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Effect of Extraction Temperature of Molecularly Imprinted Polymer in Chloramphenicol Adsorption using UV-Vis Spectrophotometry Based on Diazotation Reaction

Eka Faradila Oktaningtias, Maria Monica Sianita*

Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Negeri Surabaya, Surabaya, Indonesia
*e-mail: mariamonica@unesa.ac.id

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Abstract: Chloramphenicol is an antibiotic commonly used in aquaculture. The overuse of antibiotics poses a danger. CAP will precipitate, and the residue will accumulate in the human body, threatening human health. Efforts are made to make media to overcome the CAP problem, one of which is the manufacture of molecularly imprinted polymers. The characteristic of this polymer is a mould or template that is reacted during synthesis and then withdrawn in the extraction process to form a selective mould. One factor that affects extraction is temperature. This study aimed to determine the effect of extraction temperature on chloramphenicol adsorption. The polymerisation was carried out using the precipitation polymerization method, and extraction was done using the batch method. The test used diazotation-based Uv-Vis spectrophotometry. The temperatures used were 60,70 and 80℃, which resulted in extraction percentages of 75.64%, 89.63% and 74.08%, respectively. For the adsorption process, the concentration variation for each MIP was 10, 25, 50, 75 and 100 ppm. Higher concentrations resulted in more adsorption, but the 100 ppm concentration decreased. The test showed that temperature affected the extraction and CAP adsorption results. Polymer characterisation was carried out with FTIR Polymer NIP results showing the presence of NO_2 groups characteristic of CAP found at wave numbers 1536 cm^{-1} and 1322 $cm^{-1.}$ In MIP, there is an Imprinting Factor (IF). The IF value obtained is more than 1, so it can be said that the moulding results are good.

Keywords: Adsorption; Chloramphenicol; Extraction Temperatur; Imprinting Factor (IF); MIP.

Introduction

Molecularly imprinted polymers are a synthetic polymer that can recognise specific target molecules; compared to commonly used adsorbents such as activated carbon, molecularly imprinted polymers exhibit higher selectivity and greater reusability [1]. The MIP method continues to be developed because it is easy to make polymers and inexpensive. MIP is also used on many target molecules, known as templates. Several studies have been conducted regarding MIP with various templates, such as using nano caffeine [2] carbaryl [3] atenolol [4] template and, in this study, chloramphenicol (CAP) [5].

CAP is commonly used to treat bacterial diseases in farmed fish such as shrimp and ornamental fish. CAP antibiotics can increase the growth and production of aquatic products [6]. The continual use of antibiotics on biota can also lead to the accumulation of residues. Aquaculture products consumed by humans can cause accumulation and threaten human health. CAP antibiotics can cause bone marrow suppression, leukaemia, and aplastic anaemia in humans [7].

The creation of imprinted polymers begins with polymer synthesis to form NIP (Non-Imprinted Polymer). The synthesis process is carried out by mixing template (CAP), monomer (methacrylic acid), and crosslinker (ethylene glycol dimethacrylate) using the precipitation polymerization method [8]. The composition of materials for NIP synthesis has been optimised. In previous research, the best results were 1: 3: 18 (mmol) [9]. After that, polymer extraction was carried out. The purpose of extraction is to remove the template in the polymer so that the polymer takes the form of a template mold. The type of solvent, soaking time [3], and extraction temperature [10] are the determining factors for the success of MIP extraction. Therefore, this study varied the extraction temperature to determine its effect on chloramphenicol adsorption.

The choice of temperature variation is important because too high a temperature can damage the polymer [10]. The temperature variations used are 60 ℃, 70 ℃, and 80 ℃ extraction with variations in CAP concentrations of 10, 25, 50, 75, and 100 ppm in each MIP.

Extraction using the maceration method (batch) is by direct immersion in a solvent. The solvent used was methanol with acetic acid. The solvent was chosen because the polar solvent, with the addition of acid, can break the template bond with the monomer so that the template can be pulled from the polymer, forming a cavity according to the mold [11]. The adsorption process is carried out using the batch method, namely direct contact with the adsorbent for a specified time. The success of MIP can be seen in the value of adsorption ability and the Imprinting Factor (IF) value [12]. IF can be calculated from the results of MIP adsorption with blank polymer adsorption (PB).

In previous studies, there were several methods used to analyse CAP, namely High-Performance Liquid Chromatography (HPLC) [13], Enzyme Link Immunosorbent assay (ELISA) method [14] and Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry (LC-ESI-MS) [15]. One of the shortcomings

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of analysis with this method is that the test requires expensive costs, a long time that can cause the test to be less accurate, and the operation of the tool is difficult, which requires a specialised technician. Based on this, this study was conducted using a simpler method, namely the diazotation method, for forming azo compounds. The test was conducted using a UV-Vis spectrophotometric instrument [16].

Research Methods

Materials

Black lid Erlenmeyer (Iwaki Pyrex), magnetic hotplate stirrer (WiseStir MSH-20D), thermometer, vortex (Wizard IR Infrafed Vortex Mixer), analytical balance, micropipette, volume pipette, test tube, UV-Vis spectrophotometry (Shimadzu UV-1800), and Perkin Elmer FTIR spectrophotometry. Chloramphenicol (Sigma Aldrich), ethylene glycol dimethacrylate (EGDMA), methacrylic acid (MAA) (Sigma Aldrich), benzoyl peroxide (BPO), methanol (Merck, Germany), ethanol (Merck, Germany), acetonitrile (Merck, Germany), nitrogen gas (N_2) (UHP), acetic acid (Merck, Germany), aquabides, formic acid (Merck), Zn powder (Sigma Aldrich), 37% concentrated HCl, $NaNO_2$ (Sigma Aldrich), ammonium sulfamate (Sigma Aldrich), and N-(-1-Naphthyl)ethylenediamine dihydrochloride (Sigma Aldrich).

Methods

Synthesis of Non-Imprinted Polymers (NIP)

The NIP synthesis process followed the procedure (Sianita et al., 2019). Acetonitrile as a porogen, 1 mmol of chloramphenicol (CAP) as a template, 3 mmol of methacrylic acid (MAA) as a monomer, and 18 mmol of ethylene glycol dimethacrylate (EGDMA) as a crosslinker. To assist the polymerisation process, benzoyl peroxide (BPO) was used as an initiator and nitrogen gas was supplied. The materials were mixed, then put in a water bath at 70 ℃ with stirring until they became a paste and dried in an oven at 40 ℃. Polymer blanks were prepared in the same way without the presence of a template.

MIP Extraction

Molecularly Imprinted Polymer is made using template removal extraction. Extraction by maceration method by soaking 0.5 grams of NIP in a mixture of extractants, namely methanol and acetic acid, in a ratio of 80:20 (v/v) [11]. The mix of NIP and extractants was extracted in a bath with 60, 70 and 80 ℃ temperature variations. The extraction was carried out for 5 hours with a stirrer. The extraction results were vacuumed and washed with methanol, acetonitrile and aquabides to remove the remaining extractant on the polymer, then oven-dried at 40℃.

CAP adsorption using MIP as an adsorbent

Batch method adsorption was conducted by adding 0.05 grams of MIP to a test tube and 25 mL of 10, 25, 50, 75, and 100 ppm CAP solution. The mixture was vortexed for 20 minutes. The adsorption results were filtered, and the resulting filtrate was tested using UV-Vis spectrophotometry.

Analysis CAP by spectrophotometry UV-Vis with the Diazotation Method

Reduction of CAP with Zn and 90% formic acid. Furthermore, the formation of the diazo salt filtrate was taken 7 mL and HCl, 0.2% nitrite acid and 0.5% ammonium sulfamate were added. The solution of each 1 mL of each addition is carried out in the cooling process. The diazo salt filtrate is coupled with the addition of 0.1% Naphthyl and forms a purple colour to be tested with Uv-Vis spectrophotometry at a maximum wavelength of 567 nm.

Results and Discussion

Polymer Synthesis and Extraction

NIP synthesis is making polymers by mixing templates, porogens, monomers, crosslinkers and initiators. The synthesis is carried out using the precipitation method. Precipitation polymerization on MIP is an effective technique for synthesising MIP by understanding the basic principles and factors that influence it; then, the synthesis of MIP can be done specifically [8]. Next, the template extraction process is carried out from the polymer to form the mold cavity. The template extraction process uses a batch extraction or maceration method. This method is a simple extraction method; the solvent will be in direct contact with the polymer and then assisted with the stirring process using a magnetic stirrer. So this method has the advantage of being easy to do and can produce good MIP cavity printing [17].

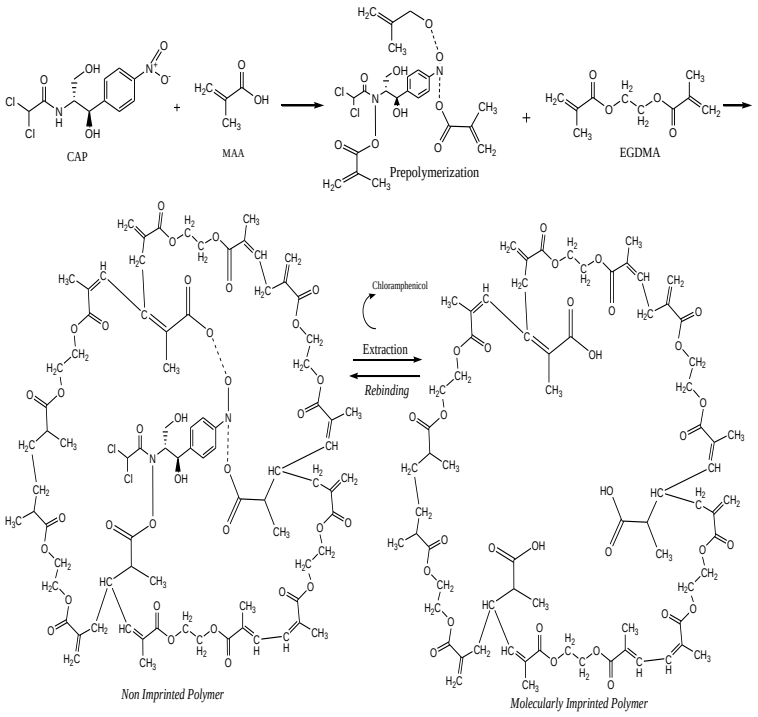


Figure 1. Prediction Mechanism of Polymer Synthesis and Extraction

Polymer Characterization with FTIR

Characterization with Fourier Transform Infa-Red (FTIR) instrument to determine the functional groups contained in the polymer. The working principle of FTIR is the interaction between energy and matter. Infrared light passes through the sample slit to control the amount of energy delivered to the sample. The sample then absorbs some infrared radiation while other infrared radiation is transmitted through the sample surface. Furthermore, the infrared light passes through the detector, and the measured signal is sent to the computer and recorded in peak form [18]. This study's characterization with FTIR aims to determine the differences in functional groups contained in blank polymers (PB), NIP, and MIP. Pure Chloramphenicol (CAP) testing was also carried out to compare the presence of CAP in the polymer.

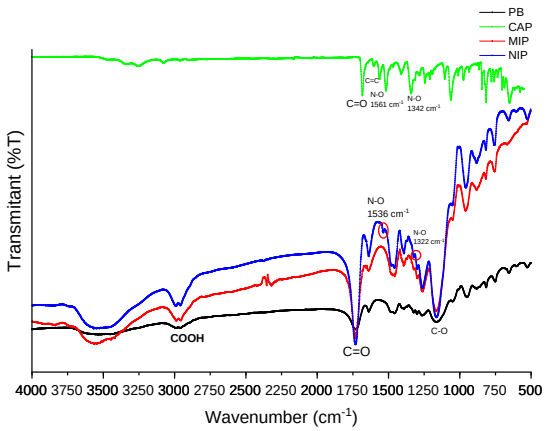


Figure 2. Spectrum FTIR

The figure compares FTIR spectra of blank polymer (PB), NIP, MIP and CAP. In the NIP spectrum, the -NO_2 group was found at wave numbers 1536 cm^{-1} and 1322 cm^{-1}. The presence of nitro groups indicates that there is CAP in the NIP polymer. The -NO_2 group is a typical group of CAP groups that can be proven in the CAP spectrum. The -NO_2 group is found at 1561 cm^{-1} and 1342 cm^{-1} wave numbers. As in the research [9] on NIP, the -NO_2 group was found at wave numbers 1521 cm^{-1} and 1348 cm^{-1}. Furthermore, the MIP and PB spectra did not find the -NO_2 group. MIP indicates that CAP has eluted during the polymer extraction process, while PB indicates that PB was successfully made as a control polymer that does not contain CAP.

Analysis with UV-Vis Spectrophotometry

CAP is a white powder that, when dissolved in ethanol, is a colourless solution, generally tested using HPLC. Alternatively, testing using UV-Vis spectrophotometry can be performed. The assay is based on a diazotation reaction. The reaction procedure starts by reducing CAP. The purpose of the reduction is to convert the nitro group on CAP with an; the amine group will be used for the diazotation reaction. Reduction optimization has been carried out in the research [19] using formic acid as a source of H^+ and Zn as a reductant. The reaction is using catalytic hydrogenation in an acidic atmosphere.

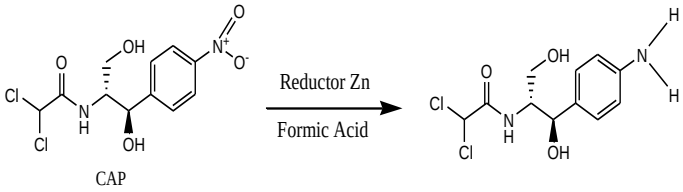
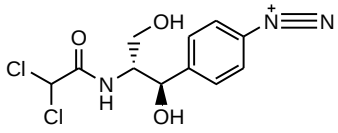


Figure 3. Prediction of CAP Reduction Reaction

Next is the reaction of diazonium salt formation, namely the reaction of primary amines that have been formed from reduction with the addition of nitrite ($NaNO_2$) and hydrochloric acid (HCl), which will form nitric acid as a precursor to nitrosonium ions (NO^+). Then, ammonium sulfamate was added to remove the nitrite gas produced so as not to interfere with the reaction of forming azo compounds [19]. The diazotation reaction has low stability, so it must be carried out at low temperatures (0-5 ℃) and in stages [20]. The final diazonium salt structure is shown in Figure 4.



Diazonium Salt

Figure 4. Structure Diazonium Salt

Additionally, to create a colour that can be tested using uv-vis spectrophotometry, coupling with the compound N-(1-Naphtyl) ethylenediamine dihydrochloride (NEDA) is carried out to form an azo compound that will have a violet purple colour as in Figure 6. The following is a prediction of the reaction of azo compound formation using the NEDA coupling agent:

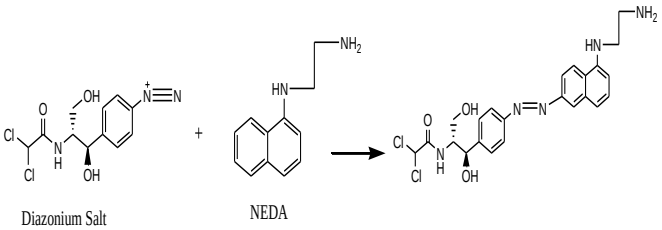


Figure 5. Prediction Coupling Reaction

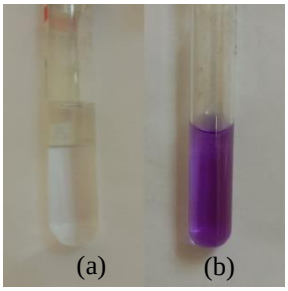


Figure 6. Differences before and after reacting.

Figure 6 shows the difference in the diazo salt solution, which was previously colourless (Figure 6(a)) and, after coupling with NEDA, produces a violet-purple colour (Figure 6(b).

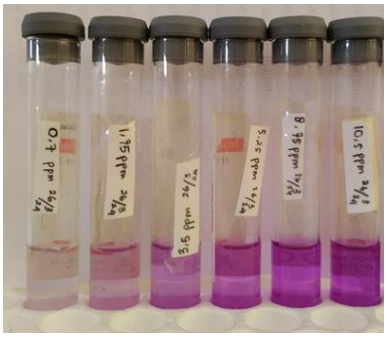


Figure 7. Standard CAP in Different Concentrations

Figure 7 shows the result of making a standard solution of CAP for calibration curves. The concentration of CAP is directly proportional to the intensity of the color formed. A high concentration will produce a concentrated color.

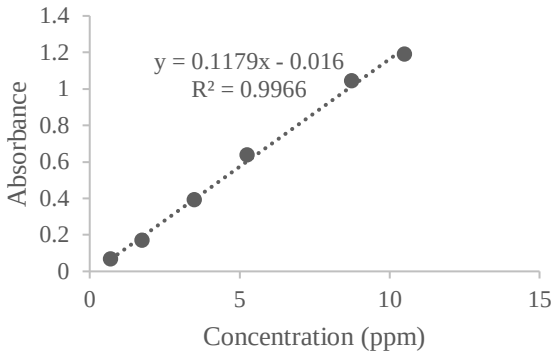


Figure 8. The Calibration Curve of CAP

The CAP calibration curve results in linear regression coefficient R^2 =0.9966 with the equation y=0.1179x-0.016. Linearity is one of the test parameter requirements because it can show the accuracy of the analysis method based on the linear regression coefficient value (R^2) with the equation y = ax + b [21]. The equation will be used to calculate the concentration of CAP in samples of unknown value.

Effect of MIP Extraction Temperature

The extraction process in MIP removes the template to form a cavity mold in the polymer. The cavity formed will be imprinted like the template molecules used [22]. Increasing the temperature can reduce the solvent's viscosity so that the solvent can enter the polymer matrix to disrupt the sample matrix relationship caused by hydrogen bonds, Van Der Waals forces and dipole interactions [23]. As a result, template release from the polymer is easier and can increase the extraction yield. The effect of increasing temperature will decrease the quality of the extraction at a certain point. High temperatures can cause polymer decomposition and decrease the molecular weight of the polymer [10].

Figure 9. shows the difference in the percentage of MIP extraction in every variation temperature at a temperature of 70 ℃, and shows the highest per cent extraction between temperatures of 60 ℃ and 80 ℃. The result is 75.64%, 89.63%, and 74.08%. Based on this diagram, 70 ℃ has reached the optimum point of extraction temperature on MIP because at 80 ℃, there is a decrease in extraction results. Furthermore, adsorption is carried out on in each MIP to determine the adsorption ability of the MIP.

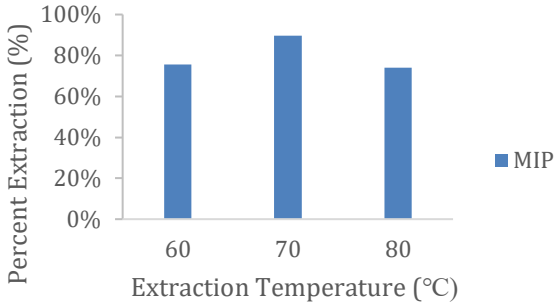


Figure 9. Percent Extraction of MIP

Chloramphenicol Adsorption with MIP

The adsorption process of MIP on CAP was carried out using the batch method. Batch adsorption is an adsorption process in which the adsorbent is in direct contact with the sample solution in one container with a stirring process within a certain time [24]. This simple adsorption process allows the adsorbent to adsorb more analysts in the sample because of the direct contact interaction that occurs in one container.

Table 1. Adsorption of MIP

Concentration (ppm)	Q Adsorption (mg/g)		
	MIP 60	MIP 70	MIP 80
10	0.8997	1.1396	0.9997
25	1.2996	1.6995	0.9597
50	2.8592	3.5390	2.7792
75	9.3375	9.9773	8.4777
100	6.6982	9.6774	7.4380

Table 1 shows the adsorption ability of MIP in each variation. MIP with an extraction temperature of 70 ℃ has a higher value of adsorption ability at each concentration. To prove its effect, a statistical test was carried out with SPSS, namely with Two-Way Anova. This test is carried out because the data has 2 independent variables (x) to determine its effect on the dependent variable (y)[22]. Temperature and concentration are independent variables, and adsorption results are the dependent variable.

The results show normal distribution and homogeneous data with a significance value > 0.05. This indicates that Two-Way Anova testing can be done to determine the effect of temperature and concentration on the adsorption ability of MIP. The results showed that the temperature variation showed a significance value of 0.044 (Sig = 0.044 < 0.05) and a concentration variation of 0.000 (Sig = 0.000 < 0.005), so both variables had a significance value < 0.05. Both variables affect the adsorption ability of MIP on CAP.

In addition, the LSD Post Hoc Test was conducted to see significant differences in each group. The results obtained in the temperature variation showed a significant difference at 70 ℃. At 70 ℃ has a higher adsorption capacity compared to temperatures of 60 and 80 ℃. In the concentration variation, significant differences were seen at concentrations of 50, 75 and 100 ppm.

Imprinting Factor (IF)

Molecularly imprinted polymers are characterized by the creation of mold cavities in the polymer. The efficiency of successful polymer imprinting can be measured by the imprinting factor (IF). The Imprinting Factor is the ratio of adsorption results obtained from the printed polymer (MIP) to the adsorption results on the blank polymer (PB) [25].

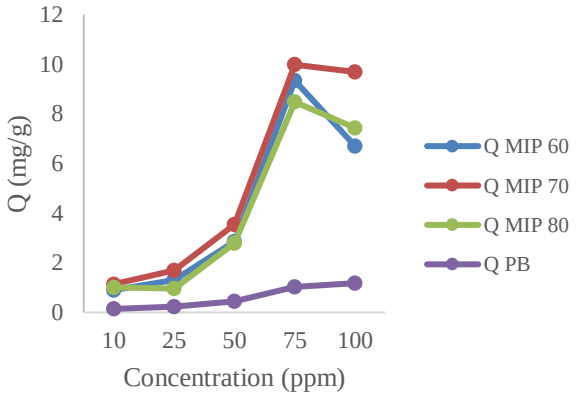


Figure 10. Comparison Q MIP and Q PB

In the figure, the adsorption capacity (Q) value of MIP is much higher than the adsorption capacity of PB. This shows that the formation of a mold cavity in MIP so that template binding occurs is characterised by MIP's high adsorption capacity value. In PB, there is no mold cavity, so the adsorption that happens is very low only on the surface of the adsorbent—f[26].

Table 2. Imprinting Factor

Concentration (ppm)	IF 60	IF 70	IF 80
10	6.4285	8.1428	7.1428
25	5.4167	7.0833	4.0012
50	6.5004	8.0455	6.3181
75	9.1568	9.7843	8.3137
100	5.6779	8.2033	6.3050

The results of the IF value are shown in Table 3.
IF values greater than one (IF>1) indicate good printing [27]. In this study, the IF value is more than one, which indicates that the MIP manufacturing successfully makes the CAP mold cavity. The best MIP extraction temperature variation is 70℃, as indicated by the higher IF value than other variations.

Conclusion

Based on the results of research on the effect of extraction temperature of Molecularly Imprinted Polymer (MIP) on the adsorption ability of chloramphenicol (CAP) based on diazotation reaction, it can be concluded that the characterization of PB, NIP and MIP using FTIR shows the similarity of groups between polymers. In NIP, the NO_2 group, a typical chloramphenicol group, was found at wave numbers 1536 cm^{-1} and 1322 cm^{-1}. The results of the per cent extraction test at each temperature variation of 60, 70, and 80 ℃ are 75.64%, 89.63% and 74.08%, respectively, directly proportional to the adsorption results, at a temperature of 70 ℃ has the highest value. The results of the Two-Way Anova test resulted in a significance of 0.044 (Sig = 0.044 < 0.05), so the extraction temperature affects the adsorption ability of CAP. The temperature of 70℃ showed the best adsorption results. Imprinting Factor (IF) values of all variations are greater than one (IF>1), indicating good imprinting. The highest IF value is shown in MIP with a temperature variation of 70 ℃.

Author's Contribution

Eka Faradila Oktaningtias: contribution to study conception and design, data collection, wrote draft manuscript, reviewed the result; Maria Monica Sianita: contribution to study conception and design, performed the analysis, reviewed the result.

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References

[1] Mustapa and M. A. Zulfikar, "Polimer Bercetakan Molekul sebagai Adsorben Zat Warna Metilen Biru," *Fullerene Chemistry*, vol. 8, pp. 1–5, 2023, doi: 10.37033/fjc.v8i1.497.

[2] Maimuna, F. Monado, and I. Royani, "Studi Awal Pengaruh Kloroform Sebagai Pelarut Pada Proses Ekstraksi Molecularly Imprinted Polymer (MIP) Nano Kafein," *Jurnal Fisika*, vol. 10, no. 1, pp. 1–7, 2020, doi: https://doi.org/10.15294/jf.v10i1.23806.

[3] L. Ratnasari, "Pengaruh Waktu Proses Ekstraksi Pada Material Moleculary Imprinted Polymer (MIP) Karbaril Terhadap Jumlah Rongga Dan Gugus Fungsi Yang Terbentuk," Universitas Sriwijaya, 2018. Accessed: Aug. 24, 2024. [Online]. Available: http://repository.unsri.ac.id/id/eprint/8177

[4] J. Tristi and M. Muchtaridi, "Molecularly Imprinted Polymer (MIP) Untuk Isolasi Atenolol Dalam Sampel Biologis," Aug. 2018, doi: 10.24198/jf.v16i1.17485.

[5] M. M. Sianita and N. Nyoman Tri, "Comparison of the method used for extraction chloramphenicol from its Molecularly Imprinted Polymer (MIP) using chloroform as porogen," 2019, doi: 10.2991/snk-19.2019.5.

[6] H. Suseno, S. Hudiyono, and M. Muslim, "Elimination of Chloramphenicol by Tiger Shrimp (Penaeus monodon) and White Shrimp (Litopenaeus vannamei)," *Hayati*, vol. 23, no. 3, pp. 117–120, Jul. 2016, doi: 10.1016/j.hjb.2016.07.001.

[7] G.-F. Pang, "Chloramphenicol," in *Analytical Methods for Food Safety by Mass Spectrometry*, Elsevier, 2018, pp. 123–141. doi: 10.1016/B978-0-12-814165-6.00004-X.

[8] S. Pardeshi and S. K. Singh, "Precipitation polymerization: A versatile tool for preparing molecularly imprinted polymer beads for chromatography applications," 2016, *Royal Society of Chemistry*. doi: 10.1039/c6ra02784a.

[9] I. Royani *et al.*, "Temperature in the extraction process: The number of cavities created in polymer based on molecularly imprinted polymer (mip)

caffeine," in *Key Engineering Materials*, Trans Tech Publications Ltd, 2020, pp. 297–302. doi: 10.4028/www.scientific.net/KEM.860.297.

[10] E. O. F. Listiadi and M. M. Sianita, "The Effect Of Variation Extractant Composition On Adsorption Capability Of Chloramphenicol Using Molecularly Imprinted Polymer (MIP)," *UNESA Journal of Chemistry*, vol. 10, no. 3, 2021, doi: https://doi.org/10.26740/ujc.v10n3.p337-347.

[11] D. A. Khuswanto, "The Effect of Variations Concentration of Chloramphenicol on the Imprinting Factor of Molecularly Imprinted Polymer," *International Journal of Current Science Research and Review*, vol. 05, no. 07, Jul. 2022, doi: 10.47191/ijcsrr/V5-i7-09.

[12] M. Juliana and M. Yulian, "Identifikasi Kloramfenikol Pada Udang Vaname (Litopenaeus Vannamei) Menggunakan High Performance Liquid Chromatography (HPLC)," *AMINA*, pp. 13–18, 2020, doi: https://doi.org/10.22373/amina.v2i1.493.

[13] E. E. Udo, S. S. Boswihi, B. Mathew, B. Noronha, and T. Verghese, "Resurgence of chloramphenicol resistance in methicillin-resistant staphylococcus aureus due to the acquisition of a variant florfenicol exporter (Fexav)-mediated chloramphenicol resistance in Kuwait hospitals," *Antibiotics*, vol. 10, no. 10, Oct. 2021, doi: 10.3390/antibiotics10101250.

[14] X. Xie *et al.*, "Quantitative analysis of chloramphenicol, thiamphenicol, florfenicol and florfenicol amine in eggs via liquid chromatography-electrospray ionization tandem mass spectrometry," *Food Chem*, vol. 269, pp. 542–548, Dec. 2018, doi: 10.1016/j.foodchem.2018.07.045.

[15] N. A. Khudzaifah and M. M. Sianita, "Determination of the optimum concentration of the coupling agent in chloramphenicol analysis," *World Journal of Advanced Research and Reviews*, vol. 15, no. 1, pp. 525–533, Jul. 2022, doi: 10.30574/wjarr.2022.15.1.0719.

[16] S. Pardeshi and S. K. Singh, "Precipitation polymerization: A versatile tool for preparing molecularly imprinted polymer beads for chromatography applications," 2016, *Royal Society of Chemistry*. doi: 10.1039/c6ra02784a.

[17] A. Sorribes-Soriano, F. Albert Esteve-Turrillas, S. Armenta, and J. Manuel Herrero-Martínez, "Molecularly imprinted polymer –stir bar sorptive extraction of diazepam from natural water," *Microchemical Journal*, vol. 186, Mar. 2023, doi: 10.1016/j.microc.2022.108354.

[18] M. Alauhdin, W. Tirza Eden, and D. Alighiri, "Aplikasi Spektroskopi Inframerah untuk Analisis Tanaman dan Obat Herbal," *Informasi Sains dan Kesehatan*, 2017, doi: 10.15294/.v0i0.15.

[19] F. F. Azizah, M. M. Sianita, and G. Supriyanto, " Optimization Of Chloramphenicol Reduction Using Zn As Reductor By Spectrophotometry Uv-Vis," *UNESA Journal of Chemistry*, vol. 4, no. 2, 2015, doi: 10.26740/ujc.v4n2.p%25p.

[20] A. Wafi, G. Supriyanto, and T. S. Tjahjandarie, "Method Validation of Chloramphenicol Analysis in the Shrimp Based on Diazotization Reaction," 2020. doi: https://doi.org/10.24198/ijpst.v7i2.26520.

[21] S. A. Apriliyani, Y. Martono, C. A. Riyanto, M. Mutmainah, and K. Kusmita, "Validation of UV-VIS Spectrophotometric Methods for Determination of Inulin Levels from Lesser Yam (Dioscorea esculenta L.)," *Jurnal Kimia Sains dan Aplikasi*, vol. 21, no. 4, pp. 161–165, Nov. 2018, doi: 10.14710/jksa.21.4.161-165.

[22] M. Garg and N. Pamme, "Strategies to remove templates from molecularly imprinted polymer (MIP) for biosensors," Jan. 01, 2024, *Elsevier B.V.* doi: 10.1016/j.trac.2023.117437.

[23] A. T. Getachew, S. L. Holdt, A. S. Meyer, and C. Jacobsen, "Effect of Extraction Temperature on Pressurized Liquid Extraction of Bioactive Compounds from Fucus vesiculosus," *Mar Drugs*, vol. 20, no. 4, Apr. 2022, doi: 10.3390/md20040263.

[24] F. Wijayani and G. Supriyanto, "Karakterisasi Molecularly Imprinted Polymer (MIP) Hasil Polimerisasi Presipitasi Sebagai Adsorben Kloramfenikol," vol. 17, no. 2, 2015, Accessed: Sep. 24, 2024. [Online]. Available: http://repository.unair.ac.id/id/eprint/49811

[25] M. N. Azalina, K. L. Chua, and I. Noorhidayah, "Theoretical and experimental study of valine molecular imprinted polymer using sol-gel process on silica microparticles surface," in *IOP Conference Series: Materials Science and Engineering*, Institute of Physics Publishing, Dec. 2018. doi: 10.1088/1757-899X/458/1/012012.

[26] S. Bakhtiar, S. A. Bhawani, and S. R. Shafqat, "Synthesis and characterization of molecular imprinting polymer for the removal of 2-phenylphenol from spiked blood serum and river water," *Chemical and Biological Technologies in Agriculture*, vol. 6, no. 1, Dec. 2019, doi: 10.1186/s40538-019-0152-5.

[27] A. N. Hasanah, N. Safitri, A. Zulfa, N. Neli, and D. Rahayu, "Factors affecting preparation of molecularly imprinted polymer and methods on finding template-monomer interaction as the key of selective properties of the materials," Sep. 02, 2021, *MDPI*. doi: 10.3390/molecules26185612.