WJARR

World Journal of Advanced Research and Reviews

World Journal Series
INDIA

(RESEARCH ARTICLE)

Check for updates

Calculation of enrichment factor in chloramphenicol analysis in shrimp with variation of concentration using molecularly imprinted polymer

Gora Fahman and Maria Monica Sianita *

Department of Chemistry, Faculty of Mathematic and Natural Sciences, Universitas Negeri Surabaya, Indonesia.

Abstract

This research aims to determine the effect of chloramphenicol concentration in shrimp toward the value of enrichment factor (EF) in the adsorption-desorption process using Molecularly Imprinted Polymer (MIP) as adsorbent. The enrichment factor describes how much analyte concentration is transferred from the sample to the solvent. In this research, the highest enrichment factor (EF) value was obtuined for chloramphenicol concentration of 50 ppm which was 120,086%. The Scanning Electron Microscope (SEM) analysis showed that the sizes of Blank Polymer (PB), Non-Imprinted Polymer (NIP), and Molecularly Imprinted Polymer (MIP) were 12, 18, and 23 nm, respectively. The detection limit value (LOD) was 0.0981 μg/mL, and the quantitation limit value (LOQ) was 0.3273 μg/mL. At this limit of detection (LOD) no chloramphenicol was detected in the analyzed shrimp.

Keywords: chloramphenicol; EF; Recovery; MIP; SEM

1. Introduction

Shrimp is one of the most economically valuable fishery commodities. According to the Central Bureau of Statistics, the average shrimp production in Surabaya in 2017 was 6,799 tons/ha, while the average shrimp production in East Java province in 2017 was 311,666 tons/ha. Fishery commodities (shrimp and fish) are currently spreading about the use of chloramphenicol in the local market, regional, and international markets, hindering and even preventing exports, particularly of shrimp from Indonesia to various countries around the world. Using chloramphenicol as an antibiotic can inhibit disease development in shrimp farming while simultaneously increasing the shrimp's weight. Indirectly, the bodies of shrimp that consume antibiotics throughout their lives will contain antibiotic residues. The antibiotic residue will enter the human body, can accumulate if the shrimp is consumed and caused many health problem [1].

Eventhough the Minister of Health Regulation banned the use of chloramphenicol, facts showed that in another research Saputra and Arfi in 'Analysis Chlorampenicol residue in shrimp' found 0,2 ppm chloramphenicol residue in shrimp [2], [3]. So the sophisticated method was needed to analyze the presense of Chloramphenicol in aquaculture, such as High Performance Liquid Chromatography (HPLC), ELISA, etc. This research will analyze the residues of Chloramphenicol in shrimp using HPLC. On of it is, since the amount of this chloramphenicol in shrimp was very small, such a method preconcentration was needed used was molecularly imprinted polymer (MIP).

Molecularly Imprinted Polymer (MIP) is a method designed to create a porous polymer through an extraction (leaching) [4]. This pore identifies the target molecule (template) with identical size, structure, and physicochemical properties as the analyte [5]. As an absorbent, MIP is utilized for separation, concentration, analysis of the target substance (template), and elimination of unused target substances [6]. Several factors, including the type of adsorbent, the type of substance being absorbed, the surface area, the concentration of the adsorbed substance, and temperature [7], can influence the adsorbing capacity of this MIP. Given the previous description, the researcher wishes to calculate the

---

* Corresponding author: Maria Monica Sianita
Department of Chemistry, Faculty of Mathematic and Natural Sciences, Universitas Negeri Surabaya, Indonesia.

enrichment factor of chloramphenicol in shrimp applications utilizing MIP with varying chloramphenicol concentrations

2. Material and methods

2.1. Material and Equipment

Shrimp (obtained at a fish auction in Surabaya), Chloramphenicol (CAP) (Sigma Aldrich), ethanol (Merck), methanol (Merck), acetonitrile (Sigma Aldrich), glacial acetic acid, formic acid (Merck), trichloroacetic acid (TCA) (Merck), aquabidest. High-Performance Liquid Chromatography (HPLC) (Shimadzu LCsolution Analysis), analytical balance (Ohaus), magnetic stirrer, hotplate stirrer (Daihan Scientific), vortex mixer (Velp Scientifica), Scanning electron microscope (SEM) (FEI Inspect-S50), chemical glass (Iwaki pyrex), test tube, tube rack, dark bottle, erlenmeyer, vial, volume pipette, dropper, Whatman filter paper, volumetric flask, 100 ml measuring cup (Iwaki).

2.2. Method

2.2.1. Sample Preparation

The sample used in this research was a sample of shrimp. The sampled shrimp meat was cleaned and peeled, then weighed to 100 g. After being weighed and mashed to a homogeneous shrimp meat consistency, the meat was put into a beaker, then 100 ml of 15% TCA was poured on it, and allowed to stand for 1 night until the filtrate and residue were obtained

2.2.2. MIP Synthesis

MIP is produced by extracting a Non-Imprinted Polymer (NIP). NIP was synthesized by dissolving 1 mmol of CAP in 25 ml of acetonitrile porogen and subsequently adding 3 mmol of methacrylic acid (MAA). MIP synthesis was taken from 1 g of NIP with a mixture of methanol and acetic acid solution with a volume ratio of 85:15 (mL) by maceration for 5 hours at a constant temperature of 70°C. The extracted solution was subsequently filtered and rinsed with aquabides, methanol, and acetonitrile . The obtained MIP was then oven-dried at 40 °C. until a constant weight was achieved.

2.2.3. MIP Application on Shrimp for Standart Addition

The blank solution of shrimp filtrate was obtained at 10 mL, then put into a 50 mL volumetric flask, and methanol was added to the limit mark. The mixing of the solution produced filtrate and residue. The blank filtrate was then analyzed using High Performance Liquid Chromatography (HPLC).

Variations concentration of CAP in shrimp, in which the filtrate from each shrimp sample was transferred to a separate 50 mL volumetric flask, followed by the addition of CAP at concentrations of 10, 25, and 50 ppm. Then, methanol was added to mark the limit, and the mixture was shaken. From this mixing, filtrate and residue were produced, the obtained filtrate was then analyzed HPLC.

2.2.4. CAP Adsorption on Shrimp using MIP

The concentration of CAP in shrimp at 10, 25, and 50 ppm was taken up to 25 mL and placed in an Erlenmeyer before 0.05 g of MIP was added. The solution was vortexed for 20 minutes until homogeneous, then separated between the filtrate and residue, was then used to analyze the filtrate using HPLC.

2.2.5. CAP Desorption on Shrimp using MIP

After the adsorption phase, the residue is carried out by the desorption procedure helpful in recovering the released CAP. To each concentration variation's residue, 10 mL of ethanol was added, and the solution was vortexed for 20 minutes until it became homogenous. It was filtered immediately after being vortexed to separate the filtrate from the residue. HPLC was used to analyze the filtrate results and calculate the enrichment factor value.

3. Results and discussion

3.1. Shrimp Sample Preparation

The samples of cleaned shrimp were then weighed at 100 grams. Then 100 ml of 15% TCA was added. The addition of 15% TCA to the sample can disrupt the hydrogen bonds in the water, preventing protein molecules from dissolving and allowing them to be recovered via centrifugation. The addition of acid can result in pH changes that alter the structure of the protein, the protein becomes positively charged when acid is added because the amino groups on the protein capture protons, resulting in a decrease in pH and precipitation of the protein [8].
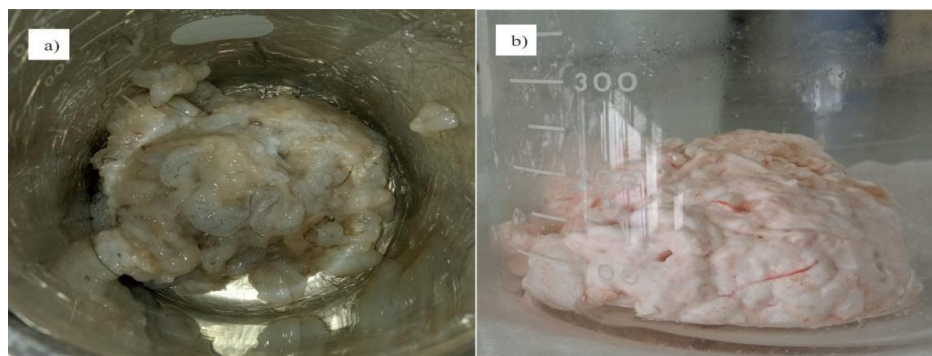

Figure 2 Shrimp samples (a) before the addition of 15% TCA and (b) after the addition of 15% TCA

3.2. MIP Synthesis

Maceration was used to extract the Non-Imprinted Polymer (NIP) in order to remove the template MIP. The methanol solvent was selected because it is polar and can break the hydrogen bonds between chloramphenicol and the MAA template due to the hydrogen bonding force between the analyte and MIP being replaced by more intensive hydrogen bonds between methanol and MIP [9]. Acetic acid was chosen because it can affect the hydrogen bonds between the templates so that the template will be easily separated [10]. After the MIP extraction process was complete, methanol, aquabiosis, and acetonitrile were used to rinse the residue. Aquabides are used in the rinsing process to remove any remaining acetic acid, while methanol attracts CAP so that it does not come off [11]. Meanwhile, rinsing with acetonitrile increased MIP pore size [10].

3.3. LOD and LOQ Value

In a research, both a limit of detection (LOD) and a limit of quantization (LOQ) will be required. LOD is the smallest limit test parameter owned by the instrument for detecting a certain number of analytes in a sample with an absorption value, whereas LOQ is the smallest amount of analytes in a sample that can be accurately measured by the instrument [12].

In this research, the LOD was 0.0981 µg/mL, and the LOQ was 0.3273 µg/mL. The values were derived from the linear calibration curve for chloramphenicol over the concentration range of 0.5 ppm to 5 ppm, with the line equation $y = 14828x - 503.16$ and a regression value of 0.9998. The LOD value can detect the presence of chloramphenicol in shrimp if the concentration is greater than or equal to 0.0981 µg/mL. The resulting detection limit is the minimum for detecting chloramphenicol levels in shrimp.

In addition, the research [13] revealed that the detection limit value (LOD) for chloramphenicol in beef was 0.5 µg/mL and the quantity limit value (LOQ) was 1.2 µg/mL. In contrast, the LOD and LOQ values in the chicken liver were 0.7 µg/mL and 2.7 µg/mL, respectively. In the research [14], the LOD value for milk was determined to be 0.17 µg/mL. Based on some of these studies' findings, the value of the detection limit is higher than in this research, so this research yielded better results than previous ones.

3.4. MIP Application on Shrimp for Standart Addition

In the application of MIP to shrimp, there were blank samples and samples of varying concentrations of 10, 25, and 50 ppm. The prepared filtrate was then added with standard CAP with variations of 10, 25, and 50 ppm, and then the addition recovery value was calculated. The addition method is adding an analyte of a specific concentration to an analysis sample. The percentage of analyte present in the sample can be used to calculate the recovery rate.

Based on table 3. calculation of addition recovery value, the recovery results obtained are 90.02%-123.45% indicating that the method used is quite accurate. The research explained that the standard range of allowable addition recovery values ranged from 80-110% [15].

Table 3 Percent Recovery Addition to MIP against CAP

Ca	C1	C2	C3	% Recovery
Blank	0	0	0	0
10	7.33	0.36	0.94	123.45%
25	23.32	1.17	6.22	101.61%
50	49.98	2.49	2.98	90.02%

Description: Ca: Initial Concentration (ppm); C1: Concentration of adsorbed CAP; C2: Mass of adsorbed CAP; C3: Concentration of CAP desorbed

This research concludes that MIP has an excellent ability to inhibit chloramphenicol in shrimp. In this research, the blank sample revealed that the samples analyzed by HPLC contained no chloramphenicol because they did not reach the set detection limit (LOD) of 0.0981 µg/mL.

3.5. Enrichment Factor

Subsequent testing of this concentration variation using MIP adsorbent, by taking as much as 25 mL of each sample then vortexed for 20 minutes. The filtrate was then separated from the residue, which was analyzed with HPLC.

After adsorption, the desorption stage of the MIP was performed to determine the CAP in the sample. MIP was added with 10 ml of ethanol to retract the adsorbed CAP. Because ethanol is an organic solvent and CAP can be dissolved in ethanol, ethanol was used as the solvent. The Enrichment Factor (EF) value was obtained through HPLC analysis of the desorption filtrate. The adsorption-desorption test aims to determine the EF value so that the concentration factor of the standard CAP solution can be applied to the sample. The formula for calculating EF is as follows

$$EFth : \frac{Vs}{Ve}$$

Description:
EF_{tr} = EF true
V_s = Analyte volume
V_e = Solvent volume

As for the true EF, it is obtained from multiplying the theoretical EF with recovery (R) or the average accuracy of the calibration curve, which is formulated as follows:

$$EF_{tr} = EF_{th} \times R$$

Description :
Eftr = EF True
Efth = EF teori
R = Recovery

Table 2 Calculation result of EF Value

Concentration (ppm)	Adsorped CAP	Adsorped Mg CAP	EF True	% EF True
Blanko	0	0	0	0
10	0.94	0.009	0.025	105.521
25	6.22	0.062	0.158	116.927
50	2.97	0.029	0.048	120.086

Table 2 shows that the EF value in the shrimp application is proportional to the concentration used, with the recovery range of chloramphenicol concentration variations of 105.521-120.086%. The highest %EF value in this research was found at a concentration variation of 50 ppm. In this instance, recovery addition refers to the concentration of standard

chloramphenicol after it has been added to the sample, whereas the enrichment factor (EF) measures the concentration factor that has occurred in the standard solution after it has been added to the sample [16].
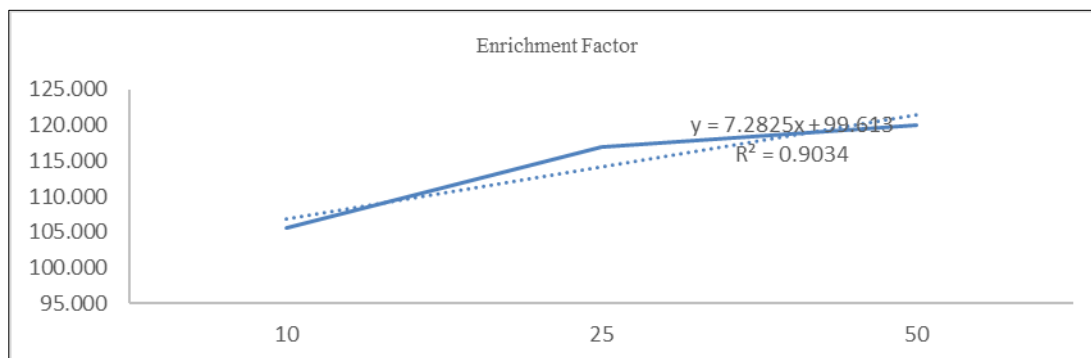

Figure 3 Value enrichment factor

Graph 1 shows an increase in the results enrichment factor (EF) because it is in accordance with other research [17] which explain that if the value enrichment factor (EF) increases, it corresponds to an increase in concentration which is useful in recovering for chlorampenicol. While in graph 2 shows a decrease in the value of addition recovery, according to [16] in calculating the value of addition recovery the higher the concentration, the higher the value of %recovery addition obtained but in this research it is not in accordance with previous researchers. This is due to several factor that affect the sample.
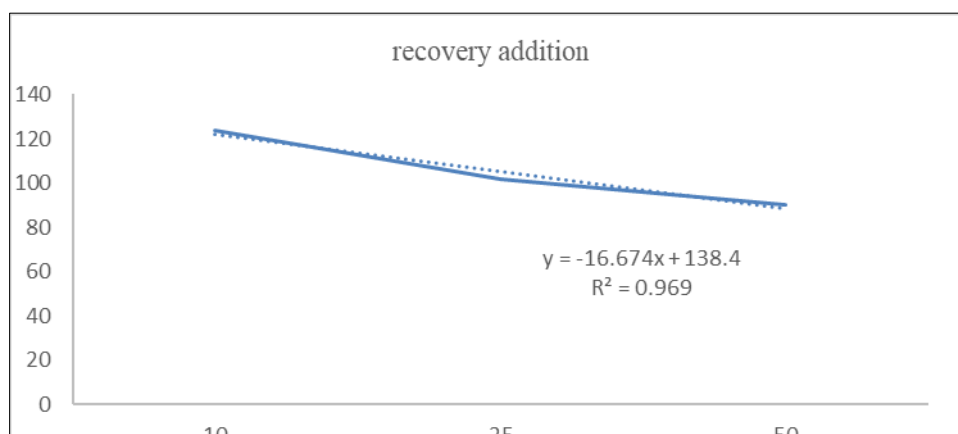

Figure 4 Value %recovery addition

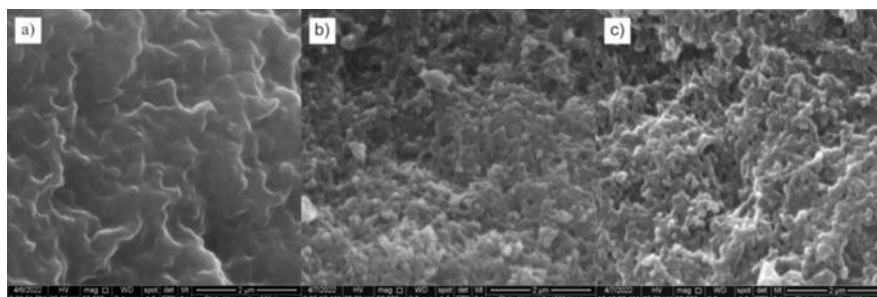### 3.6. Characterization of PB, NIP, and MIP using SEM

Figure 5 Characterization of (a) PB, (b) NIP, (c) MIP using 50.000x . magnification

The Scanning Electron Microscope (SEM) is commonly used to examine the morphology of the surface structure of samples under high magnification and can provide information on the structure's state [18]. Blank Polymer (PB), Non-

Imprinted Polymer (NIP), and Molecularly Imprinted Polymer (MIP) must be characterized in order to determine the particle's surface area, which influences the adsorption capacity.

4. Conclusion

Based on the research, it can be concluded that the value of %recovery addition to chloramphenicol in shrimp with variations in CAP concentrations between 90.02 and 123.45% has a high degree of precision and is acceptable because it falls within the standard addition range of 80-110%. The enrichment factor was calculated to determine how much the concentration factor of the standard solution was, and the %EF results were 105.521-120.086%. In this research, the detection limit (LOD) was determined to be 0.0981 µg/mL, while the quantity limit value (LOQ) was 0.3273 µg/mL.

The suggestion further research needs to be done with a concentration lower than 10 ppm to determine the enrichment factor (EF) value and percent recovery.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

References

[1] Alghifari D, Kuswandi B, Pratoko DK. Pengembangan Sensor Kloramfenikol Berbasis Imobilisasi Bovine Serum Albumin (BSA) pada Selulosa Asetat dengan Metode Spektroflorometri (The Development of Chloramphenicol Sensor Based on Bovine Serum Albumin (BSA) Immobilization on Cellulose Acetate using Spectrofluorometry Method). Pustaka Kesehat. 2017;5(1):40–5.

[2] Saputra SA, Arfi F. Analisis Residu Kloramfenikol Pada Udang Windu (Penaeus Monodon) Menggunakan High Performance Liquid Cromatography (HPLC). AMINA. 2019;1(3):126–31.

[3] Nurrokhimah M, Nurerk P, Kanatharana P, Bunkoed O. A nanosorbent consisting of a magnetic molecularly imprinted polymer and graphene oxide for multi-residue analysis of cephalosporins. Microchim Acta. 2019;186(12):1–9.

[4] Yusuf TM, Sianita MM. The Effect Of Amount Of Porogen On MIP (Molecularly Imprinted Polymer) Synthesis Toward The Adsorption Of MIP-Chloramphenicol. UNESA J Chem. 2022;11(1):53–60.

[5] Aprilia R. Analisis Kadar Residu Antibiotik Kloramfenikol Dalam Ikan Mas (Cyprinus Carpio) Dari Budidaya Ikan Daerah Rancaekek Dengan Metode Kromatografi Cair Kinerja Tinggi. [S.Si. thesis]. Universitas Al-Ghifari; 2018.

[6] Sianita MM, Puspaningsih NNT, Khazanah M, Supriyanto G. Comparison of the method used for extraction chloramphenicol from its Molecularly Imprinted Polymer (MIP) using chloroform as porogen. In: National Seminar on Chemistry 2019 (SNK-19). Atlantis Press; 2019. p. 77–8.

[7] Wijayanti IE, Kurniawati EA, Solfarina S. Studi Kinetika Adsorpsi Isoterm Persamaan Langmuir dan Freundlich pada Abu Gosok sebagai Adsorben. EduChemia (Jurnal Kim dan Pendidikan). 2019;4(2):175–84.

[8] Niu L, Zhang H, Wu Z, Wang Y, Liu H, Wu X, et al. Modified TCA/acetone precipitation of plant proteins for proteomic analysis. PLoS One. 2018;13(12):e0202238.

[9] Mirzajani R, Keshavarz A. The core–shell nanosized magnetic molecularly imprinted polymers for selective preconcentration and determination of ciprofloxacin in human fluid samples using a vortex-assisted dispersive micro-solid-phase extraction and high-performance liquid chromatography. J Iran Chem Soc. 2019;16(11):2291–306.

[10] Shafqat SR, Bhawani SA, Bakhtiar S, Ibrahim MNM. Synthesis of molecularly imprinted polymer for removal of Congo red. BMC Chem. 2020;14(1):1–15.

[11] Chrisnandari RD. Sintesis dan Karakterisasi Molecularly Imprinted Polymer Untuk Kloramfenikol Menggunakan Polimerisasi Fasa Ruah. J Pharm Sci. 2018;3(1):40–6.

[12] Sumarno D, Kusumaningtyas DI. Penentuan Limit Deteksi Dan Limit Kuantitasi Untuk Analisis Logam Timbal (Pb) Dalam Air Tawar Menggunakan Alat Spektrofotometer Serapan Atom. Bul Tek Litkayasa Sumber Daya dan Penangkapan. 2019;16(1):7–11.

[13] Rahimi Z, Shahbazi Y, Ahmadi F. Polypyrrole as an efficient solid-phase extraction sorbent for determination of chloramphenicol residue in chicken liver, kidney, and meat. Food Anal Methods. 2017;10(4):955–63.

[14] Qin D, Wang J, Ge C, Lian Z. Fast extraction of chloramphenicol from marine sediments by using magnetic molecularly imprinted nanoparticles. Microchim Acta. 2019;186(7):1–10.

[15] AOAC. Official methods of analysis. Vol. 222. Association of Official Analytical Chemists Washington, DC; 2016.

[16] Sianita MM. Pengembangan Metode Analisis Kloramfenikol Dalam Akuakultur Secara Fotometrik Menggunakan Molecular Imprinted Polymer (MIP) Sebagai Adsorben. [Doctral Dissertation]. Universitas Airlangga; 2018.

[17] Najafi A, Farajmand B, Sharafi HR, Yaftian MR. A fast and sensitive detection of low-level chloramphenicol in food samples using the IMS/homogenizer assisted DLPME combination. J Food Compos Anal. 2022;105:104204.

[18] Adhika DR, Anindya AL, Tanuwijaya VV, Rachmawati H. Teknik pengamatan sampel biologi dan non-konduktif menggunakan scanning electron microscopy. In: Prosiding Seminar Nasional Instrumentasi, Kontrol dan Otomasi. 2018. p. 53–8.