; 0.2; 0.05; and 0.01 mmol with the maximum ratio of $AgNO_3$:MOF-808 is 1:1 w/w. The MOF-808 used is MOF-808 (72h) because it produces the best crystallinity, nano-MOF-scale

particle size, and has a high surface area and pore volume. The impregnation method with the reduction process of Ag^+ silver ions to AgNPs in MOF was chosen because the growth of silver nanoparticles that occurred in the MOF pores would be able to adjust to the MOF pore size, compared with impregnation by post-synthesis method of AgNPs and MOF.

AgNPs formation on MOF-808 framework confirmed using UV-Vis spectrophotometer characterization (Figure 1). UV-Vis spectroscopy measures the absorbance of a chemical compound to light emission in the wavelength range of 200-800 nm, where the maximum absorbance value obtained at a certain wavelength can be specifically detected for a compound product.

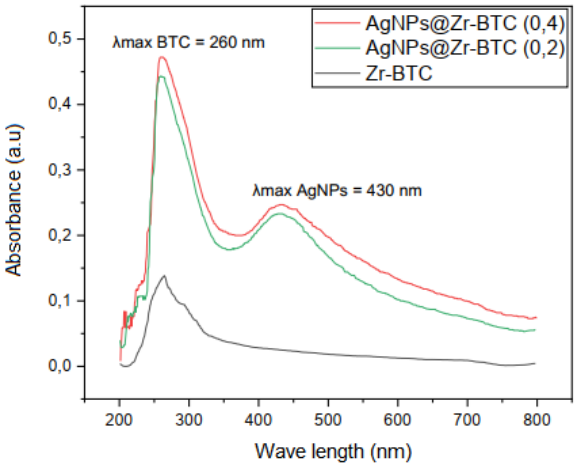

Figure (1) Absorbance spectrum of Zr-BTC or MOF-808, AgNPs@MOF-808 (0.2), and AgNPs@MOF-808 (0.4)

The maximum Localized Surface Plasmon Resonance (LSPR) of AgNPs is seen at a wavelength of 430 nm in the UV-Vis absorption spectrum of AgNPs@MOF-808. In the absence of AgNPs in the MOF, there is no absorbance peak in the 350–800 nm wavelength range. On the other hand, there was no peak at other max indicating no formation of other compounds in the synthesis product. This proves the reduction of silver ions Ag^+ to AgNPs and impregnated on MOF-808. max 260 nm is the absorption of the benzene ring on the BTC ligand. This is in accordance with the research of Han and co-workers who impregnated AgNPs on UiO-66 for the application of cancer cell apoptosis induction [6].

XRD characterization also carried out to observe the crystallinity of the AgNPs@MOF-808 structure. Figure (2) shows the AgNPs@MOF-808 diffractogram which is also compared with the synthesized MOF-808 diffractogram.

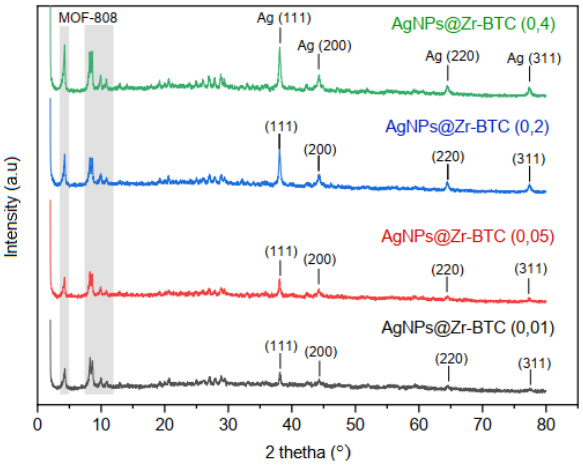

Figure (2) Diffraction pattern or diffractogram of AgNPs@MOF-808 and MOF-808

In the diffractogram, it is observed that there is no change in the diffraction pattern in the Zr-BTC (MOF-808) structure, namely at 2θ 4.3°, 8.6°, and 10° angles. This proves that the reduction process by DMF does not damage the MOF structure [11]. The intensity of the MOF-808 diffraction pattern is relatively lower than before due to the increase in collisions between particles. Diffraction of metallic silver which was later identified as AgNPs appeared at 2θ 38.1°, 44.3°, 64.4°, and 77.4° (according to JCPDS no. 84-0713) with slightly widened peaks and low intensity. This indicates the presence of Ag in the MOF with nanoparticle size [6]. There was no addition or change in the diffraction pattern of MOF-808 or AgNPs, which means that the two particles were mixed, there was no chemical bonding, explaining that the two interacted physically to form nanocomposites.

The drug loading process is carried out by encapsulating biomedical agents through the post-synthesis method on MOF-808 and AgNPs@MOF-808. Substances carried by MOF can be drugs and active targeting agents. The drug encapsulation strategy can be carried out in three ways, namely surface adsorption, pore encapsulation, and covalent bonding [12]. The presence of covalent or non-covalent interactions that are formed, makes MOF can be release the drug slowly and continuously. The drug loading capacity of the MOF material is highly dependent on the pore size, particle size, and stability of the MOF. MOF with a particle size of about 100 nm as a drug delivery system is relatively easily targeted to cancer tissue and functions well, this aims to avoid the clearance mechanism of white blood cell macrophages by the liver [13].

Drug loading is done by immersing MOF-808 and AgNPs@MOF-808 (2.5 mg/mL) each in 1000 ppm TMC drug stock solution for 72 hours. Ethanol is added as a solvent which helps the drug loading process into the MOF pores, impregnation of porous

materials in ethanol solution is proven to be able to incorporate drugs efficiently [1].

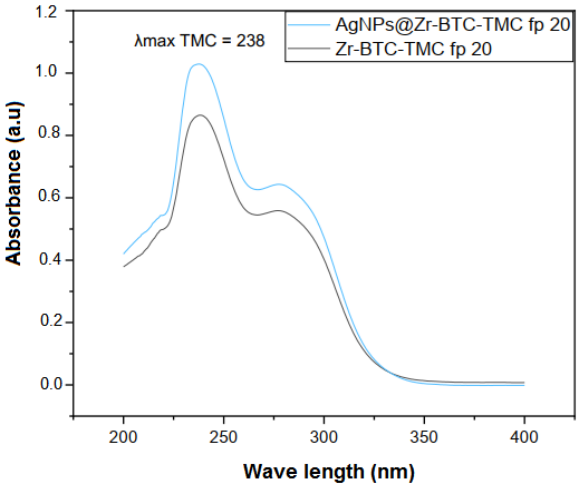

Figure (3) Absorbance spectrum of drug loading supernatant from MOF-808-TMC and AgNPs@MOF-808-TMC with the dilution factor of 20

Figure (3) shows the UV absorption spectrum at a wavelength of 200-400 cm^{-1} as a result of UV-Vis spectrophotometer characterization on TMC supernatant. TMC was observed to be at 238 nm. This value is close to the results of research by Albert and co-workers[7] with max TMC which is 236 nm. Maximum absorbance obtained from the sample supernatant AgNPs@MOF-808-TMC is higher than MOF-808-TMC, this indicates that the concentration of unabsorbed TMC AgNPs@MOF-808 is higher than MOF-808. It can be interpreted that MOF-808 absorbs more TMC drugs than AgNPs@MOF-808, because the presence of impregnated AgNPs in the MOF partially fills the pores of the MOF thus providing a smaller space for the TMC loading process. Comparison of concentration and mass of drug loading of the two samples (per 2.5 g/l of material) can be seen in Table (1).

Drug release is carried out in vitro with the dialysis technique using a dialysis tube as a reservoir for samples of MOF-808-TMC and AgNPs@MOF-808-TMC containing acetate buffer solution pH 5 (2 mg/mL) as a cell membrane simulation. Cell environment simulation using phosphate buffered saline (PBS) pH 7.4 at 37 °C. Dialysis was carried out for 36 hours and drug release samples (PBS solution) were taken every 6 hours. Samples of PBS solution resulting from drug release every time interval characterized by UV-Vis spectrophotometer at a wavelength of 200-400 nm. Figure (4) shows the UV absorption spectrum in the drug release process of MOF-808-TMC and AgNPs@MOF-808-TMC. max TMC in PBS pH 7.4 is known to be at 208 nm. TMC absorbance was observed to increase with increasing dialysis time.

Table (1) Drug loading capacity

Material	TMC loading (mg/L)	TMC loading mass (g)	TMC loading capacity (%)
Zr-BTC-TMC	552.53	0.0138	55.25
AgNPs@Zr-BTC-TMC	449.37	0.0112	44.94

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The release of TMC from the dialysis tube into the cell medium (PBS solution pH 7.4) took place slowly (slow release). This is due to the diffusion process of PBS solution. During the dialysis process, the PBS solution will diffuse from the simulated cell medium as an external environment into the dialysis tube and then into the core of the MOF material to achieve equilibrium conditions. The release of TMC through two physical barriers, namely MOF and cell membrane simulation (dialysis tube), so it takes a little more time to get to the cell medium. When dialysis was stopped at 36 hours, the drug release capacity reached 73.5% (10.15 mg) and 77.1% (8.66 mg) of MOF-808-TMC and AgNPs@MOF-808-TMC, respectively. The concentration of TMC in PBS increased with increasing dialysis time (Figure 5).

Release of TMC from MOF-808-TMC and AgNPs@MOF-808-TMC at the time interval difference takes place continuously (sustained release). The release of TMC in the first 6 hours was observed to be fast, 58.7% (8.11 mg) by MOF-808-TMC and 64.73% (7.27 mg) by AgNPs@MOF-808-TMC.

The release capacity of TMC by AgNPs@MOF-808 is higher than that of MOF-808, due to the presence of AgNPs which reduces the interaction of TMC with MOF and the presence of TMC attached to

the surface of nanoparticles may accelerate the efficiency of TMC release. Burst effect or this rapid release occurs in the initial release, caused by the TMC faction adsorbed on the surface or close to the surface of the MOF or nanoparticles [14].

Burst release can also be caused by the small particle size of the material so that it has a larger surface area compared to the volume ratio, which then stimulates a rapid release [15].

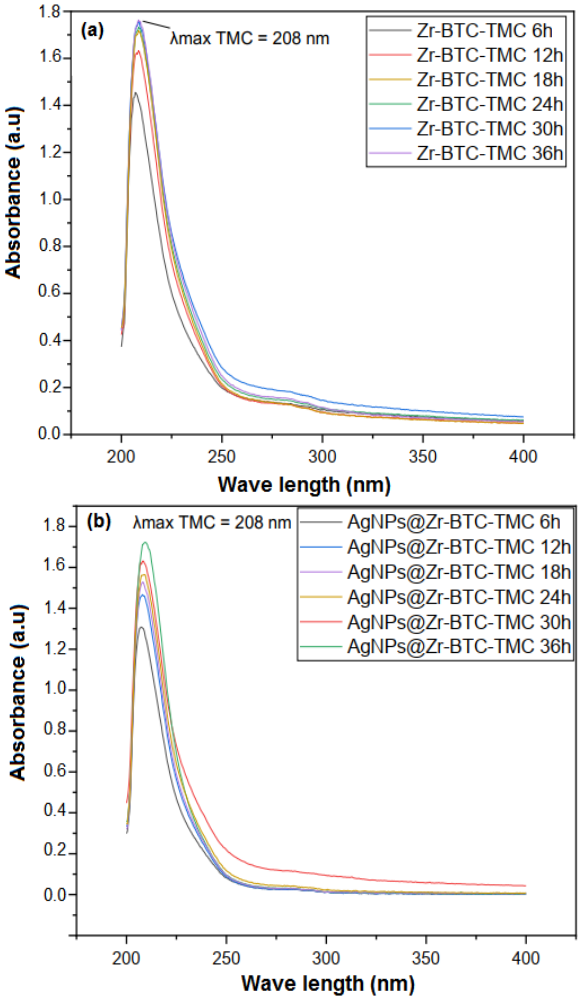

Figure (4) Drug release absorbance spectrum of: a) MOF-808-TMC and b) AgNPs@MOF-808-TMC

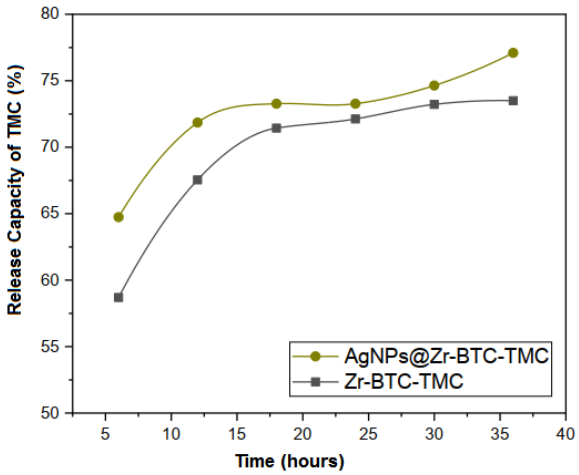

Figure (5) Drug release profile of MOF-808-TMC and AgNPs@MOF-808-TMC

Over time, TMC release occurs more slowly and controlled during dialysis. This is because the TMC diffusion process from inside the MOF pores, exits the material and is in the dialysis tube, then to the cell medium, which initially interacts chemically and physically with MOF-808 and AgNPs@MOF-808. % Drug release capacity based on the calculation, the comparison of MOF-808-TMC and AgNPs@MOF-808 data obtained during the TMC release period of 6, 12, 18, 24,30, and 36 hours as shown in Table (2) below.

Table (2) Drug release capacity

Material	TMC realease capacity (%)					
	6h	12h	18h	24h	30h	36h
Zr-BTC-TMC	58.70	67.54	71.43	72.12	73.22	73.50
AgNPs@Zr-BTC-TMC	64.73	71.85	73.27	73.27	74.64	77.10

4. Conclusion

To sum up, variations in reaction time affect the crystallinity and particle size of MOF, where MOF-808 (72h) has the highest crystallinity with a nano-MOF particle size, which is ± 77-227 nm, the size is in accordance with the characteristics as an anticancer drug delivery system. Synthesis of AgNPs@MOF-808 with the impregnation method of Ag+ into AgNPs was carried out with various concentrations of AgNO3. The largest concentration of $AgNO_3$, AgNPs@MOF-808 (0.4), obtained more widely impregnated AgNPs in the MOF pore with spherical AgNPs particles of size ±5 nm and MOF-808 in the form of an octahedron measuring ±130 nm. The application of the anticancer drug delivery system tamoxifen citrate was carried out in vitro. The drug loading capacity of MOF-808-TMC and AgNPs@MOF-808-TMC were 55.25% and

44.94% respectively at 72 hours immersion. The drug release capacity of Zr-BTC-TMC reached 73.5% and AgNPs@MOF-808-TMC reached 77.1% for 36 hours dialysis. The presence of AgNPs in MOF reduces the amount of loading of tamoxifen citrate and maintains good release efficiency.

5. Acknowledgement

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