International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Molecularly Imprinted Polymer Nanoparticles Synthesis for Penicillin G Antibiotic Detection

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Abstract

Penicillin G is one of the antibiotics found contaminating the water bodies leading to antibiotic resistant issue. In order to overcome this problem, we study a method employing polymer as the antibiotic capture agent. Nanomolecularly imprinted polymers was synthesized custom for Penicillin G detection by miniemulsion polymerization technique. Two different monomers were used and characterized for its morphology, elemental and binding. Both monomers, NAEMA and MAA produced nanoMIP with the average size of 39.7 nm and 15.4 nm, respectively. EDX elemental analysis confirmed the presence of carbon, nitrogen and oxygen which present in the polymer compound. FTIR analysis shows the removal of template during acetone wash. The synthesis technique successfully formed a nanoparticle polymer structure and by the FTIR result indicates that the template has been removed thus the nanoMIP synthesized can be used for further Penicillin G antibiotic detection studies.

Keywords: MIPs, NanoMIPs, Penicillin G, antibiotics, biomimetic

Full length article *Corresponding Author, e-mail: azahy@upm.edu.my

1. Introduction

Molecularly imprinted polymers (MIPs) are synthetic biomimetic polymers synthesized basically from monomer, crosslinker and template molecules. MIPs mimic the ability of biorecognition units such as antibodies, aptamers, enzymes, etc. in detecting its preordained target. MIP structure comprise of cavities with the ability to detect the target molecules depending on the template molecules used during synthesis. There are plenty of MIPs implementation in biosensor [1,2], chemical sensor [3,4], drug delivery[5,6], catalysis [7,8] and molecules separation [9,10]. MIPs are attracting the interest of researchers due to its specificity and selectivity, easy synthesis method and high yield as well as its affordability. They are frequently used for application in environmental, medical, pharmaceutical and food industry. MIPs can be synthesized in several formats which include nanoparticles, microparticles, film, and membrane [11]. MIP nanoparticles (NanoMIPs) exhibit more advantages over other types of MIPs. The nano size range of nanoMIP signify that it has a wide surface to volume ratio. This increases the accessibility for analyte of target to bind on the imprinted cavities of nanoMIP [12].

Antibiotics pollution in environment have been a major issue worldwide. With more than 100,000 tonnes of antibiotics usage per year, significant amount of it ended up in the water system with concentration over 450 µg/mL have been detected around Asia Pacific countries [13]. Antibiotics found in the water system possess major threat not only to the aquatic life, but to other animals consuming the water including human. According to World Health Organization, antibiotic resistance has become one of the biggest threats to the global health. Bacteria related diseases such as tuberculosis, pneumonia, salmonellosis and gonorrhea are becoming harder to treat since the antibiotic medicine used to treat the diseases has become less effective [14]. In 2019, more than 2.8 million antibiotic resistant infection with more than 35 thousand fatalities has been recorded in the US [15]. In the long run, the antibiotics that we currently use will no longer be able to treat bacterial infection. More potent, expensive medicine and longer treatment duration will be used thus will increase the cost of healthcare treatment. Therefore, antibiotics pollution in water should be addressed and water treatment solution for antibiotics pollution should be applied to avoid severe antibiotics resistance issue.

In this work, we are investigating the synthesis of nanoMIP for Penicillin G (PenG) detection using two different monomers. The synthesized nanoMIPs were characterized morphologically and chemically to ensure the formation of nanoMIP. The nanoMIP synthesized in this work will function to capture the PenG contamination in water thus helps to reduce the antibiotic pollution.

2. Materials and methods

2.1. Materials

NanoMIP synthesized in this work were produced using chemicals purchased from Sigma Aldrich, USA. Deionized water was used unless otherwise specified.

2.2. PenG-NanoMIP synthesis

There are two methods used to synthesis the PenG-nanoMIP. Inverse miniemulsion polymerization technique (inverse MiniEP) was used for both synthesis methods [16]. All the chemicals used are the same except for the monomer used in aqueous phase. Figure 1 illustrated the preparation process of the nanoMIP.

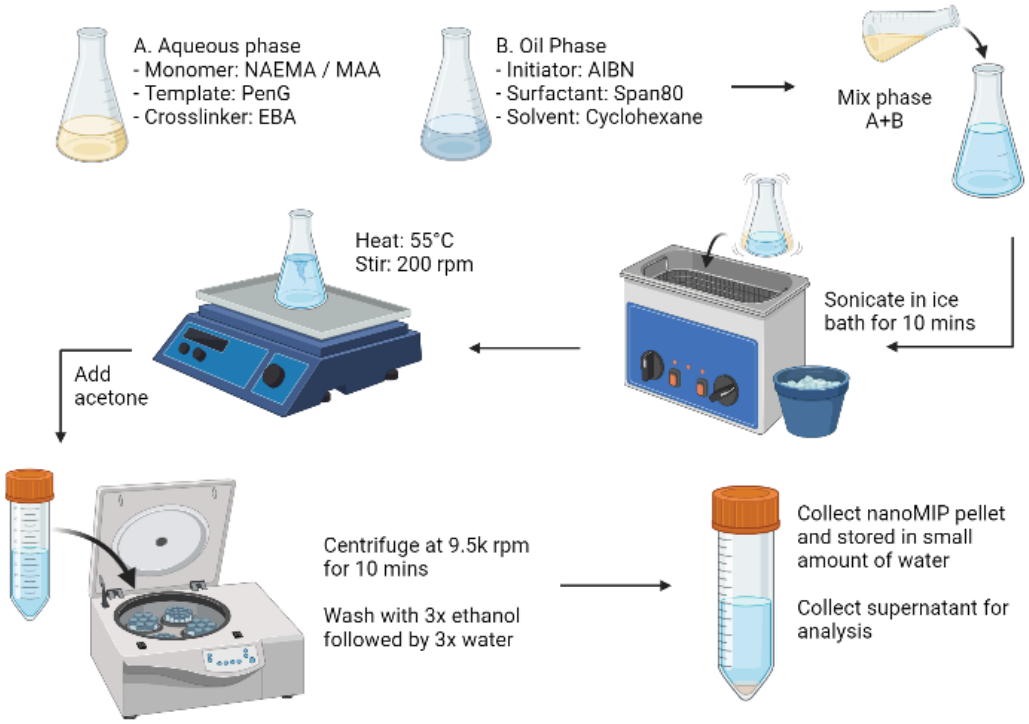

Fig. 1. PenG NanoMIP synthesis method using miniemulsion polymerization technique

For Method I, disperse phase (aqueous phase) was prepared using monomer; 0.715 g NAEMA (N-(2-aminoethyl) methacrylamide hydrochloride, 4.36 mmol), template; 0.036 g PenG (0.1 mmol ≙ 2.3 mol % relative to NAEMA) and crosslinker; 0.168 g EBA (N,N'-Ethylenebisacrylamide, 1.0 mmol). Then, the chemicals were dissolved in 750 µL PBS-Buffer (0.001 M phosphate, total ion concentration= 0.013 M) resulted in a slightly yellow colored transparent solution. For method 2, the monomer was replaced with MAA (Methacrylic acid, 4.36 mmol). The required continuous phase (oil phase) was prepared by dissolving 2.43 ml Span 80 and 26 ul AIBN (2,2'-Azobis(2-methylpropionitrile)) in 45 mL of cyclohexane. These two solutions were combined in a flask and thoroughly shaken by hand. Subsequently the desired emulsion was formed by sonication for 10 minutes. During sonication, an ice bath cooling was applied to prevent emulsion destabilization and premature initiation of polymerization. To prevent freezing of the emulsion (cyclohexane Tm ≈ 7°C) the ice cooling was removed immediately after completion of the sonication. The resulting emulsion was placed in a container containing a magnetic stir bar. The hot plate was set to a temperature of 55 °C to initiate the polymerization. At this temperature, the emulsion was agitated overnight and stirred with a speed of 200 rpm. The PenG-MIPs were purified by centrifugation. For this purpose, the emulsion was transferred into two polycarbonate centrifugation tubes (35 mL), broken by adding a sufficient amount of acetone and centrifuged at 9.5k rpm for 10 minutes. The supernatant was collected for analysis; the resulting pellets were redispersed in ethanol (35 mL) via sonication, shaken for 10 minutes and centrifuged. This procedure was repeated three times with ethanol and three times with water. The PenG-MIPs were stored in a small amount of water.

2.3. NanoMIP characterization

The synthesized nanoMIP was analyzed using FEI Nova Nanosem 230 Field Emission Scanning Electron Microscopy with Energy Dispersive X-Ray Spectroscopy (FESEM-EDX) for its morphological and elemental characteristics. The samples were deposited on the metal stub and sputter coated with titanium for analysis. Then, the nanoMIP was characterized using Nicolet 6700 Fourier Transform Infrared spectroscopy (FTIR).

3. Results and discussion

3.1. Synthesis of PenG-NanoMIP

PenG has been used as the template for nanoMIP synthesis for predestined PenG target detection in environmental sample. Miniemulsion polymerization technique has been used as the nanoMIP synthesis method. The synthesis mechanism of nanoMIP has been illustrated as in Figure 2. Monomer, crosslinker and template are the main chemicals used for synthesis.

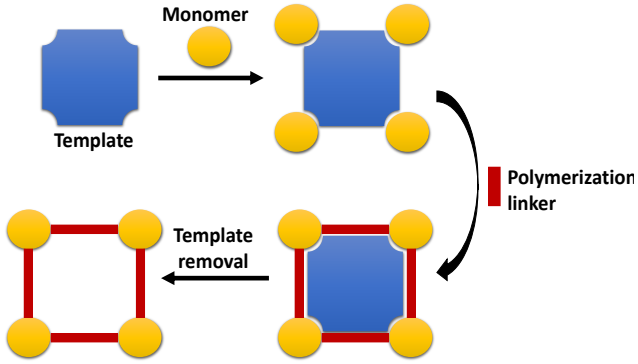

Fig. 1. Schematic of nanoMIP formation

3.2. FESEM-EDX characterization

NanoMIP was dispersed in DI water and left for the undispersed polymer to precipitate at the bottom. The remaining well dispersed polymer was dropped (10 µl) on metal stub and air-dried producing thin layer of nanoMIP sample. EDX analysis was conducted by direct analysis on the sample to confirm the elemental composition present on the sample. For analysis using FESEM, the sample was coated with a thin layer of titanium via sputtering deposition method to reduce charging during analysis since the polymer is a non-conductive sample. The nanoMIP was synthesized using miniemulsion technique. Firstly, to confirm the size of the polymer, samples were sent for nanosizer size characterization. The results from nanosizer are not consistent and exceed our estimated size. Then, the samples were sent for confirmation analysis using FESEM-EDX to measure the size and to check the elemental composition of the nanoMIP. Morphological characterization using FESEM shows presence of round shape nanoMIP particles with the average size of 39.7 nm using Method I and 15.4 nm using Method II. The nanoMIP present abundantly on the surface of the subtrate. NanoMIP produced using Method 2 shows uniform size less than 20 nm which indicates monodispersed synthesized polymer as exhibited by nanosizer analysis (though this analysis did not show accurate size of nanoMIP). EDX was used to confirm the elemental components of the analyzed samples to make sure the analysis was done on the desired nanoMIP samples (Not on the stub, carbon tape or any interference). It can be observed that the elemental composition of carbon, nitrogen and oxygen presence in both nanoMIP samples which comes from the monomer molecules. Though, aluminum element that was observed came from the stub that hold the sample.

(a) (b)

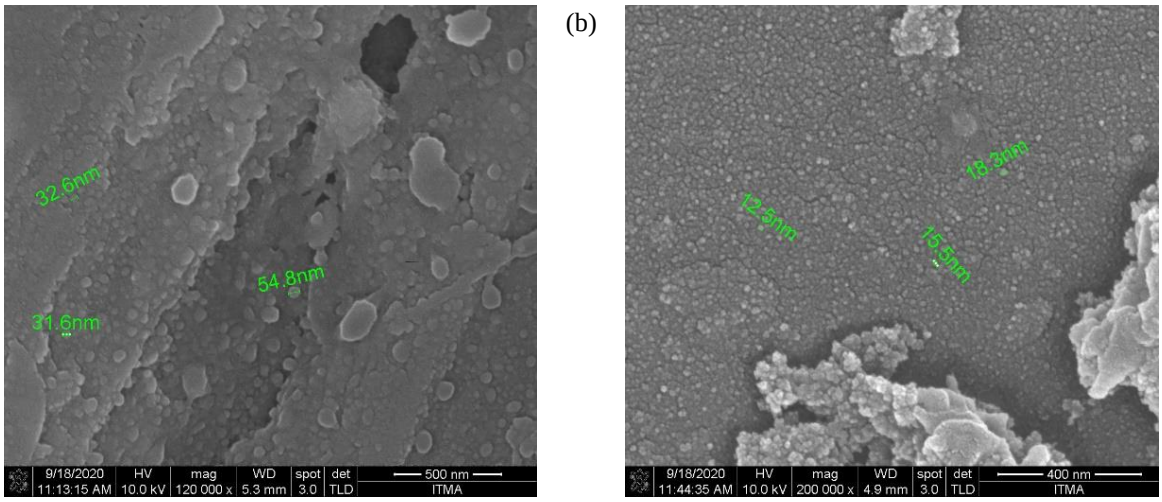

Fig. 3. FESEM image of nanoMIP synthesized using (a) Method I and (b) Method II

Table 1: Elemental composition of elements present in the synthesized nanoMIP characterized using EDX

	Element	C	N	O	Al
Method I	Weight%	52.62	10.73	21.86	14.79
	Atomic %	62.04	10.85	19.35	7.76
Method II	Weight%	43.42	6.88	11.32	38.38
	Atomic %	57.97	7.88	11.34	22.81

3.3. FTIR analysis

Method 2 shows more uniform size of nanoMIP thus it has been selected for FTIR analysis. FTIR characterization has been conducted on the supernatant discarded after the washing steps. It is to confirm the successful removal of the PenG template. NanoMIP was first added with acetone to separate the oil and water phase and centrifuged to separate the nanoMIP from the solution. The supernatant was discarded out and sent for FTIR analysis. Then the nanoMIP was dispersed in ethanol and water for washing, centrifuged and the supernatant was collected for analysis. Sample synthesized using the latter method was sent for FTIR analysis to ensure complete removal of template by analyzing the supernatant discarded after washing steps. The spectra for washing steps using ethanol and DI water did not show any signs of the presence of PenG bond. However, the acetone separation step supernatant shows presence of C-C ring binding and C-N stretch which might belong to PenG. Further characterization needs to be done to confirm the presence of the antibiotic imprinted cavities and the ability of the nanoMIP for effective nanoMIP detection.

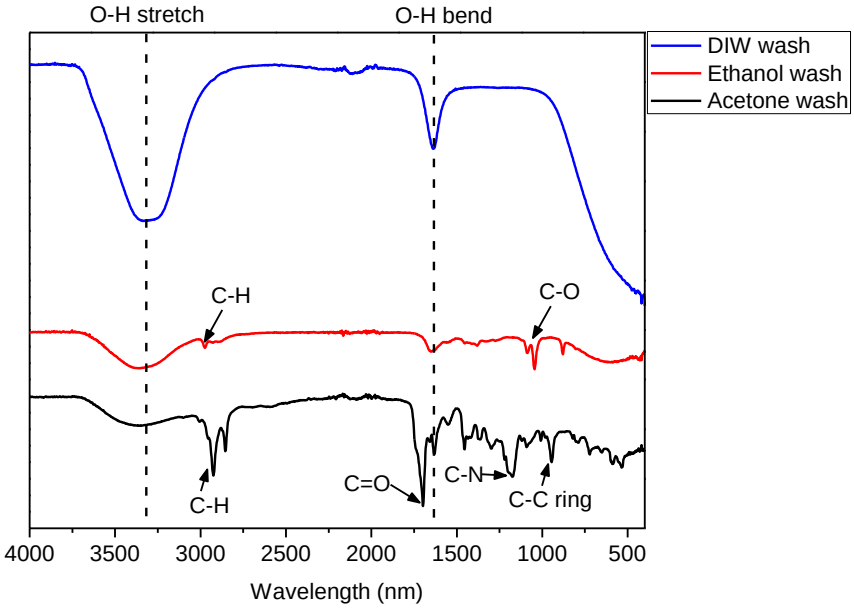

Fig. 4. FT-IR spectrum of nanoMIP after washing with acetone, ethanol and followed by water

4. Conclusions

In general, the miniemulsion polymerization technique used to synthesized the PenG specific nanoMIP has proven to produced uniform particles in the nano size range. Both methods used produced round shaped nanoparticles but method II using MAA as monomer has been selected for further use due to the ability of this monomer to produce slightly smaller nanoMIP. The nanoMIP has been washed to remove the PenG template and the FTIR shows the removal of the template during acetone washing step. This nanoMIP produced can be used for further study of PenG antibiotic detection.

Acknowledgements

The author would like to express gratitude to Ministry of Higher Education Malaysia for support from Fundamental Research Grant Scheme (FRGS) 2019 [276140-310514].

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