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Bulk and Precipitation Polymerization Techniques in Alpha Mangostin Imprinted Polymers Synthesis

Neena Zakia[1*], Muhammad Ali Zulfikar[2], and Muhammad Bachri Amran[2]
[1]Department of Chemistry, Universitas Negeri Malang, Jalan Semarang 5, Malang, 65145, Indonesia
[2]Department of Chemistry, Institut Teknologi Bandung, Jalan Ganesha 10, Bandung, 40132, Indonesia
[*]Corresponding author (e-mail: neena.zakia.fmipa@um.ac.id)

Alpha mangostin is a xanthone compound in the mangosteen rind, which has pharmacological activities such as antioxidant, anticancer, and antiinflammatory. There are many xanthone derivatives in the mangosteen rind, but each compound has different benefits. An effort is needed to obtain alpha mangostin compounds selectively. The use of a molecular imprinted polymers material as a sorbent is able to effectively separate the target compound from a sample with a complex matrix. The aim of the research was to synthesize imprinted polymers with alpha mangostin as a molecule template by bulk and precipitation techniques. In this study the synthesis of alpha mangostin imprinted polymers using methacrylic acid as a functional monomer, ethylene glycol dimethacrylate as a crosslinker, benzoyl peroxide as an initiator, and acetonitrile as a porogen. The volume of porogen used in the bulk technique is 10 mL and the precipitation technique is 20 mL. As a comparison, a control polymer was synthesized using the same procedure, but without the presence of a template molecule. Physical characterization by FTIR and SEM, and retention characterization included adsorption capacity and imprinting factor (IF). The results obtained indicate that the synthesis of molecular imprinted polymers has been successfully carried out using bulk and precipitation techniques. The adsorption capacity of the bulk technique is 11.214 $mg.g^{-1}$ and the IF value is 1.78, while the adsorption capacity of the precipitation technique is 11.767 $mg.g^{-1}$ and the IF value is 2.87.

Keywords: Alpha mangostin; molecular imprinted polymers; bulk polymerization; precipitation polymerization

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Mangosteen (*Garcinia mangostana* L.) is a tropical plant that can be found in several countries in Southeast Asia, including Indonesia. Mangosteen is one of Indonesia's commodities that has a high selling value. Mangosteen fruit has been widely used for traditional treatment of several diseases such as diarrhea, fever, dysentery, and skin diseases. The rind of the mangosteen fruit has been scientifically proven to contain bioactive compounds and has been widely produced as herbal medicine [1, 2].

The potential of the mangosteen fruit as a herbal medicine is due to the content of compounds in the skin of the fruit that have pharmacological activity. Phytochemical studies show that the mangosteen rind contains xanthone compounds and their derivatives, benzophenone, flavonoids, anthocyanins, tannins, polysaccharides, and pigments [3-5]. Xanthone compounds are major secondary metabolites and as many as fifty types of derivative compounds are found in the mangosteen rind. One of the xanthone derivatives with the greatest abundance and known pharmacological effects is alpha mangostin [2, 6]. The chemical structure of alpha mangostin is shown in Figure 1. These xanthone compounds and their derivatives actually have different pharmacological properties, thus providing different benefits. Therefore, an effort is needed to obtain one of the active compounds, to obtain the maximum potential of the pharmacological effects of each compound.

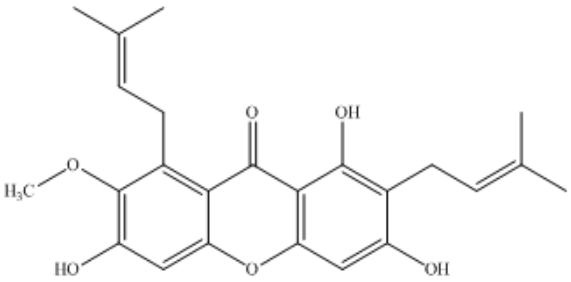

Figure 1. Chemical Structure of Alpha Mangostin.

To obtain compounds selectively requires a complex separation process. The steps taken in sample preparation to analysis require procedures that are not easy, chemicals, and a long time so that they are not effective and cause many errors that occur during the analysis process [7-9]. Various separation methods to obtain the target compound are commonly used, including solvent extraction, column chromatography, and solid phase extraction [10-12]. Solid phase extraction provides efficiency in removing complex matrices.

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However, the sorbent used in solid phase extraction has a drawback in terms of selectivity. Therefore, a material has been developed as a sorbent that has high selectivity towards the target analyte, namely a molecular imprinted polymers [7, 9, 13].

Molecular imprinted polymers (MIPs) are polymers that have a template as a recognition of the target molecule. In the synthesis, in the initial stage there is prepolymerization between template molecules and functional monomers through non-covalent interactions to form template-monomer complexes. The next step is the polymerization of the template-monomer complex by crosslinking in the presence of the initiator in a suitable porogen solvent. The molecular imprinted polymers will be produced by a leaching process using a suitable solvent, to produce a selective cavity [7, 14, 15]. To produce MIPs that have good retention capabilities, an adequate synthesis technique is needed. MIP can be prepared by several techniques, such as bulk, precipitation, emulsion, surface, and core shell polymerization [16-18].

The bulk polymerization technique is the simplest technique and does not require special skills. The resulting polymer is then ground and sieved to obtain the desired particle size. Precipitation polymerization is more economical, with the advantages that it does not require grinding and a more uniform size is obtained. In this study, the synthesis of alpha mangostin imprinted polymers with bulk and precipitation techniques will be compared, to determine the effectiveness of the polymers produced in the binding study for alpha mangostin compounds. Non-imprinted polymers (NIPs) as control polymers were synthesized by following the same procedure without the template.

EXPERIMENTAL

Materials

The chemicals used have a pro-analytical degree. The ingredients used were Alfa mangostin (97%, ChengDu biopurity, China), 98% ethylene glycol dimethacrylate (Sigma), methacrylic acid (Merck), benzoyl peroxide (Merck), glacial acetic acid (Merck), methanol (Merck), and acetonitrile (Merck).

The equipment used in this study were a number of common glassware, Thermolyne electric bath, Fourier transform infrared spectrophotometer (FTIR Shimadzu Prestige-21), scanning electrom microscope (SEM) FEI brand type ISPECT-S50, HPLC Infinity Agilent Tech 1260 Series column C18 (Phenomenex, 150 x 4.6 mm), Agilent 8453 G1103A UV-Visible Spectrophotometry.

Synthesis and Characterization of Alpha Mangostin Imprinted Polymers by Bulk and Precipitation Polymerization Techniques

Molecular imprinted polymers (MIPs) were synthesized by bulk and precipitation polymerization techniques. In the bulk polymerization, MIPs was performed by mixing alpha mangostin (1 mmol) and methacrylic acid (4 mmol) in 10 mL acetonitrile for 24 hours at room temperature. Furthermore, ethylene glycol dimethacrylate, EGDMA (20 mmol) and benzoyl peroxide (1%), in the synthesis bottle were added. The mixture was run with nitrogen gas for 10 minutes, to remove oxygen gas. The polymerization was carried out on an oil bath for 8 hours at 60°C.

After polymerization, the removal of the mold molecules was carried out under reflux using a Soxhlet extractor. The solvent used was a mixture of methanol: acetic acid (9:1) for 24 hours. The remaining number of template molecules was analyzed by UV-Visible spectrophotometry. For control polymers, non-imprinted polymers (NIPs) were synthesized. The procedure for synthesizing NIPs is the same as for MIPs, but without the addition of a template molecule. In the precipitation technique, the procedure is the same, but the volume of porogen solvent added is 20 mL. In the precipitation technique, NIPs were also synthesized as control polymers.

MIPs before and after extraction were characterized by functional groups using the FTIR instrument. The resulting spectra were compared with the functional group spectra of NIPs. The surface morphology of MIPs was analyzed by SEM instrument.

Batch Rebinding Study

The rebinding study was carried out in batch, 10 mg of MIPs were put in vials, 5 mL of alpha mangostin 25 $mg.L^{-1}$ solution was added in 1:1 methanol:water (%v/v) solvent. Adsorption process was carried out for 24 hours using a shaker at 150 rpm and room temperature. After decantation, the supernatant was analyzed using HPLC with 95:5 (%v/v) methanol: water eluent and a flow rate of 1 $mL.min^{-1}$. Binding studies were carried out on MIPs and NIPs, which were synthesized by bulk and precipitation polymerization techniques, respectively. The adsorption capacity (q_e) of alpha mangostin was calculated by the amount of analyte remaining in the solution against the weight of the adsorbent using Equation 1. The imprinting factor (IF) is the value of the analyte adsorption capacity on the MIPs to the value of the analyte adsorption capacity on the NIPs, was calculated using Equation 2.

$$q_e = \frac{v(C_i - C_e)}{w} \quad (1)$$

Where q_e is the adsorption capacity or total binding amount ($mg.g^{-1}$), v is the volume of solution (L), Ci and Ce are the initial concentration and the final concentration of alpha mangostin ($mg.L^{-1}$), respectively, and w is the sorbent mass (g).

$$IF = \frac{q_{MIP}}{q_{NIP}} \quad (2)$$

Where q_{MIP} and q_{NIP} are the adsorption capacity of MIPs and NIPs, respectively.

RESULTS AND DISCUSSION

One of the factors that can affect the performance of molecular printed polymers other than the polymer components, is the polymerization technique. Polymerization is carried out using a non-covalent approach, namely the interaction of template molecules and functional monomers through non-covalent interactions. Non-covalent interactions can occur through Van der Waals forces, electrostatic interactions, dipole-dipole, and hydrogen bonds [7, 15, 19, 20]. In this study, the type of polymerization for the synthesis of MIPs was free radical polymerization. Different MIPs synthesis techniques for alpha mangostin imprinted polymers are bulk and precipitation polymerization have been carried out.

The polymerization technique can improve the morphology and shape of the desired particles, so that it will improve the retention capacity of the MIPs material. The bulk polymerization technique has the advantage that it is simple, easy, fast, and does not require special skills. However, the drawbacks of the bulk technique are that the shape and size of the resulting particles are generally irregular and less homogeneous. The precipitation polymerization technique was improving the shortcomings of the bulk technique, due to the shape of the resulting particles and does not require grinding that polymers [17, 18].

The results of the synthesis of the alpha mangostin imprinted polymer from the bulk and precipitation polymerization techniques shown in Figure 2 are MIPs before leaching, after leaching, and NIPs as a control polymer. Physically, the shape of the polymer from the two polymerization techniques were not different, but the hardness of the polymer when ground showed that MIPs with the bulk technique was harder than MIPs with the precipitation technique.

The characterization of the synthesized polymer using IR analysis is shown in Figure 3. The IR spectra of the synthesized polymer using the bulk technique and the precipitation polymerization technique is not different, because the polymer constituents in both polymerization techniques use the same compound. IR spectra used to determine the success of polymer formation by comparing the IR spectra of MIP components. IR spectra shown functional group component constituents in MIPs before and after removal template (leaching) that compare with NIPs as a control polymer.

The IR spectra of the MIPs before and after leaching showed that there were some differences in the absorption bands and the band shift was at wave numbers 1605 and 1638 cm^{-1}. In MIP before leaching the functional group showed that there was a missing functional group of the alpha mangostin compound (it has been reported in previous studies [21]. The absorption band at 1605 cm^{-1} was the C=C stretching vibration of the alpha mangostin molecule in the MIPs before leaching, which was not found in the MIPs after leaching. The shift of the absorption band from 1638 cm^{-1} in MIP before leaching to 1667 cm^{-1} in MIP after leaching indicates that there is a change in the bond after the removal process. The peak profile spectra after extraction were similar to the spectra of NIPs indicating that some functional groups were lost due to the leaching process of template molecules. This indicates that the template removal extraction has been successfully carried out, so it can be expected that the mold cavity in the MIPs has been formed.

Another physical characterization is the surface morphology analysis using the SEM instrument, which is shown in Figure 4. It can be seen from the SEM analysis, that the MIPs with the bulk particle technique are smaller and irregular shape, while the MIPs with the precipitation technique are larger and more spherical. Similar results were obtained from research by Suwanwong who explained that polymer materials synthesized in bulk have a tendency of non-uniform shape and size when compared to precipitation methods [17]. The spherical shape of the particles can improve the retention performance of MIPs, because the cavity which has an active site for the target molecule is more available than in the MIPs synthesized by the bulk technique.

Batch Rebinding Study

Rebinding studies were used to determine the performance of the synthesized MIPs against a target molecule having a suitable shape, size and functional group in the cavity. The results are shown in Table 1 and Figure 5, regarding the adsorption capacity of MIPs and NIPs of the two synthesis techniques, as well as the value of the imprinting factor (IF) of the two MIPs synthesized in bulk and precipitation polymerization.

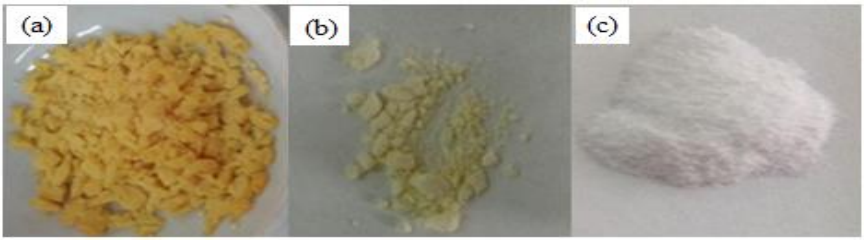

Figure 2. Synthesis Result of MIPs and NIPs: (a) MIPs Before Leaching, (b) MIPs After Leaching, and (c) NIPs.

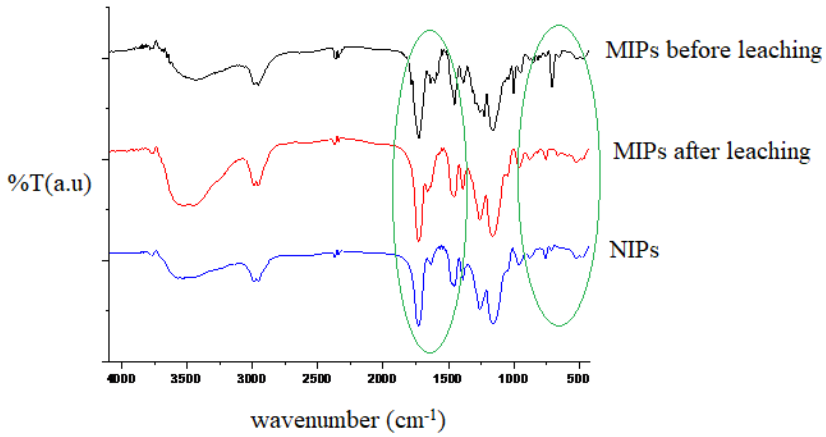

Figure 3. Spectra IR for MIPs Before and After Removal Template (Leaching), and NIPs.

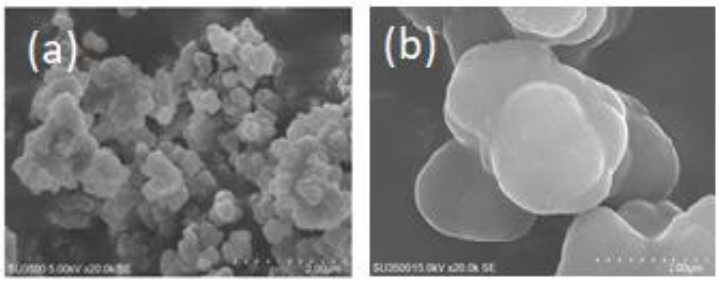

Figure 4. Product of Polymers Synthesized by (a) Bulk Polymerization and (b) Precipitation Polymerization.

Table 1. Rebinding Study on Alpha Mangostin Imprinted Polymers (MIPs) and NIPs, and IF Value of MIPs in Variation Polymerization Techniques.

Polymerization Technique	Adsorption Capacity (q_e, mg.g^{-1})		IF Value
	MIPs	NIPs	
Bulk	11.214	6.3	1.78
Precipitation	11.767	4.1	2.87

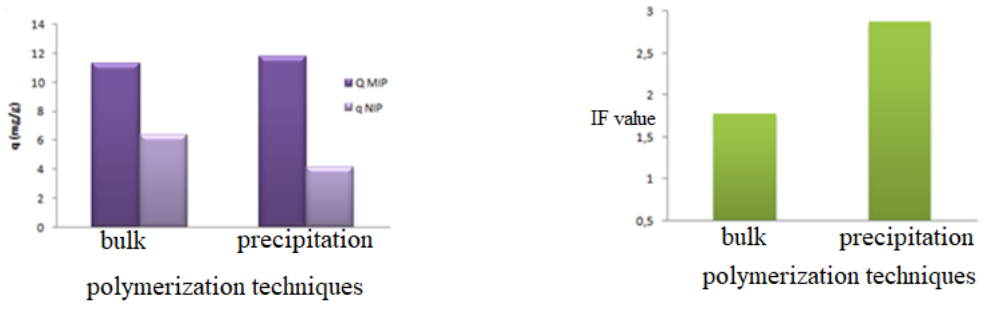

Figure 5. Rebinding Study on Alpha Mangostin Imprinted Polymers (MIPs) from Bulk and Precipitation Polymerization Techniques (a) Adsorption Capacity of MIPs and NIPs, (b) IF Value of MIPs.

The rebinding study showed that the adsorption capacity (q_e) of the two MIPs with different polymerization techniques were similarity, but the MIPs of the precipitation technique (11.767 mg.g^{-1}) was better than the MIPs of the bulk technique (11.214 mg.g^{-1}). The adsorption capacity of both MIPs (bulk and precipitation polymerization) was greater than that of NIPs as the control polymer, this indicates that MIPs were binding more to the analyte due to their template cavity (Figure. 5a). Both MIPs bind to alpha mangostin through their specific interaction, due to in MIPs there is a cavity that has a shape, size, and

functional groups that suitable with that template. Whereas in NIPs the bond that occurs is actually a non-specific interaction because it does not have a template cavity in accordance with the target molecule.

Based on the IF value, it can also indicate the presence of a specific interaction type possessed by the template cavity in the MIPs (Figure 5b). The precipitation technique has a higher IF value (2.87) than the bulk technique MIPs (1.78). This is possible because in the preparation with the bulk technique, the synthesized MIPs were ground to obtain a uniform size, so that the binding site could be damaged. This resulted in a decrease in the loading capacity of the MIPs. Meanwhile, MIPs with precipitation techniques do not require grinding so that the active side of the template cavity is not damaged and the loading capacity is increased.

CONCLUSION

Alpha mangostin imprinted polymers synthesis using the bulk and the precipitation polymerization technique were successfully carried out. Characterization of MIPs and NIPs by IR analysis showed that MIPs had been successfully synthesized. Surface morphological analysis showed that the MIPs of the precipitation technique gave a homogeneous and more spherical particle size, as well as providing a better retention capacity. The adsorption capacity and the imprinting factor value of the MIPs precipitation technique were greater than the bulk technique, due to the relatively large number of template cavities containing the active binding site of the MIPs precipitation polymerization technique.

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