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Selectivity of Molecularly Imprinted Polymer-based Chitosan Membrane for Caffeine Separation by Filtration

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Abstract

Caffeine is a chemical compound that can be isolated from coffee beans. The isolation and purification of caffeine compounds have been successfully carried out using a filtration method using chitosan biomaterial synthesized by molecularly imprinted polymer (MIP). Chitosan as a functional polymer is dissolved in acetic acid (2% v/v) with stirring at 60°C overnight until a clear and homogeneous solution is formed. Furthermore, caffeine as a template molecule is added to the polymer solution and stirred for 60 minutes. The polymer solution is poured into a petri dish until evenly distributed and dried in an oven at 60°C for 12 hours. The dried polymer solution is then soaked in sodium tripolyphosphate as a crosslinking solution and dried again in the oven until the membrane sheet separates from the petri dish. The membrane sheet is then washed using distilled water until the pH is neutral and continued washing with chloroform to remove caffeine from the membrane pores to form an MIP membrane. The formed MIP membrane is characterized using FTIR and SEM. The MIP membrane was then cut into a circle, inserted into an empty syringe, and used to filter samples containing caffeine. The filtration results were analyzed using a UV-Vis spectrophotometer which showed that caffeine filtered with the MIP membrane contained less interference than caffeine filtered with a non-imprinted polymer membrane (NIP). Quantitative analysis was also carried out using the spiked sample method to determine the recovery value of the filtration results. From this study, the percentage recovery value (% recovery) was obtained at 96.16 - 103.80%.

Keywords: *Caffeine, Chitosan, Filtration, Molecularly imprinted polymer (MIP)*

Introduction

Caffeine (1,3,7-trimethylxanthine) is a purine alkaloid and is one of the most widely consumed pharmacologically active substances. Naturally, caffeine is found in more than 60 plant species including coffee, cocoa, tea, kola nuts, and guarana fruit [1]. Caffeine is also contained in several dietary supplements for weight loss and improving sports performance in combination with p-synephrine. Caffeine in the health field acts as a central nervous system stimulant, and mild diuretic, increases heart rate and blood pressure, and stimulates gastric secretion. Various painkillers (such as aspirin and acetaminophen) are used in combination with caffeine to treat migraine headaches [2,3].

Several extraction methods have been developed to isolate caffeine from several samples of ground coffee with soxhlet extraction, microwave-assisted extraction, solid phase extraction, and other related techniques[3,4]. In this study, the results obtained were a % yield in each sample of 1.381% in sample A, 1.363% in sample B, 1.233% in sample C, 1.010% in sample D, 1.013% in sample E, and 0.953%. Liquid-liquid extraction was also applied in a study conducted by [2] which used samples in the form of several brands of tea in

India with measurement of levels using Thin Layer Chromatography [2]. In this study, the results obtained were yields in various brands of tea, namely Darjeeling tea as much as 0.67%; Temi tea as much as 0.72%; and Green tea as much as 0.65% [2]. The technique of isolating caffeine through liquid-liquid extraction is the most frequently used technique because it can eliminate interfering matrix components, concentrate the analyte, and change the solvent so that the targeted analyte is in accordance with the analytical technique used [5]. Meanwhile, the weakness of this method is the use of a large amount of solvent, because in this method there is strong shaking which will cause an emulsion, and its selectivity is low [5].

Based on these problems, a more selective extraction method with higher yield is needed, namely solid phase extraction (SPE). This extraction is carried out by flowing the solution through a solid phase as a column filler [3]. This technique has advantages, namely a more perfect extraction process, separation of analytes from interfering matrices becomes more efficient, reduces the amount of organic solvents used, and is easier to automate [5]. In this solid phase extraction there are stages of conditioning, retention and, elution. While in this study, a modification of the SPE process stages was carried out, namely at the retention stage to filtration. This is because in this study a membrane was used that has certain pores to filter the caffeine that passes through it. This membrane is known as a molecularly imprinted polymer (MIP) membrane.

MIP is a synthetic polymer made using the molecularly imprinted (MIT) technique to produce polymers that have specific binding sites according to the target molecule [6]. Molecularly imprinted polymer (MIP) has several advantages, namely high selectivity and affinity for the target molecule (template) used in the printing procedure, high physicochemical stability, low production costs, and its service life can reach several years at room temperature. Some synthetic monomers used in the synthesis of MIP include ethylene glycol dimethacrylate (EGDMA) and tetraethyl orthosilicate (TEOS)[7,8]. The use of these synthetic monomers requires hazardous organic solvents, so in the synthesis of MIP in this study chitosan was used as a natural biopolymer. Chitosan has several advantages such as having an effective adsorption capacity for heavy metal ion cations and cations from organic substances (proteins and fats) due to the presence of hydroxyl and amino groups along the polymer chain, non-toxic, the ability to form excellent film membranes so that it can be used as an adsorbent for the binding process of organic and inorganic substances [6,9–11]. Based on the background above, this study conducted the development of filter material for caffeine isolation using molecularly imprinted chitosan polymer with sodium tripolyphosphate crosslinker. The developed material was then applied to isolate caffeine from coffee beans. Qualitative and quantitative analyses were carried out to determine the selectivity of caffeine separation using chitosan-sodium tripolyphosphate MIP from coffee beans.

Experimental

Equipment

The tools used in this study include a UV-Vis spectrophotometer (Thermo Scientific Genesis 10S), ultrasonicator (Lokal), analytical balance (Ohaus PA214), cuvette (Hellma), magnetic stirrer (Kartell), vortex, Whatman 42 filter paper, plastic wrap, aluminum foil, mortar, stamper, 60 mesh sieve, FTIR (Shimadzu IRSpirit-T), and SEM (Jeol JED-2300).

Material

Some of the research materials used have a pro-analysis purity level (p.a) and are Merck products, Germany, including caffeine, sodium tripolyphosphate (Na-TPP), chloroform, and glacial acetic acid, ethyl acetate, sodium bicarbonate, methanol, TLC plates, and ammonium hydroxide (Merck, Germany). While the chitosan used is food grade and medical grade chitosan (DAC> 85%, flake form 30-50 mesh) (PT. Biokitosan Indonesia). Aquades (Brataco), coffee sample without sugar (Kapal Api).

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Methods

MIP Membrane Synthesis

The MIP membrane synthesis process carried out in this study is a modification of the method carried out by Bagheri and Ghaedi (2019). The modification of the method carried out in this study is the use of caffeine as a template and Na-TPP as a cross-linker [9].

The preparation of (MIP) begins with the preparation of chitosan solution. A total of 0.42 grams of chitosan is put into a 50 ml beaker and added to 20 ml of 2% acetic acid solution. The mixture is then stirred using a magnetic stirrer for 24 hours at a temperature of 60 ° C until a clear suspension is formed. Then added with 0.042 grams of caffeine and continued stirring for + 1.5 hours at a temperature of 60 ° C. Then the solution is poured into a petri dish and dried in an oven at a temperature of 60 ° C for 12 hours. The crosslinker in the chitosan polymer that has been added with caffeine is soaked in 0.5 mol L-1 sodium tripolyphosphate as much as 20 ml for 30 minutes. After 30 minutes, the polymer formed is dried again using an oven at a temperature of 60 ° C until it peels off to form a chitosan membrane sheet. After drying, the membrane is rinsed with deionized water until a neutral pH is obtained and dried again in the oven. The dried membrane was soaked in chloroform and stored in a refrigerator for 10 minutes. Furthermore, to remove the template from the pores of the membrane, the membrane that had been soaked in chloroform was ultrasonicated for 5 minutes several times. The soaking and ultrasonication process was repeated until the residual soaking filtrate was not detected as containing caffeine on the UV-Vis spectrophotometer. The preparation of non-imprinted polymer (NIP) was carried out with the same procedure as MIP, except that the caffeine template was not added to the polymer solution.

Characterization of MIP Membranes Using Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (SEM)

The physical characterization of the formed MIP and NIP powders was carried out by determining the functional groups using an infrared spectrophotometer (FTIR) and a scanning electron microscope (SEM) to observe the surface morphology and pores of the material.

Sample Preparation and Filtration on MIP Membranes

Before filtration, the coffee sample was extracted using a microwave. Sample preparation began by weighing 75 grams of coffee and then dissolved in 500 mL of distilled water, then the extraction process was carried out using a microwave for 5 minutes at a temperature of 70°C. Then the extract obtained was left to warm first, then the extract was added with 60 grams of sodium bicarbonate, the mixture was stirred and left for 30 minutes. The extract results were filtered using a Buchner funnel with Whatman filter paper. Furthermore, the coffee sample extract was diluted 100 times. The prepared sample was then filtered to isolate caffeine from the interference of other compounds. The series of sample preparation and filtration processes are shown in **Figure 1.**

The filtration process for caffeine isolation begins with preparing 1 sheet of MIP membrane with a diameter of 1 cm and a mass of ±10 mg as a filter in a syringe and coated with polyethylene frit on the top and bottom of the MIP membrane. The conditioning process is carried out using 1 mL of distilled water which is flowed into the syringe. The filtration process is carried out by flowing 3 mL of coffee extract into the syringe. The results of this process are collected and analyzed qualitatively and quantitatively using a UV-Vis spectrophotometer at a maximum wavelength of 274 nm to determine its selectivity. At this stage, the mass of the MIP membrane used for filtration is also optimized.

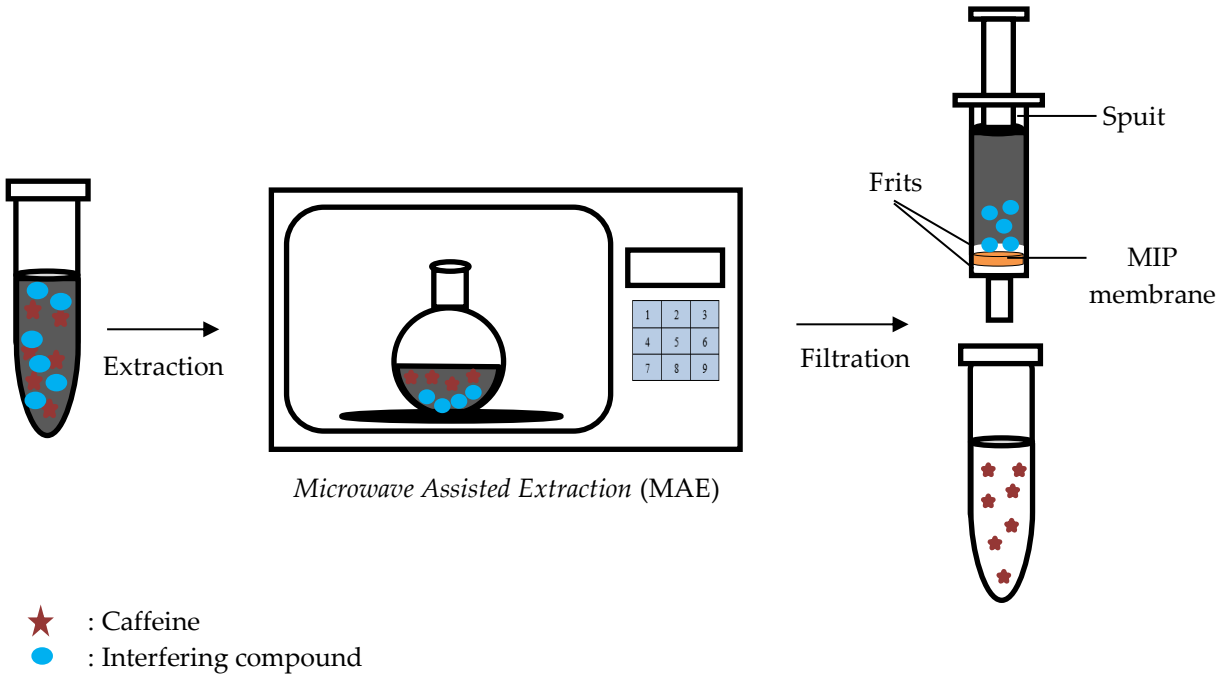

Figure 1. Set-up of Filtration Process with MIP/NIP Membranes

Accuracy determination (% Recovery) was carried out using the standard addition method. Preparation of standard caffeine addition solutions with five concentration variations, namely 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm was carried out by taking 5 mL, 10 mL, 15 ml, 20 ml, and 25 mL of 10 ppm caffeine working solution. The solution that had been taken was then put into a 25 mL measuring flask and ethanol was added to the boundary mark. Furthermore, the preparation of standard addition solutions was carried out by mixing standard caffeine solutions with a ratio of 1:1 (2 ml of standard solutions 2, 4, 6, 8, and 10 ppm: 2 ml of coffee extract from the extraction that had been diluted 100x). Furthermore, filtration was carried out in the same way as the sample filtration process. The results of this process were collected and analyzed using a UV-Vis spectrophotometer.

Results and Disscusion

Synthesis of MIP and NIP Membranes

The reaction mechanism of MIP formation begins with the combination of chitosan material with caffeine as a template. The reaction that occurs in the prepolymerization of chitosan and caffeine is a hydrogen bond that occurs at the hydrogen atom of the NH_2 group in chitosan which reacts with the C=O and –N- groups owned by caffeine. The hydrogen bond is a non-covalent interaction that is expected to facilitate the process of releasing the template during leaching. Meanwhile, the reaction between the H_2SO_4 crosslinker and the chitosan polymer occurs as an ion bond at the NH_2 group owned by chitosan which reacts with the O group owned by H_2SO_4. The polymer reaction mechanism that occurs is shown in **Figure 2.**

In addition to MIP membranes, this study also conducted the synthesis of NIP membranes. The MIP and NIP membranes produced from the synthesis are shown in **Figure 3.**

The formed MIP and NIP membranes have the same appearance. Therefore, to see the differences in their characteristics, characterization was carried out using FTIR and SEM.

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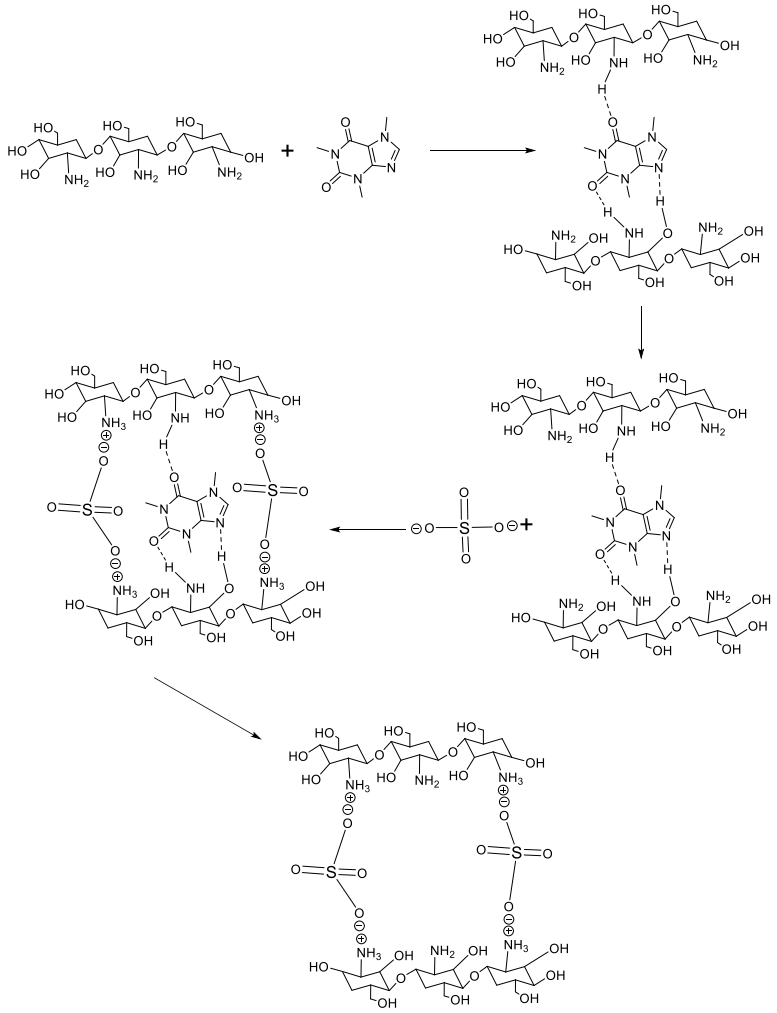

Figure 2. Reaction Mechanism of MIP Formation



(a) (b)

Figure 3. (a) MIP membrane, (b) NIP membrane

Characterization using Fourier Transform Infra Red Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM)

Characterization of MIP and NIP membranes using FTIR was carried out to determine the differences in functional groups of MIP and NIP. The spectrum of chitosan was chosen as a comparison. The results of the characterization of MIP and NIP powders using FTIR are shown in **Figure 4.**

Characteristics of FT-IR Spectra of Chitosan, MIP, and NIP in Figure 4. In chitosan compound has a wave number of 3455.71 which shows the

O-H functional group is widened, then wave number 1641.57 shows the C = O functional group is curved, at wave number 1317.82 shows the C-N functional group. The wave number shows the structure of chitosan without MIP or NIP synthesis which has a greater difference in O-H peak intensity, perhaps due to the addition of acetic acid solution as a chitosan solvent in the synthesis of MIP and NIP and the addition of sulfuric acid as a crosslinker and the number 2337.72 which is the sulfate peak owned by the crosslinker that has been added to the synthesis of MIP and NIP. While in NIP and MIP there is no difference, only MIP has a small transmission value compared to NIP because in the MIP process caffeine has been added as a template[10,12].

Scanning Electron Microscopy with Energy Dispersive X-Ray Spectroscopy (SEM-EDX) is a type of microscope that uses electrons instead of light to view objects with high resolution and to determine the composition contained in a material. Characterization using SEM was carried out to determine the morphological form of MIP Na-TPP and NIP Na-TPP particles used in this study. Characterization using EDX was carried out to determine the elemental and oxide composition of MIP Na-TPP and NIP Na-TPP as samples used in this study. The results of SEM analysis are shown in **Figure 5** and **Table 2**.

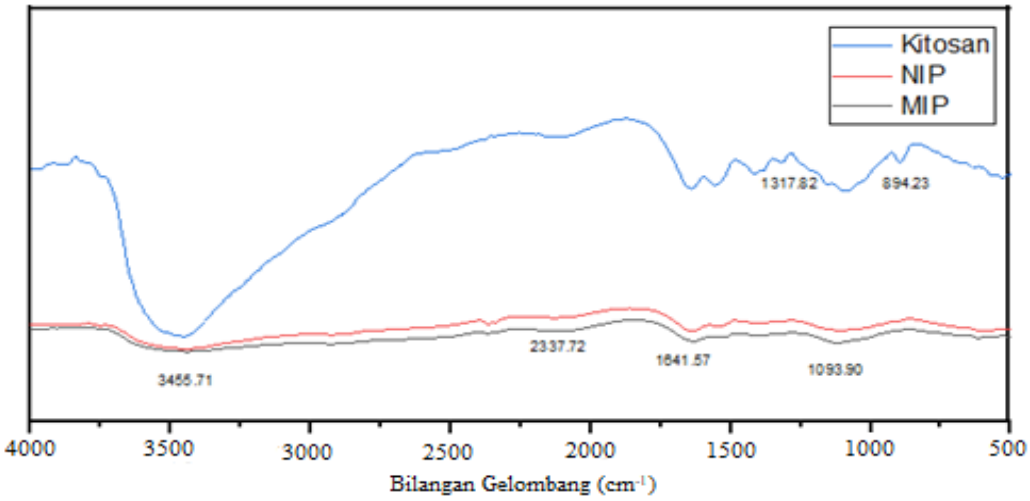

Figure 4. Spektrum FTIR

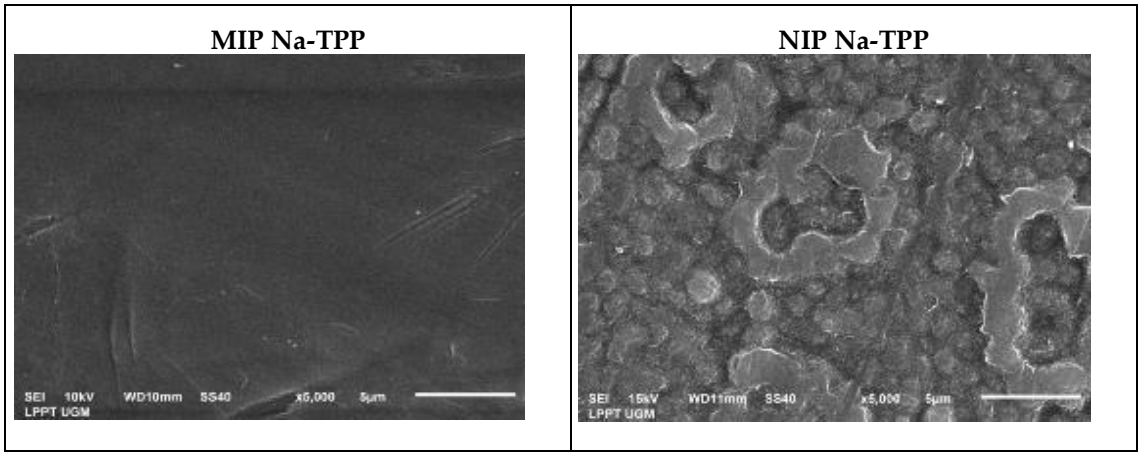

Figure 5. Comparison of SEM Characterization of MIP Na-TPP and NIP Na-TPP

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Table 2. Comparison of Atoms in MIP and NIP

Atoms	% atom in MIP	% atom in NIP
C	34.82	40.37
N	15.37	17.07
O	49.71	42.27
Na	0.11	0.28
Total	**100.00**	**100.00**

Table 3. Optimization of MIP Membrane Mass for Caffeine Filtration

Mass (mg)	Absorbance*
10	0.749 ± 0.0011
20	0.786 ± 0.0014
30	0.913 ± 0.0102
40	0.813 ± 0.0225
50	0.811 ± 0.0073

* Obtained from 3 time of replications

Table 2 shows that the majority of the number of atoms in NIP has a larger percentage of the number of atoms when compared to MIP. This happens because in NIP there is no bond between chitosan and caffeine while in MIP there is a bond between chitosan and caffeine.

Filtration and Selectivity Tests on MIP and NIP Membranes

Mass optimization was performed to see at what time the highest and purest caffeine was obtained. The results of mass optimization data can be seen in Figure 6. and the absorption results in **Table 3.**

Density was measured to assess the film's ability The results obtained in the optimization of the filter material mass showed that at a mass of 30 mg, the highest caffeine isolate was obtained. At a mass of 10 mg and 20 mg, it was very little so only a little caffeine isolate was filtered. While at a mass of 40 mg and 50 mg, less caffeine was obtained, perhaps because at that mass the MIP powder in the syringe was too much so that the caffeine filtration took longer compared to a mass of 30 mg so that caffeine might be left in the pores that were too many for the caffeine compound to pass through.

The results of the analysis of coffee samples using the microwave extraction method and filtration using MIP membranes are shown in **Figure 6.**

From the Figure 6, it can be concluded that the peak of the sample before filtration experienced an absorbance shift to a shorter wavelength region when compared to the peak of the sample after filtration using MIP or NIP. This is known as a hypsochromic shift. This can be caused because the matrix in the sample before filtration has a complex matrix so filtration using MIP or NIP is needed to obtain a peak that is close to the literature to confirm the presence of caffeine.

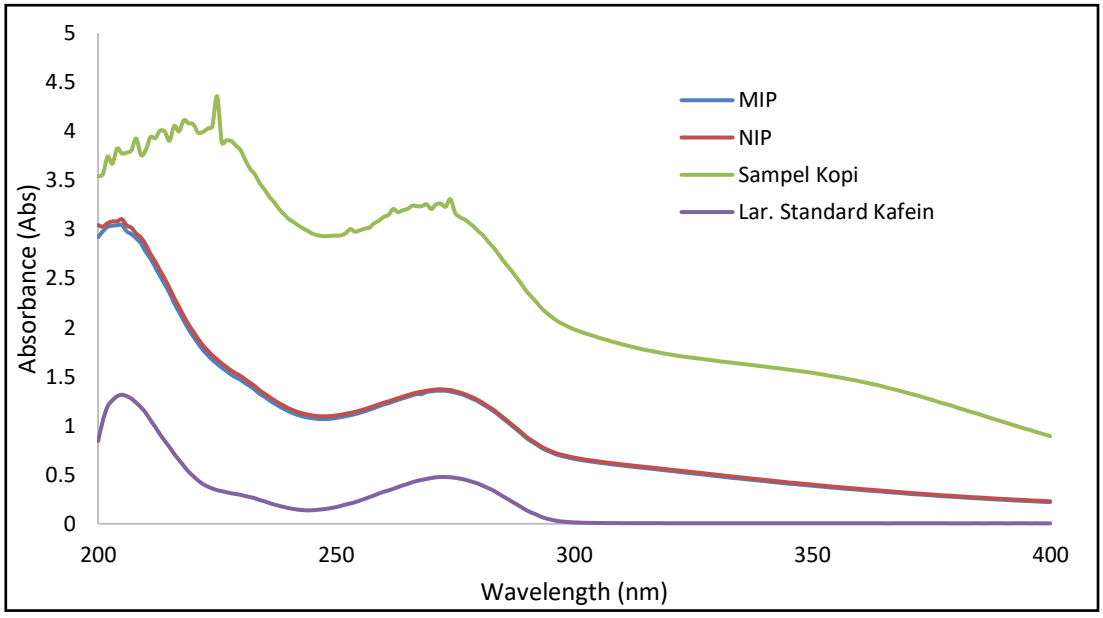

Figure 6. Comparison of Wavelength Scan Results of Samples, MIP, NIP, and Standard Caffeine Solution

Table 4. Results of % Recovery of Standard Caffeine Addition

Concentration (ppm)	% Recovery	
	MIP Filtration	NIP Filtration
2	103.80	103.15
4	99.25	91.70
6	96.16	105.73
8	99.41	96.78
10	101.59	99.23
Averages	100.04	99.32

Determination of the percent recovery (% Recovery) in this study was carried out quantitatively using a UV-Vis spectrophotometer with the standard addition method. The results obtained from the standard addition method are shown in **Table 4.**

From the table above, it can be seen that the % Recovery of the MIP filtering results is greater than the NIP filtering results. However, the difference in the % Recovery values is not much different. This is because, in MIP and NIP filtration, interactions occur through hydrogen bonds and phi (π) bonds so they are able to absorb caffeine so that some of it is left behind in the MIP and NIP filter materials. However, because the MIP pores are more numerous, the % recovery of MIP is slightly larger. In addition, the presence of MIP pores reduces the filtered interference.

Conclusions

Caffeine filtered with MIP membranes contained less interference than caffeine filtered with non-imprinted polymer membranes (NIP). Quantitative analysis was also carried out using the spiked sample method to determine the % recovery value of the filtration results. From this study, the % recovery value was obtained at 96.16 - 103.80%.

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Declaration of Generative AI and AI-Assisted Technologies

The authors declare that no generative AI or AI-assisted technologies were used in the preparation of this manuscript.

Author Contributions

E.R.A.: Conceptualization, corresponding author responsibilities and supervision. L.W.O.: Methodology, investigation, data collection, formal analysis, data curation, visualization, and writing —original draft preparation. M.N.A.: Methodology and investigation C.I.A.: Data collection, formal analysis, data curation, and visualization. L.N.: Methodology, validation, and writing —review and editing.

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Declaration of Conflicting Interests

The authors declare no conflict of interest.

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