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The Effect of Variations Concentration of Chloramphenicol on the Imprinting Factor of Molecularly Imprinted Polymer

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ABSTRACT: This research aimed is to determine the effect of chloramphenicol concentration on the imprinting factor (IF) value of the Molecular Imprinted Polymer (MIP). Imprinting Factor is a standard of the interaction power between printed polymer and template molecule. The IF value was calculated based on the adsorption capacity value between MIP and blank polymer (BP). MIP was synthesized from non-imprinted polymer (NIP) using the precipitation method with chloramphenicol as a template, methacrylic acid (MAA) as a monomer, and ethylene glycol dimethacrylate (EGDMA) as a crosslinker. The results showed that the optimum concentration was at 10 ppm with the IF value of 5,005. The isothermal adsorption result of Chloramphenicol using MIP can best be described by the Langmuir model. The limit of detection (LOD) value was 0.098 and the limit of quantification (LOQ) value was 0.327.

KEYWORDS: Adsorption, Chloramphenicol, Imprinting factor, Isothermal, MIP.

INTRODUCTION

Antibiotics are widely used to treat disease and stimulate growth in animals. One of the most used antibiotics is chloramphenicol (CAP). Chloramphenicol is a broad-spectrum antibiotic with excellent antibacterial properties. Generally, it is used to inhibit gram-positive and gram-negative bacteria [1]. However, it is also one of the drugs that often have side effects. Thus, foods containing chloramphenicol have the potential to damage human health. The European Union establishes the required limit of chloramphenicol in food-producing animals at 0.15 ppb [2].

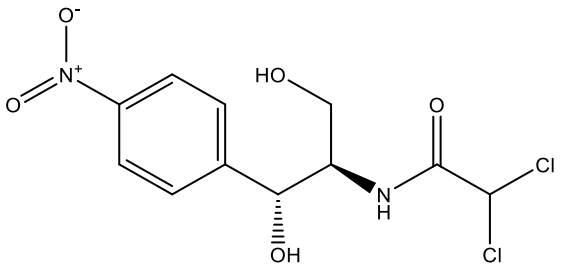

Figure 1. Structure of chloramphenicol

The CAP analysis can be determined by the High-Performance Liquid Chromatography (HPLC) method [3]. However, if the amount of CAP is very small and difficult to detect, that is why a preconcentration method was needed. There are some preconcentration methods, one of them is molecularly imprinted polymers (MIP) [4]. MIP is a remarkable polymer adsorbent in which the molecular target was involved in the synthesis process as a template. The template then be removed from the polymer, remains the cavities on the sites which are suitable only for the target molecule [5]. In this research, MIP was synthesized using chloramphenicol as a template, methacrylic acid (MAA) as a monomer, ethylene glycol dimethacrylate (EGDMA) as a crosslinker and acetonitrile as a porogen [6].

The template will operate as a template to create the template-complementary binding site. Normally, MIP formation involves copolymerization of template complexes and functional monomers in either covalent or non-covalent interaction with crosslinking agent on the appropriate porogen. After removal of the molecular template, a rigid three-dimensional cavity will appear related to the target analyte [7].

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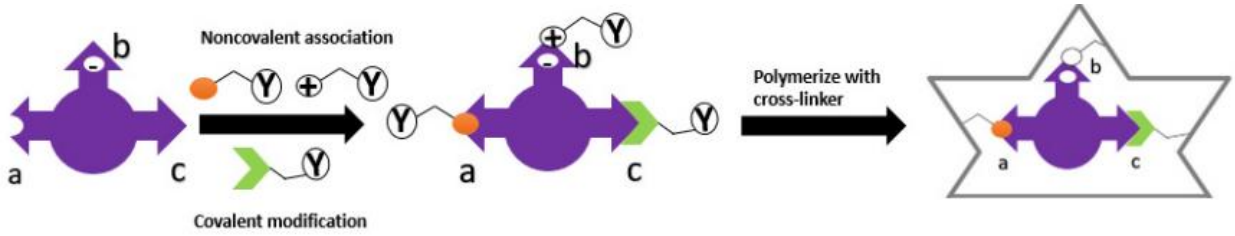

Figure 2. Types of interactions between covalent and non-covalent [7]

Currently, methodologies for producing MIP include bulking, suspension, two-step swelling, precipitation and emulsion, and core-shell polymerization. The manufacture of MIP by bulking method shows high selectivity, but there are disadvantages such as heterogeneous distribution of binding sites, interpolation as binding sites size, and poor site accessibility for template molecules. Meanwhile, the manufacture of MIP using the precipitation polymerization particle method from the precipitation method produces more particles and the particle size is homogeneous. Also, the crushing and sifting steps are not required [8].

MIP is a useful adsorbent and plays an important role in the decontamination of antibiotics in the aquatic environment. The use of this adsorbent has many benefits such as excellent affinity, non-toxicity, low-cost regeneration, large quantities and high surface area [9], [10]. The adsorption technique was identified as the most common method for the decontamination of antibiotics in the discharged effluent. Some of the advantages include cheap, easy to operate, profitable, efficient and effective than other techniques [11].

Research [12] showed that the results of imprinting factor (IF) using homogeneous MIP gave well results and more consistent properties than MIP with mass-produced heterogeneity (bulk polymerization). This research aimed to determine the effect of variations in the chloramphenicol concentration toward the IF of the MIP adsorption results with the extractant composition of methanol: acetic acid 85:15(v/v) %.

MATERIALS AND RESEARCH METHODS

Material and Tool

The research materials were chloramphenicol (CAP) (Sigma Aldrich), ethylene glycol dimethacrylate (EGDMA), methacrylic acid (MAA) (Sigma Aldrich), benzoyl peroxide (BPO), methanol (Merck, Germany), ethanol (Merck, Germany), acetonitrile (Merck Germany), aquabidest, and nitrogen gas (N_2) (UHP).

The research tools were analytical balance (Ohaus), magnetic stirrer, hot plate stirrer (Daihan Scientific), vortex mixer (Velp Scientifica), thermometer, buchner funnel, Erlenmeyer flask with side tube, vacuum pump, measuring cup of 100 ml (Herma), measuring flask of 100 ml (Iwaki), beaker of 100 ml (Iwaki), measuring pipette, volume pipette, High-Performance Liquid Chromatography (HPLC) (Shimadzu LC solution Analysis), and Scanning Electron Microscope (SEM) (FEI Inspect-S50).

Research Methods

Non-Imprinted Polymer (NIP) Synthesis

1 mmol of CAP dissolved in 25 ml of acetonitrile porogen then 3 mmol of methacrylic acid (MAA) was added. Afterward, the mixture was vortexed until it was clear and completely mixed, then closed and allowed to stand. The mixture was added with 18 moles of ethylene glycol dimethacrylate (EGDMA) and vortexed until completely mixed. Then, the mixture has flowed with nitrogen gas (N_2) for 5 minutes. After that, the mixture was added with 0.05 grams of benzoyl peroxide (BPO) then vortexed and flowed with nitrogen gas (N_2). Then, the mixture was put into a waterbath with a temperature of 70°C and a speed of 350 rpm to be a paste. The paste was washed and dried in an oven to obtain a constant weight. This paste was called Non-Imprinted Molecular (NIP).

Blank Polymer (BP) Shyntesis

The synthesis of blank Polymer is the same as the synthesis of NIP but without CAP.

Molecularly Imprinted Polymer (MIP) Synthesis

The NIP was extracted and weighed 0.5 grams. Then, it was added to 100 ml of the 85:15(v/v) % methanol mixture: acetic acid and put into a water bath at a constant temperature of 70°C and a speed of 350 rpm for 5 hours. The result was a filtrate and a

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precipitate called Molecular Imprinted Polymer (MIP). Then, MIP from the extraction was washed and dried in an oven at 40°C until dry to obtain a constant weight.

CAP adsorption using MIP and PB

Each variation of CAP with concentrations of 2, 5, 10, 15, and 20 ppm was put into a different 25 ml container and 0.05 grams of MIP were added. The mixture was vortexed for 20 minutes and separated between the filtrate and the MIP precipitate. Then, the filtrate was analyzed using High Performance Liquid Chromatography (HPLC) to estimate the adsorption capacity and Imprinting Factor (IF) value, while the MIP precipitate was used for the preconcentration stage. The CAP adsorption amount was measured by the difference between the initial and final concentrations of CAP in the filtrate.

The Imprinted Factor value can be estimated using the formula:

$$IF = \frac{q_{MIP}}{q_{BP}}$$

Where q is the adsorption capacity. The value of q could be estimated using the formula:

$$q = \frac{(C_0 - C_e)\,V}{W}$$

V is the adsorption volume while C_0 is the initial substance concentration and C_e is the substance concentration released [11].

RESULTS AND DISCUSSION

NIP and PB synthesis

The synthesis of NIP and BP was conducted by the precipitation method because this method had many advantages, namely more polymer yields, similar particle size, and no crushing and sieving steps. [8].

The preparation of NIP used a non-covalent approach by reacting the template and monomer in the acetonitrile porogen (figure 2). Porogens with high polarity tend to interfere with the interaction between template and monomer, so it is necessary to choose a porogen that did not affect the interaction between template and monomer. The template was chloramphenicol where the active site can bind to the monomer, namely MAA. So, there was an interaction between chloramphenicol and MAA through hydrogen bonding. Hydrogen bonding is caused by chloramphenicol with -NO_2, -NH, and -OH functional groups interacting with carboxylate groups in MAA [13]. Then EGDMA is added as a crosslinker followed by N_2 gas. The addition of nitrogen gas aims to remove oxygen and is carried out before the addition of an initiator so that it does not react with oxygen, and added BPO as an initiator to create a polymer. Then it is flowed with N_2 gas again to remove oxygen which can interfere with the polymerization process [6]. The polymerization process was conducted at a temperature of 70°C and a speed of 350 rpm. Polymerization is usually carried out under an inert atmosphere. The formation of blank polymer (BP) has the same steps as NIP, but CAP was not added.

The last step of the polymerization process was characterized by the formation of a white paste. Then, this paste was washed and dried in the oven until a constant weight was obtained. An illustration of the NIP and BP synthesis process was shown in Figure 3.

MIP Synthesis

MIP is created by polymerizing polymers and functional group with a crosslinker so that they can interact with the functional groups of the template molecule. After polymerization, the template molecule is extracted to create the required shape, size, and function [14]. MIP extraction was conducted using maceration extraction. The researchers chose the maceration method because the extraction result was better than the traditional method [15].

MIP extraction aimed is to remove the template, so the polymer conforms to the template. The method of removing this template was to break the hydrogen bonds between the template and the monomers present during the polymerization process. The bond could be broken using a polar solvent with a high polarity and a small of alkali or acid additives, so the interaction between the template and monomer was disrupted and broken [16].

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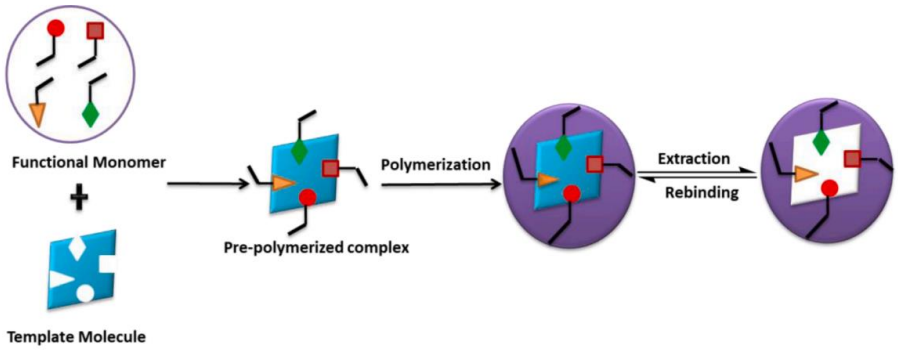

Figure 3. Polymerization and extraction procedures for MIP [17]

MIP was extracted by maceration by immersing 0.05 grams of NIP in a mixture of methanol: acetic acid 85:15(v/v) %. Extraction used methanol because it is a polar solvent and can dissolve and elute CAP perfectly. The extraction method was heating and stirring to accelerate the extraction process. Extraction was conducted at a constant temperature of 70°C with a stirrer speed of 350 rpm.

After extraction, MIP was washed to remove residual acetic acid. The washing solvent should be correct. If there were differences in solvent, there was an unrealistically high selectivity factor of MIP [18]. Several conditions during the template removal process were shown in Figure 4. These conditions could cause unrealistic MIP extraction results.

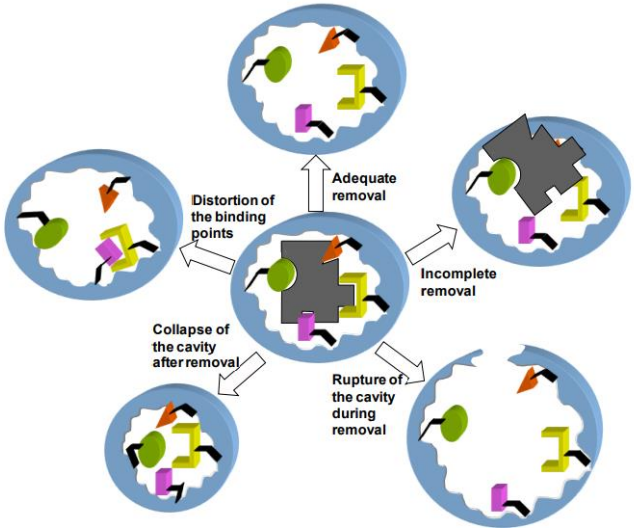

Figure 4. Template release process scheme [18]

CAP adsorption on MIP and BP to IF value

Adsorption on MIP and PB aimed to determine the interaction (rebinding) between chloramphenicol to MIP and BP. The adsorption method was to measure 0.05 grams of adsorbent and add several variations in chloramphenicol concentration. The adsorption filtrate was analyzed using HPLC and the adsorption capacity value was calculated.

Graph 1. showed that the adsorption capacity of MIP was higher than PB. It was because the polymerization of BP did not add CAP as a template, so the polymer did not have a cavity as in MIP. It caused the lack of effective rebinding when the concentration of CAP was added, so very little CAP was adsorbed on BP.

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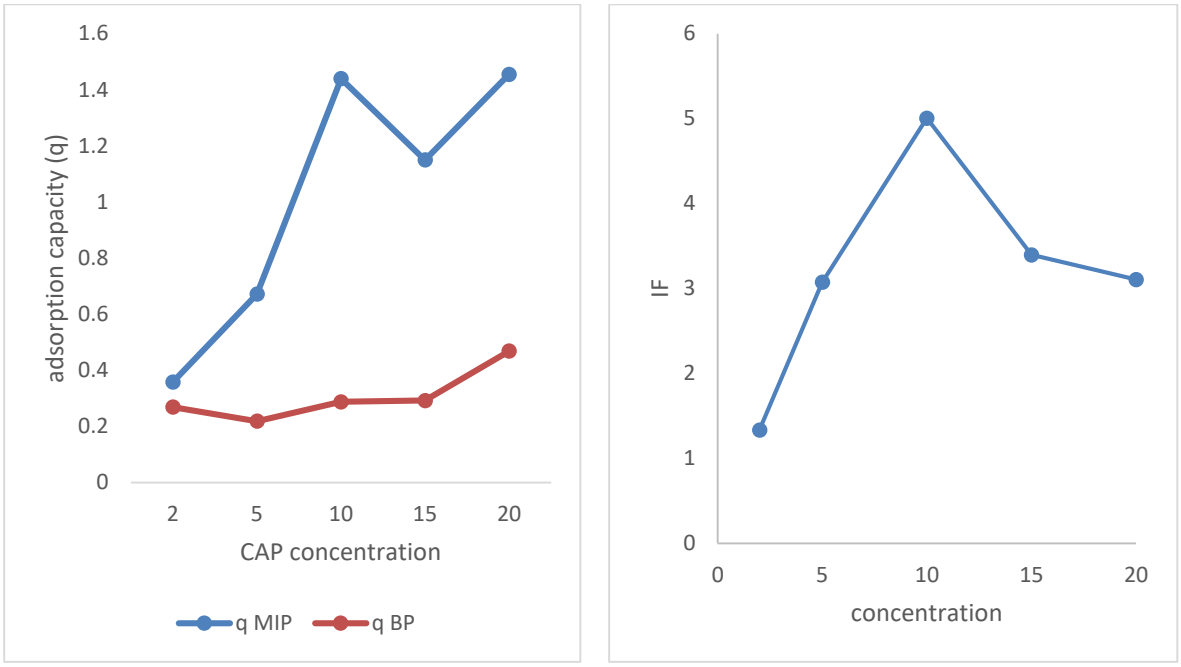

(1) (2)

Figure 5. Graph (1) Comparison between the adsorption capacity of MIP and BP, (2) The correlation between concentration and IF value

The imprinting factor (IF) value could be calculated from the adsorption capacity results between MIP and BP. IF is a measure of the interaction strength of the printed polymer to the template molecule. High IF values interact effectively with template molecules [19]. So, the IF value on MIP was greater than BP.

Table 1. Calculation Results Of q MIP, q BP, And IF

Concentration (ppm)	q MIP	q BP	IF
2	0,3584	0,2684	1,3356
5	0,6716	0,2185	3,0734
10	1,4399	0,2877	5,0058
15	1,1498	0,2920	3,3973
20	1,4551	0,4683	3,1069

Table 1 showed that the IF values of several concentrations of CAP variations were very different, and the IF values were not directly proportional to the concentration. It was because the MIP cavity formation was more effective at certain concentrations [20]. Thus, the concentration of 10 ppm had a high IF value (graph 2) because the cavity in the MIP could receive CAP concentrations effectively. Cavities in MIP and BP can be observed using SEM (Scanning Electron Microscope).

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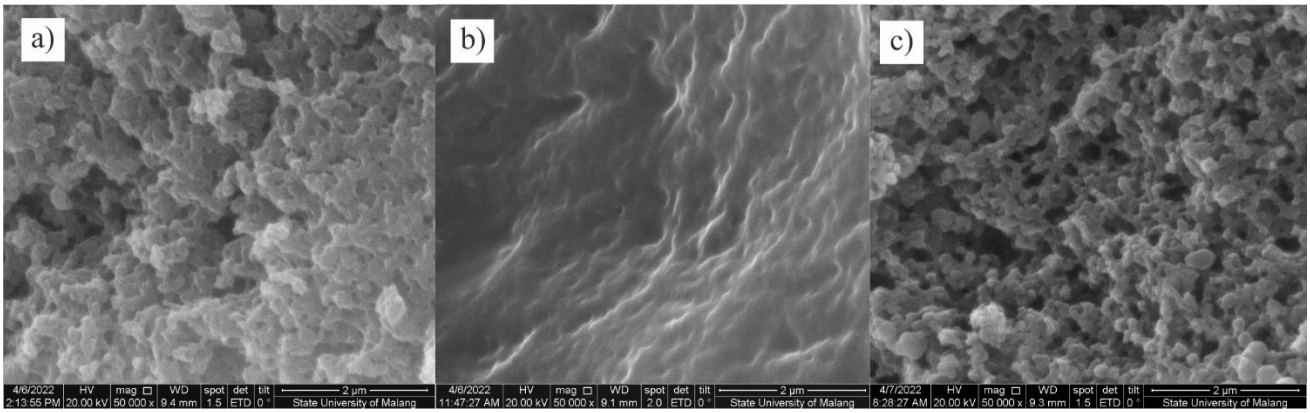

Figure 6. Cavities on a) NIP, b) BP, and c) MIP using SEM

Characterization for NIP, PB, and MIP uses a Scanning Electron Microscope (SEM) to determine their morphological properties. SEM characterization is used to explain pore size and particle size for adsorption studies. SEM analysis plays an important role in the characterization of adsorption [21]. According to the SEM results presented in (figure 6), MIP has a rougher surface when compared to NIP and BP. In NIP there is still a template while in MIP there is none. This is what causes MIP to be more rough and hollow. The results of this SEM showed the average pore size of NIP, BP, and MIP which was 22.5 nm; 15.2 nm; and 26.9 nm.

Furthermore, the adsorption calculation was conducted using isothermal parameters. The isothermal types were Langmuir and Freundlich. This test aimed to provide basic information about the adsorption mechanism, surface properties, and affinity adsorbent. It assisted to define the adsorption application as a unit operation [11]. The result was as follows.

Table 2. Adsorption Calculation Results Using Isothermal Parameters

Type/model	Parameter	Results
Langmuir	q_{max} (mg/g)	-48,5909
	K_L (L/mg)	-0,1683
	R_L	-0,5401
	R^2	0,9524
Freundlich	K_f	10,4450
	1/n	1,4102
	R^2	0,7790

In the Langmuir model, it was assumed that there was adsorption at certain homogeneous active sites on the adsorbent. This active site had an affinity for single layer adsorption but there were no intermolecular interactions between the adsorbed molecules. Meanwhile, the Freundlich model assumed that the adsorption of the adsorbate to the adsorbent through several surfaces (multi-layer) inside and outside the adsorbent layer.

Table 2 showed that the regression result of the Langmuir model was 0.9524. This result was higher than the regression result of the Freundlich model, which was 0.7790. Therefore, CAP adsorption on MIP followed Langmuir adsorption because it was conducted to a single layer of adsorbed substances.

LOD and LOQ values

The limit of detection (LOD) is the smallest limit test parameter of a tool to measure a certain analyte in a sample that shows the absorption value. While the limit of quantification (LOQ) is the smallest amount of analyte sample that can be measured by the tool accurately [22].

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The linear calibration curve for chloramphenicol was obtained in the concentration range of 0.5 ppm to 5 ppm with the line equation y = 14828x – 503.16 and the regression result was 0.9998. The calculation of the LOD and LOQ values could be determined by the formula:

$$LOD = \frac{3 \; x \; Sy/_{x}}{b}$$

$$LOQ = \frac{10 \; x \; Sy/_{x}}{b}$$

From the formula above, the LOD value was 0.098 and the LOQ value was 0.327.

CONCLUSION

Based on the research results, it can be concluded that the best result of the effect of variations in CAP concentration on the best IF value is the addition of 10 ppm chloramphenicol concentration variation with an IF value of 5,005. The limit of detection value (LOD) is 0.098 and the limit of quantification value (LOQ) is 0.327.

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