DEVELOP AND CHARACTERIZATION OF MOLECULARLY IMPRINTED CONDUCTING POLYMER (MICP) AS URIC ACID ABSORBING MATERIAL

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ABSTRACT
Molecularly Imprinted conducting polymers (MICP) from the monomer’s aniline and methacrylic acid have been successfully developed as uric acid absorbers. The method used to synthesize the MICP membrane in this study is based on photopolymerization by using ultraviolet (UV) light radiation. The electrical conductivity analysis of the resulting MICP membrane by using a four-point probe (FPP) revealed that the MICP membrane has an electrical conductivity of 1.9904 ohm^{-1} cm^{-1}. FTIR characterization of the extracted MICP membrane did not find an N-H absorption peak, whilst the re-extracted MICP membrane exhibited an absorption peak in the wavenumber region of 910-665 cm^{-1}, which corresponds to the N-H functional group of uric acid. This suggests that the as-prepared MICPs membrane is capable of absorbing uric acid in solution. The optimum absorption conditions of the transparent MICP membrane for uric acid are pH 7.5 by using a 0.003 g MICP membrane with an absorption capacity of 0.741 mg g^{-1}, and a contact time of 24 h. The uric acid MICP membrane has been successfully tested to absorb uric acid molecules in human blood by using an easy-touch GU uric acid meter with an absorption capacity of 0.57 mg g^{-1} at normal blood pH.

Keywords: Absorbance, Conducting Polymer, Molecularly Imprinted Polymer, Photopolymerization, Uric Acid.

INTRODUCTION
Conducting polymers have been widely used as the supporting materials\textsuperscript{1} in the development of highly sensitive and selective electrochemical sensors and biosensors\textsuperscript{2}, particularly molecularly imprinted polymers (MIPs) are currently being extensively studied as a chemical or biological sensing material because they are highly selective for their analytes with high mechanical and thermal stability.\textsuperscript{3} MIPs are a type of polymer designed from functional monomers and crosslinking agents in the presence of the target analyte. It is a technique developed to produce porous polymers with specific cavities designed for a target molecule through an extraction process.\textsuperscript{4} These pores function to recognize target molecules with the same size, structure, physical, and chemical properties as the analytes.\textsuperscript{5} The principle of molecular imprinting is based on the polymerization process involving crosslinkers and functional monomers in the presence of the target analyte, which acts as a template molecule.\textsuperscript{6} Several synthesis methods have been reported for the formation of MIPs, such as the cooling-heating method, hydrothermal method, bulk method, deposition method,
suspension method, and emulsion method. The suspension polymerization method involves the radical polymerization of monomers in a dispersed medium to produce a polymer suspension\(^7\). However, the existing methods in synthesizing MIPs possess several drawbacks e.g., the hazardous materials used could pose a threat to the environment, and a time-consuming, and complicated synthesis procedure. To overcome these problems and to speed up the production of MIP, a rapid and facile method in synthesizing molecularly imprinted conducting polymer (MICP) is proposed in this study via photopolymerization method by using ultraviolet (UV) light radiation for the activation of monomers into free radicals or ions in order to initiate the polymerization process.\(^8\) The basic principle of this photopolymerization process is the absorption of light by monomers when irradiated with UV light and that several activated monomers in the form of free radicals, cations, and anions will be produced, which will then chemically combine to form polymer chains. A non-toxic photoinitiator, namely 2,2-dimethoxy-2-phenyl acetophenone (DMPP) that is sensitive to UV rays was incorporated in the polymerization reaction for ease of MICP production. Uric acid was used as a template molecule during the synthesis of the MICP through a photopolymerization reaction. The proposed MICP preparation protocol is simple, eco-friendly, time, and cost-saving.\(^9\) However, this research will develop a molecularly imprinted conduction polymer (MICP) from the monomer aniline and methacrylic acid as a uric acid absorbent.

**EXPERIMENTAL**

**Materials and Instruments**

Uric acid powder (~99%) was supplied by Sigma Life Science Company. Methacrylic acid acetonitrile (C\(_2\)H\(_3\)N, 99.8%), (MAA, 99%) monomer, 2-2-dimethoxy-2-phenyl acetophenone (DMPP, 99%) photoinitiator, ethylene glycol dimethyl acrylate (EGDMA, 98%) crosslinker, and aniline monomer (99.9%) solvent were produced by Aldrich Company. All chemicals used in this study were used without purification which is an analytical grade. A UV-emitting unit at a wavelength of 350 nm was used for the photopolymerization reaction to produce MICP. An ultrasonic unit was used to homogenize the MICP precursor mixture. Perkin-Elmer Fourier Transform infrared (FTIR) spectrometer was for FTIR measurement. The electrical resistivity value of the as-prepared MICP membrane was measured with a four-point probe (FPP). By passing a current through the two outer probes of the FPP that were connected to the current source, the voltage generated through the two inner probes was measured to allow estimation of the MICP substrate resistivity.\(^10\) An Easy Touch GU Uric Acid Meter, ET-201 was used to measure the uric acid levels in the human blood sample.

**Synthesis and Characterization of Uric Acid MICP Membrane**

MICP matrix was synthesized by photopolymerization method as reported by Dedi\(^11\) but with slight modification. A total of 0.001 g of uric acid was used as a template and was mixed with 402 L methacrylic acid (MAA) monomer, 435 L aniline monomer, 900 L ethylene glycol dimethacrylate (EGDMA) crosslinker and 0.006 g 2,2-dimethoxy-2-phenyl acetophenone initiator (DMPP) via ultrasonication for 30 min at ambient conditions (25 °C) until the mixture became transparent. The resulting mixture solution was then inserted into a petri dish filled with an adequate amount of equates and underwent photopolymerization using a UV light for 5 min under a continuous stream of nitrogen gas. Finally, the synthesized of MICP membrane was air-dried at room temperature. Control MICP and MICP membranes were also prepared without using a uric acid template and aniline monomer, respectively for comparative study. Figure-1 shows the molecular structure of uric acid, aniline, and methacrylic acid as a uric acid absorbent.

![Fig.-1: Molecular Structure of (a) Uric Acid, (b) Aniline Monomer, (c) Methacrylic Acid (MAA), and (d) Ethylene Glycol Dimethacrylate (EGDMA)](image-url)
Extraction of uric acid in MICP membrane was carried out by immersing a total of 30 mg of the as-synthesized MICP membrane in 10 mL of acetonitrile solution for 24 h\textsuperscript{12} followed by an air dry at ambient conditions and denoted as extracted MICP membrane. The re-extraction of uric acid was done by immersing 20 g of the extracted MICP membrane in 3 mL of 10 mg L\textsuperscript{-1} uric acid solution for 24 h and air dried at 25 °C (re-extracted MICP membrane). The chemical structural properties of both extracted MICP membrane and re-extracted MICP membrane were characterized by FTIR spectroscopy. For conductivity testing, the resistivity of the electrical conductor materials i.e., MICP membrane, control MICPs, extracted MICP membrane, and re-extracted MICP membrane was analyzed by using a four-point probe (FPP) with a distance between probes of 0.4 cm, whereby probe 1 and probe 4 were connected to a digital multimeter, whilst probe 2 and probe 3 were connected to the MICP sample. The output voltage and current values were later recorded accordingly. The electrical conductivity and resistivity of the MICP can be determined based on the following equations, which have been previously reported by Siti Veren.\textsuperscript{10}

\[
\sigma = \frac{1}{\rho}\quad (1)
\]

Where, \(\sigma\) and \(\rho\) are conductivity (ohm\textsuperscript{-1}∙cm\textsuperscript{-1}) and resistivity (Ohm∙cm), respectively. The resistivity value is calculated using the below equation:

\[
\rho = 2 \pi s \frac{V}{I}\quad (2)
\]

Where, \(s\), \(r\) and \(I\) are radius values (\(\pi=3.14\)), the distance between probes (cm), and the value of mains voltage (volts) and current (A), respectively. While the adsorption capacity of the MICP membrane (\(Q, \mu g mg^{-1}\)) was evaluated with the below formula\textsuperscript{13,14}

\[
Q = \frac{(C_0 - C_f)V}{m}\quad (3)
\]

Where the \(Q (\mu g mg^{-1})\) symbol is the mass of uric acid adsorbed by an amount of dry MICP. \(C_f (\mu g mL^{-1})\) and \(C_0 (\mu g mL^{-1})\) symbols are the final and initial uric acid concentrations, respectively. While the \(m (mg)\) symbol is the mass of the MICP, while \(V (mL)\) is the volume of the initial solution. Additionally, the analysis of the effect of MICP amount (i.e., 0.001 g, 0.002 g, 0.003 g, and 0.004 g) and pH of the uric acid solution at 10 mg L\textsuperscript{-1} (i.e., pH 6.0-pH 8.0) on the uptake capacity of uric acid by MICP substrate with a contact period of 24 h have also been carried out.

**RESULTS AND DISCUSSION**

**Synthesis and Characterization of Uric Acid Molecularly Imprinted Conducting Polymer (MICP)**

Chemical structural elucidation of the MICP membranes was conducted using FTIR spectroscopy to determine the functional groups at the wavenumber range of 500-4000 cm\textsuperscript{-1}. The wavenumbers of the chemical functional groups for uric acid, control MICP without a uric acid template, MICP membrane without aniline, extracted MICP membrane, re-extracted MICP membrane, and aniline are shown in Table-1. The reference wavenumber ranges for different organic groups (Pavia 2008) are also provided in Table 1 for comparison purposes. FTIR data analysis shows that all the samples possessed the C-H group except uric acid. The C=O group is also found in all the samples except for aniline. The C=N group is also found in all the samples except for aniline. The C-N group is not detected for each sample except for aniline, and the C-O group is not detected in both uric acid and aniline, whilst the C=C group is determined in all the FTIR samples. The FTIR analysis revealed that the functional groups present in all the samples are in accordance with the functional groups available in the monomers used to form the respective MICP matrices. The N-H group is not detected in both control MICP and extracted uric acid MICP membranes as uric acid is not present in both these samples, whereas it is detected in re-extracted uric acid MICP, this indicates that the uric acid in MICP membranes can be extracted and re-extracted again by the proposed MICP membrane. This confirms that the fabricated MICP as the absorbent material for uric acid has been successfully developed and further exploited from the study previously reported by Jihan.\textsuperscript{12}

The electrical conductivity test carried out using the FPP instrument for several samples, namely uric acid MICP membrane, MICP membrane without aniline, control MICP membrane without uric acid, extracted MICP membrane, and re-extracted MICP membrane, and producing the respective electrical conductivity values are presented in Table-2. It is found that the MICP membrane without the addition of aniline has not produced electrical conductivity signal.
The methacrylic acid (MAA) monomer itself has a very low conductivity of 23.3 pS m\(^{-1}\) (30 °C), which will produce a polymer with very low conductivity and its electrical conductivity signal was not detected by the FPP instrument. Uric acid MICP membrane, which possesses aniline moiety was capable of generating a significant electrical conductivity signal at 1.9904 ohm\(^{-1}\) ∙ cm\(^{-1}\). This is because the chemical copolymerization of aniline could yield a conductive polymer\(^{16}\) and that the addition of aniline monomer in the preparation of uric acid MICP membrane in this study has increased the electrical conductivity of the molecularly imprinted polymer from 0 ohm\(^{-1}\) ∙ cm\(^{-1}\) to become 1.9904 ohm\(^{-1}\) ∙ cm\(^{-1}\). However, the membrane’s conductivity decreased to 1.0877 ohm\(^{-1}\) ∙ cm\(^{-1}\) when the MICP membrane was synthesized without the presence of uric acid. This is because uric acid is an electrochemically active compound with many lone pairs of electrons on the oxygen (O) and nitrogen (N) atoms as the conductors of electricity. Generally, a conducting material has high electron mobility with many free electrons on the valence orbit, and the material has the properties associated with an electrical conductor.\(^{17}\) The extraction of uric acid from the MICP membrane upon soaked with acetonitrile has caused some of the aniline to dissolve, thereby reducing the electrical conductivity response of the resulting extracted MICP membrane, and no considerable difference in electrical conductivity response was observed when the uric acid was being re-extracted to the MICP membrane. A similar observation has been previously reported by Zhang Jun-Ling.\(^{18}\)

### Table-2: The Electrical Conductivity Values of the MICP Samples Used In This Study

<table>
<thead>
<tr>
<th>MICP sample</th>
<th>Electrical Conductivity (ohm(^{-1}) ∙ cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric acid MICP membrane</td>
<td>1.9904</td>
</tr>
<tr>
<td>MICP membrane without aniline</td>
<td>0.0000</td>
</tr>
<tr>
<td>Control MICP membrane without uric acid</td>
<td>1.0877</td>
</tr>
<tr>
<td>Extracted MICP membrane</td>
<td>0.3317</td>
</tr>
<tr>
<td>re-extracted MICP membrane</td>
<td>0.3474</td>
</tr>
</tbody>
</table>

### The Effect of MICP Amount on the Absorption Capacity Response

The optimum amount of MICP membrane used can be determined from the uric acid absorption capacity test by using different MICP membrane masses at 0.001 g, 0.002 g, 0.003 g, and 0.004 g. Figure-2 illustrates the effect of MICP amount on the absorption capacity of the uric acid. As can be seen, the amount of uric acid absorbed on the MICP membrane increased as increasing the amount MICP from 0.001 g to 0.003 g as the porous MICP polymer comprised an increasing number of specific cavities to capture for target uric acid substance through an extraction process, after which the absorption capacity response declined slightly. The decrement in the uric acid absorption was caused by the absorption concentration on the surface of the uric acid MICP membrane being greater than the concentration of remaining uric acid in the solution and resulting in the desorption of uric acid back into the solution. The same phenomenon happened when the mass of the MICP membrane in the uric acid solution exceeded the optimum amount, the MICP membrane inhibited the absorption reaction in the solution, and the amount of uric acid absorbed by the MICP membrane became reduced. This observation is inconsistent with the molecularly imprinted polymer developed before\(^{19-21}\) for the separation of cholesterol and absorption of cholesterol, respectively.

### The Effect of Uric Acid Solution pH on the MICP’s Absorption Capacity

Absorption of uric acid on the MICP membrane occurred owing to the hydrogen bonding interactions between the O atom on the uric acid (template) and the H atom on the methacrylic acid moiety of the MICP.
during extraction and re-extraction of uric acid processes. Hydrogen bonds are weak, partially covalent bonds, which are vulnerable to pH change, which induces association and dissociation of intermolecular complexes.

![Fig.-2: The effect of MICP Amount on the Uric Acid Absorption Capacity](image)

Alteration of the uric acid solution pH will affect this interaction, where the number of $\text{H}^+$ and $\text{OH}^-$ ions in the solution can compete with these interactions. Figure-3 represents the effect of uric acid solution pH on the absorption capacity of the MICP membrane. The absorption capacity of the MICP membrane for uric acid increased if the solution pH increased (pH 6.0 to pH 7.5) and decreased thereafter at pH 8.0. The acidity of the uric acid solution has rendered protonation of bases, which led to the destruction of hydrogen bonds. The increment in the absorption capacity of the MICP membrane as increasing the uric acid solution pH was attributed to the reduced number of $\text{H}^+$ ions that interacted with the $\text{O}$ atom of uric acid. This has reduced the interference of $\text{H}^+$ ions to uric acid to be absorbed by the MICP membrane. The decrement in the absorption capacity of the MICP membrane from pH 7.5 to pH 8.0 was attributed to the uric acid in an alkaline state that contained a lot of $\text{OH}^-$ ions that can interact with $\text{H}$ atoms in the MICP membrane, as a result, extraction and re-extraction of uric acid processes by the MICP membrane were disrupted. The effect of pH on the absorption of some templates (analytes) by molecularly imprinted polymers has also been previously reported by Yanli Mao\cite{22} and Azam Nadali.\cite{23} In addition, the effect of pH on the absorption of activated carbon from candlenut shells (Aleurites Moluccana) has also been reported.\cite{24} The uric acid MICP membrane has been attempted to evaluate uric acid molecules in human blood. An absorption capacity of 0.57 mg g$^{-1}$ at normal blood pH was successfully registered on the Easy Touch GU uric acid meter.

![Fig.-3: Effect of Uric Acid Solution pH on the MIPC Membrane’s Absorption Capacity](image)

**CONCLUSION**

The as-synthesized uric acid MICP membrane based on infrared spectra showed that the MICP membrane was selective for absorption of uric acid molecules at optimal absorption conditions of the MICP membrane to absorb uric acid molecules of pH 7.5, and contact time of 24 h at an absorption capacity of 0.741 mg g$^{-1}$. A significant enhancement in the MICP’s electrical conductivity value of 1.9904 ohm$^{-1}$cm$^{-1}$ was noted when an aniline monomer was added as much as 432 L per 100 mg. The uric acid MICP membrane can absorb uric acid molecules in human blood samples with an absorption capacity of 0.57 mg g$^{-1}$ at normal human blood pH.

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