Design and characterization of membrane molecularly imprinted polymer (MIP) as cholesterol absorbent

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Abstract. Molecularly Imprinted Polymer (MIP) membrane as cholesterol absorbing have succesfull synthesized. MIP membrane can absorb cholesterol molecules because have cavite (pore) and active groups that are selective and sensitive to cholesterol molecules. MIP membranes synthesized from methacrylic acid monomer, cross-linker ethylene glycol dimethacrilate (EGDMA), initiator of the 2-2-dimethoxy-2-phenylacetophenone (DMPP) and cholesterol as a template. MIP membranes are synthesized using photopolymerization irradiated with UV light (UV). Results obtained in the form of leaflets and transparent membrane can be characterized using Fourier Transform Infrared (FTIR). The results obtained by FTIR spectrum showed that the characterization of infrared spectra of MIP membrane-extraction did not reveal any peak absorption by the OH group, whereas MIP-Re-extraction contained absorption peaks in the area of 3540.58 cm\(^{-1}\). This indicates that the MIP membrane selective and sensitive to analyte. Based on the optimum absorption of MIP-cholesterol membrane can absorb the cholesterol molecules in the amount of 0.06 grams of cholesterol composition with a time of 30 minutes and a pH of 8. The average cholesterol absorption MIP membranes -cholesterol in the blood 0.5782 mg/dL.

1. Introduction
Currently health problems have shifted from infectious diseases to degenerative diseases, namely hypercholesterol [1]. Hypercholesterol is a condition where the amount of cholesterol in the blood exceeds the normal limit due to an imbalance in the intake of food and synthesis in the body. In terms of chemistry, cholesterol is a complex compound produced by the body with various functions, including making cortical hormones adrenal, vitamin D, and to make bile salts that help the intestines to absorb fat but high amounts of cholesterol can cause atherosclerosis which ultimately affects the coronary heart [2]. So, if the dose is right or normal, cholesterol is a fat that plays an important role in the body [3]. Handling is needed to control blood cholesterol levels in an effort to prevent further impacts of hypercholesterol [4].

Therefore, the need for analysis of the determination of cholesterol levels in a serum sample in terms of health control to the amount of cholesterol in order not to exceed the threshold that causes negative health impacts. There are several methods cholesterol analysis by FT-IR spectroscopy and UV-Visible. FT-IR spectroscopy method (Fourier Transform Infrared) is a physical characteristic analysis method based on the principle of molecular vibration which showed the presence of functional groups so that a compound can be identified clearly. Cholesterol simple analysis method is to use a spectrophotometer UV-Visible because of low operating costs and easy to use. UV-Visible analysis method also has the
disadvantage of minimizing interference matrix on the amount of solvent used quite a lot by using the reagent-Lieberman Burchard (LB) or called calorimetry method. Therefore, the method can be used in a molecularly imprinted polymer (MIP) which is designed in the form of solids can tie transparent membrane selectively target molecule. MIP (molecularly imprinted polymer) is one of the polymers that can be used for the absorption process [5]. MIP method has the ability to produce prints that are specific to the analyte in the sample [6]. At the end of the process template molecule is released back to form a cavity (cavities) that is similar to the template molecule as the analyte is then used for adsorption of analyte molecules by size and physical properties similar to those formed [7].

MIP is resistant to high temperature and pressure [8] and the main advantage of the MIP is to have high selectivity for the template used in printing, in addition to the MIP cheap synthesized and simple manufacturing procedure [9]. Based on this, the researchers are interested in doing research on "Design and Characterization of Membrane Molecularly Imprinted Polymer (MIP) as the absorbent material in Cholesterol" In this study, MIP is designed in the form of a membrane by reacting template cholesterol with monomer, crosslinker, and initiator in fotopolymerization.

2. Instrument and Materials
The equipment used in this study is fotopolymerization, sonication (ultrasound), FT-IR, Spectrophotometer UV-Vis and Autocheck. Materials used in this study are cholesterol, photoinitiator 2-2-dimethoxy-2 phenylacetophenone (DMPP), methacrylic acid (MAA), ethylene glycodimethacrylate cross linker (EGDMA), acetonitrile (C$_2$H$_3$N) where manufactured by Sigma-Aldrich, reagent Lieberman-Burchard (LB), chloroform (KGaA) and blood.

3. Methods
3.1 Preparation of membrane MIP (molecularly imprinted polymer)

3.1.1 Sintesis membrane MIP and characterization. MIP synthesized following the method performed by Dedi Futura et al, 2013, with slight modifications which do not use the SDS and 17b-estradiol template molecule are replaced with cholesterol. Mixing cholesterol as much as 0.002 grams, methacrylic acid (MAA) as monomers 3.39 mL, ethylene glycol dimethacrylate (EGDMA) as crosslinker 6.30 mL and 2-2-dimethoxy-2-phenylacetophenone (DMPP) 0.06 grams, in the sonicated at time 10 minute. Then petridisk poured into the filled bit aquabidest. Polymerised using UV light for 300 seconds under a nitrogen gas flow in contynue. MIP membrane then dried at room temperature. Characterization of MIP membranes was examined by using FT-IR.

3.2 MIP Membrane Absorption Test against cholesterol and optimization

3.2.1 The extraction of cholesterol from the membrane analysis MIP. A total of 30 mg of membrane MIP soaked into a solution of 10 ml of acetonitrile for 24 hours. MIP membrane then dried. Furthermore, MIP membrane tested by FTIR.

3.2.2 Cholesterol Absorption Analysis. MIP membrane that has been extracted cholesterol, 20 mg from membrane MIP is mixed into a solution of 3 mL cholesterol with a concentration of 10 ppm for 24 hours. Dried. Tested by FTIR. As used control membrane MIP synthesized without using cholesterol.

3.3 Optimization of Absorption MIP Membrane against Cholesterol

3.3.1. Preparation of Cholesterol Calibration Curve. In this study, analysis of cholesterol followed the method that has been done by Yogesh Saini, 2007, with minor modifications. Before the making of a standard curve, using the Liebermann-Burchard reagent made by mixing acetic acid anhydride (CH$_3$CO)$_2$ with sulfuric acid H$_2$SO$_4$ with a ratio of 20: 1 standard curve is made with varying concentrations are
1, 5, 10, 30, 50, 100, 250, 500, 750 and 1000 ppm in the parent stock solution 1000 ppm, then add 4 ml of reagent Liebermann-Burchard (LB) in each concentration.

3.3.2. Determining of Amount Cholesterol Optimum for MIP Membrane. 3 ml solution of cholesterol and 100 mg MIP membranes with varying amounts of cholesterol composition (0.01, 0.02, 0.03, 0.04, 0.06, 0.08 and 0.10 grams) soaked for 24 hours. Furthermore, MIP membrane cholesterol removed from the solution. Cholesterol reagent solution is added to the Lieberman-Burchard (LB) and cholesterol levels were analyzed using spectrophotometry UV-Vis. The final result is taken highest absorption efficiency by the formula:

Absorption efficiency MIP = Σ cholesterol absorbed/mg of MIP membrane

\[ \Sigma \text{cholesterol absorbed} = A - A^* \]

Where A is the concentration of initial cholesterol solution and A* is the cholesterol concentration left in solution.

3.3.3. Determination of Time Optimum Absorption for MIP Membrane. 100 mg of MIP membrane with amount of cholesterol the optimum amount to be incorporated into each of 3 ml solution of cholesterol by varying the immersion time (5, 10, 20, 25, 30, 40 and 55 minutes). MIP separated from the membrane solution is then added the reagent LB. Cholesterol levels were analyzed using spectrophotometry UV-Vis. Said to be the optimum contact time if obtained the greatest absorption efficiency. Calculate using the formula absorption efficiency MIP.

3.3.4. Determination of Ph Optimum Absorption Solution for MIP Membrane. 100 mg of MIP membranes with the optimum absorption time put into each of 3 ml solution of cholesterol with various pH (5, 6, 7, 8, and 8.5). After adsorption at that time, MIP membrane removed, and the solution was added with the reagent LB. Cholesterol levels were analyzed using spectrophotometry UV-Vis. Said to be the optimum solution pH if obtained the greatest absorption efficiency. Calculate using the formula absorption efficiency MIP.

3.4. Adsorption of Cholesterol in Blood Samples

Blood supply of 5 ml is provided from a patient who has high cholesterol. Taken 100 mg of MIP membrane with the optimum amount of cholesterol and soaked in 1 ml of blood during optimum absorption time. Blood solution is tested for cholesterol content (A), as a comparison of the cholesterol content test in the blood that is not mixed with the MIP membrane (B). Calculate the efficiency of absorption of membrane MIP in blood. Cholesterol tests were analyzed using the Autocheck for cholesterol.

Absorption efficiency MIP = (B – A)/B

Where A is the blood soaked in the MIP membrane with optimum absorption and B is the blood that is not mixed with MIP membranes.

4. Results and Discussion

4.1 Sintesis MIP membrane and characterization

In this study the synthesis of MIP is done by following the method performed by Dedi Futura et all [10] and Smith F, 2016 [11] with a slight modification that is without the use of surfactants and 17 β-Estradiol is replaced by the cholesterol molecule as printing molecules synthesized with methacrylic acid, EGDMA and initiator. The synthesis of MIP produced without the use of surfactant-like membrane that is transparent solids (MIP-cholesterol membrane).
MIP membrane of methacrylic acid is a free radical polymerization reaction by using initiator DMPP photopolymerization method that goes by several stages of initiation and propagation. Initiation function as activators of monomers prior to the polymerization process, the initiation phase lasts radical formation C atom that has a double bond then undergoes reaction propagation process is repeated or elongation, in which monomers with reactive chain ends will undergo chain elongation. Propagation reaction followed by termination reaction that free radicals react with other free radicals resulting in a more stable product. There are several stages of polymerization reaction that occurs in methacrylic acid (MAA) of initiation; the initiation stages, Propagation stage and Termination stage [12]. In the first stage reaction is initiated, R’ free radical initiator is from DMPP, R’ attack monomer so that the double bond in the monomer was cut into a single bond to form free radicals from MAA, the next stage of propagation is the lengthening of the chain by the incorporation of monomer [12]. The following synthesis of design estimates membrane MIP-cholesterol similar with report before [10]. Absorption Test Against MIP Membrane Cholesterol and Optimization

4.2. Analysis of extraction and absorption of cholesterol from the membrane MIP
To determine the molecular characterization and functional groups, MIP membranes can be tested by an infrared spectrophotometer (FTIR). In this experiment, the wavelength 4000-500cm\(^{-1}\) to each sample of pure cholesterol, non-MIP membrane cholesterol, MIP membrane-cholesterol, MIP membrane extraction and MIP-re-extraction.

Results of analysis of the MIP membrane extraction of cholesterol absorption and MIP membrane against cholesterol can be seen from the analysis of the FTIR spectrum in Figure 1.

![Figure 1. Results of analysis of the infrared spectrophotometer (FTIR)](image)

Based on analysis of this instrument shows the difference of a character of pure cholesterol, non-membrane cholesterol MIP, MIP-cholesterol membrane, membrane and membrane extraction MIP-MIP-re-extraction. In the 3700-3200 cm\(^{-1}\) region formed band widening in a characteristic peak of the OH group is accompanied by the appearance of C = O groups (carboxylic acid) in the frequency range 1740 to 1700 cm\(^{-1}\) and CO-OH from 1280 to 1250 cm\(^{-1}\) tandem the C = C in the region 660-1000 cm\(^{-1}\) is assumed to be derived from the functional group of methacrylic acid (MAA). In the spectra of MIP membrane-non cholesterol, MIP membrane-cholesterol, MIP membrane-extraction and MIP
membrane-re-extraction of the characteristics that there are similarities in the CO group in the region 1150-1020 cm\(^{-1}\) which interpreted the characteristics of the ether that is crosslinker EGDMA, besides there are differences in their infrared absorption spectra before the extraction of membrane MIP-cholesterol and after soaking solution of cholesterol (MIP membrane-re-extraction) that is characterized by loss of OH groups in the area of 3371.55 cm\(^{-1}\) significantly after extraction, then the OH group reappears in the 3401.55 cm\(^{-1}\) in the process of re-extraction. It thus shows that, MIP membranes can bind the analyte that has the same characteristics and properties of the template molecules that have been synthesized and their response to the active site templates that are able to absorb back the template molecule. Then the OH group reappeared in the area 3401.55 cm\(^{-1}\) in the process of re-extraction. It thus shows that, MIP membranes can bind the analyte that has the same characteristics and properties of the template molecules that have been synthesized and their response to the active site templates that are able to absorb back the template molecule [13]. Then the OH group reappeared in the area 3401.55 cm\(^{-1}\) in the process of re-extraction. It thus shows that, MIP membranes can bind the analyte that has the same characteristics and properties of the template molecules that have been synthesized and their response to the active site templates that are able to absorb back the template molecule [14].

4.3. Optimization of Membrane Absorption MIP against Cholesterol

4.3.1. Preparation of Standard Curve Cholesterol with Spectrophotometer UV-Vis. In this study, prior to the determination of the optimum conditions of the membrane MIP precise method is needed in order to be analyzed with good cholesterol that is using colorimetric method with the Liebermann-Burchard reagent (LB). Determination of cholesterol levels referenced from methods that have been carried out by Yogesh Saini, 2007.

![Absorbance vs. Cholesterol (ppm)](image)

**Figure 2.** The calibration curve of standard solution of cholesterol

According to the Lambert-Beer law, that is, if the concentration increases, the number of molecules which passed a beam increases, so uptake will also increase. This, together with the results of the calibration curve below shows that the greater the concentration of the absorbansi additions will increase. The linear regression equation \(Y = 0.002x + 0.030\) with a value of \(R^2 = 0.998\) can be used as the determination of cholesterol in this study[15].

4.3.2. MIP Membrane Optimum Composition. In this study, the optimum conditions required to obtain the amount of cholesterol composition adsorption good results and as a reference in the subsequent adsorption process. Optimization is done with mass-MIP membrane cholesterol of 100 mg with varying amounts of cholesterol of the MIP membrane-cholesterol which had been in the extraction solution used in adsorption of cholesterol. The variations in the amount of cholesterol composition of 0.01, 0.02, 0.03, 0.04, 0.06, 0.08 and 0.1 grams. The volume of solution used cholesterol 3 ml with a concentration of 100 ppm (theoretical). This adsorption process performed with 24 hours of contact time.
Figure 3. Curve absorption efficiency optimum amount of cholesterol MIP membrane-cholesterol.

The figure shows that the greater the amount of the composition of cholesterol in the membranes of the MIP, the greater the efficiency of the power absorbed on cholesterol, this is due to the process of extraction of membrane MIP greater the composition of cholesterol membrane MIP, the more cavities (cavite / pore) is formed so that the uptake MIP-cholesterol membrane increases. Based on the image above to use the amount of 0.06 grams of membrane cholesterol MIP-cholesterol as the optimum adsorption conditions for the next process.

4.3.3. Determination of Optimum Absorption Time. Optimization of the contact time is used to demonstrate the adsorption process from time to time. In addition to knowing the longer the contact time provide a loophole to the adsorbent to bind the adsorbate. Massa membrane used MIP-cholesterol of 100 mg with cholesterol optimum amount of the composition is 0.06 grams. The concentration of the solution used is 100 ppm (theoretical) with variations of immersion time is 5, 10, 20, 25, 30, 40 and 55 minutes.

Figure 4. Curve optimum time efficiency of absorption of MIP-cholesterol membrane.

The picture above shows that with time, the efficiency of absorption cholesterol adsorbed by the membrane MIP also increased so obtained optimum adsorption time is 30 minutes with the result of absorption efficiency gains amounting to 1,107.
4.3.4. **pH Effect to Cholesterol Absorbance.** Optimization cholesterol pH of the solution is used to determine the effect on the pH of the solution adsorption process. Massa MIP membrane-cholesterol of 100 mg with a total amount of cholesterol 0.06 grams of cholesterol namely, the time of immersion for 30 minutes. The concentration of the solution used is 100 ppm (theoretical) with a variation of pH is 5, 6, 7, 8 and 8.5. The solution is used to determine the concentration of the pH is NaOH (alkaline) and HCl (acid).

![Figure 5](image.png)

**Figure 5.** Curves absorption efficiency optimum pH of MIP membrane-cholesterol.

The picture above shows that the greater the pH increase absorption efficiency of the membrane MIP-cholesterol greater in the solution so obtained pH optimum solution for the highest adsorption at pH 8 with the efficiency of absorption of 0.1075. This is due to the influence of the OH group of NaOH and H⁺ ion from HCl that can provide loopholes to MIP methacyric acid groups interact to form hydrogen bonds with the hydroxyl group on cholesterol.

4.4. **Adsorption of Cholesterol in Blood**

The results of the study blood cholesterol that is absorbed by the membrane MIP-cholesterol extraction and without the addition of MIP-cholesterol membrane that has been extracted done in order to compare the percentage of cholesterol that is absorbed by the membrane MIP-cholesterol. The results of the total cholesterol levels without the addition of MIP-cholesterol membrane obtained from Autocheck tool that is 298 mg/dL cholesterol in the blood. While the blood that was mixed with cholesterol MIP membrane that MIP-1, MIP-2 and MIP-3 respectively 129 mg/dL, 124 mg/dL and 124 mg/dL. In the present study the average absorption is 0.5782. This is because the MIP membrane-cholesterol that has been extracted has a pore cavity due to the disposal template cholesterol molecule.

5. **Conclusion**

The MIP membrane design has been successfully synthesized as a cholesterol absorber with the photopolymerization method. MIP is synthesized from MAA monomers with cholesterol as a template. Under optimum conditions, MIP can absorb cholesterol well. This MIP can absorb cholesterol in the blood with an efficiency of 0.5782 mg/dL if this MIP is used to absorb cholesterol in the blood as a real sample.

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