The Optical Sensor Membran based on Polymer Poly(Methyl Methacrylate) (PMMA) for Methampyrone Detection in Herbal Medicine

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Abstract

Methampyrone is one of drug frequently added in herbal medicine (jamu) to enhance the effect as an analgesic. Herbal medicine should not contain a chemical drug due to toxic effect. The optical sensor membrane based on polymer Poly(methyl methacrylate) (PMMA) was synthesized by phase inversion method and was used to detect the methampyrone in jamu. PMMA was made in different concentration (5, 7.5 and 10%) and mixed with specific colorimetric reagent for methampyrone detection [ferric chloride ($FeCl_3$), folin-ciocalteu and silver nitrate ($AgNO_3$)]. The ratios of solvent and reagent were 6:4; 7:3; and 8:2.

The result shows that $FeCl_3$-PMMA 7.5% (7:3), Folin-PMMA 5% (6:4) and $AgNO_3$-PMMA 5% (6:4) give the best performance with the lowest measurable detection as 0.98 mg/ml, 1.25 mg/ml and 0.99 mg/ml respectively. Jamu as a real herbal medicine samples was analyzed to demonstrate the practical application of this sensor and the results showed good agreement with UV-Vis Data. This optical sensor membrane is applicable for metampyron detection in jamu.

Keywords: Optical sensor membrane, Methampyronee, Poly(methyl methacrylate) (PMMA).

Introduction

The Indonesian traditional herbal medicine called *jamu* is still very popular in the Indonesian community to maintain good health and to treat disease[1]. Herbal medicine differs from conventional medicine, it contains plant extract or herb combining[2]. The growing use of *jamu* makes the producer add some chemical substance to improve therapeutic effects. One of drug that is usually added to *jamu* is methampyrone. Methampyrone (synonym: metamizole, dipyrone) is a non-opioid drug that commonly used in human as analgetic medicine[3].

Meanwhile, the Indonesian Ministry of Health set the regulation for traditional herbal medicine that it should not contain synthetic chemicals or medicinal isolation results, as well as no hard drugs or narcotics or psychotropic substances and animal or plant to be protected[4]. Hence, monitoring methampyrone in *jamu* is important to safety control of *jamu*.

Numerous analytical methods such as spectrophotometry[5], high performance liquid chromatography (HPLC)[6] and liquid chromatography-mass spectrometry LC-MS)[7] are used for methampyrone detection. These methods are selective and sensitive, however they require expensive instrumentation, highly trained personnel and cannot be used for on-site analysis. Therefore, analytical methods for methampyrone analysis that are simple, reliable and selective are still needed. The optical sensor for sample analysis has been developed based on electrochemical or colorimetric detection[8,9]. Colorimetric methods have shown great potential to be developed as methampyrone detection.

The colorimetric reagents for methampyrone detection are ferric chloride, folin-ciocalteu, Lieberman reagent and silver nitrate[10]. One of the potential substrate materials for optical sensor is polymer. It has many advantages for a lab-on-chip device since it is simple, cost-effective and disposal material[11]. In this work, the optical sensor membrane has been developed for methampyrone detection using poly(methyl methacrylate) (PMMA) as a membrane material. PMMA is chosen due to its high thermal stability, mechanical strength and chemical inertness[12]. The specific colorimetric reagent for methampyrone detection was mixed into a polymer solution. A phase inversion method was applied to synthesize polymer membrane as a straightforward and rapid fabrication method[13]. The application of this optical sensor membrane to *jamu* was also investigated. The result shows that this system is applicable to methampyrone detection in *jamu*.

Material and Methods

All material used were of analytical grade and used without further purification. All solutions were made using distilled water. Methampyrone was obtained from Medialabs. Ibuprofen, acetosal, paracetamol, ferric chloride ($FeCl_3$), folin-ciocalteu, sodium hydroxide (NaOH), sodium nitrite ($NaNO_2$), sulfuric acid (H_2SO_4) and silver nitrate ($AgNO_3$) were purchased from Merck. Ethanol 96% and methanol were obtained from Emsure. Ethyl acetate was obtained from Bratacho Chemistry. Poly(methyl methacrylate) (PMMA) was obtained from Aldrich Chemistry. The characteristic of polymer membrane was observed by Scanning Electron Microscope-Energy Dispersive X-ray (SEM-EDX) (Jeol JSM-651OLA). The absorbance measurement was recorded by UV-Visible spectrophotometer (Analytic Jena Specord 200).

Colorimetric reaction of reagent and methampyrone: The folin-ciocalteu and lieberman reagent were prepared as reported in literature[10]. Ferric chloride ($FeCl_3$) and silver nitrate ($AgNO_3$) reagent were prepared as a United State Pharmacopea. For colorimetric sensing, each of reagent was reacted with variation concentration of methampyrone.

Fabrication of optical sensor membrane: The optical sensor membrane was made by using PMMA in three different concentrations 5%, 7.5% and 10%. Each concentration was dissolved in mixture solvent containing ethyl acetate as a solvent and a specific reagent for methampyrone detection (Folin-Ciocalteu reagent, Lieberman reagent, Ferric chloride and silver nitrate). The ratio of the solvent and reagent was 6:4, 7:3 and 8:2. The PMMA solution was coated on a glass plate and allowed to dry at room temperature. The polymer membrane was cut into 1 cm^2. After the fabrication was complete, the optical sensor membrane was characterized using Scanning Electron Microscope-Energy Dispersive X-Ray (SEM-EDX). The sensitivity, stability and selectivity were tested to know the performance of optical sensor membrane.

Aplication for real sample: To demonstrate the applicability of the optical sensor membrane for real sample analysis, 15 herbal medicines were collected from Bandung, West Java, Indonesia for analysis using spectrophotometry UV-Visible and optical sensor membrane. The samples (7 g) were extracted using methanol (2x70 mL) by liquid-liquid extraction method. The solvent was removed by evaporation and the residue was dissolved in methanol. The absorbance spectra were recorded at 234 nm. For the optical sensor membrane analysis, 2 g of sample was dissolved in 10 ml methanol and then the sample was added to the membrane.

Results and Discussion

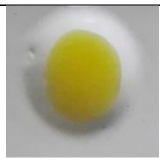
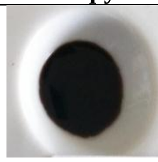
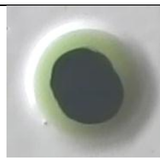
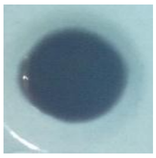
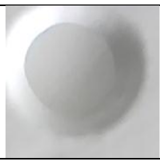
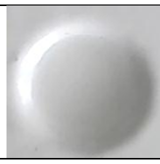
Characterization of Colorimetric reagent and methampyrone: In order to investigate the colorimetric sensing ability of reagent of methampyrone, the colorimetric reagents (ferric chloride, folin-ciocalteu, lieberman reagent and silver nitrate) were reacted with methampyrone.

As shown in table 1, the solution color changed after methampyrone was added into the $FeCl_3$, $AgNO_3$ and folin-ciocalteu reagent. $FeCl_3$ and $AgNO_3$ reagent can detect fenilpyrazolon moiety in methampyrone and give a dark-purple and dark-blue color respectively. Folin-ciocalteu gives a dark blue color when this reagent is reacted with methampyrone. The color of Lieberman reagent will change if it is reacted with methampyrone in heated condition.

In this work, the heating procedure is avoided because it is not applicable to optical sensor membrane. Therefore, this reagent did not continue to be used in optical membrane sensor.

Design and fabrication of optical sensor membrane: The optical sensor membrane was made by phase inversion method as a well-known process for the preparation of membrane[13]. In this method, the polymer solution will transform from a liquid to solid phase[14]. The PMMA was prepared in the concentration of 5%, 7.5% and 10% of the ratio of the solvent and reagent is 6:4, 7:3 and 8:2 for each concentration. This concentration is chosen based on the optimization study that shows the concentration of PMMA is bellowed 5% produces a fragile membrane while the concentration above 10% makes it dense and difficult to absorb the reagent.

Table 1
Colorimetric reaction between reagent and methampyrone (100 mg/ml)

Reagent	Reagent solution	Added methampyrone
$FeCl_3$		
Folin-ciocalteu		
$AgNO_3$		
Lieberman		

Blank: reagent solution + ethanol as a paracetamol solvent

Table 2 shows the different characterization of optical sensor membrane when metamphyron is added. All of optical sensor membranes give a positive result with different reaction time and homogeneity. In the high concentration of polymer, the membrane will be more dense and the sample needs more time to react with the reagent in the membrane. Therefore, the reaction time increased as the concentration of PMMA increased.

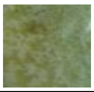
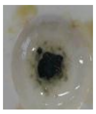
The best result of this sensor is $FeCl_3$-PMMA 7,5% 7:3; Folin-PMMA 5% 6:4; and $AgNO_3$-PMMA 5% 6:4. The optical sensor of $FeCl_3$-PMMA 7,5% 7:3 shows the shortest reaction time with the homogeneous membrane. It also gives a suitable color change from yellow to brown[10] as shown in table 3. The color of Folin-PMMA changed from green to dark blue and the $AgNO_3$-PMMA color changed from grey to dark purple.

Table 2
Characterization of reaction of optical sensor membrane with methampyrone

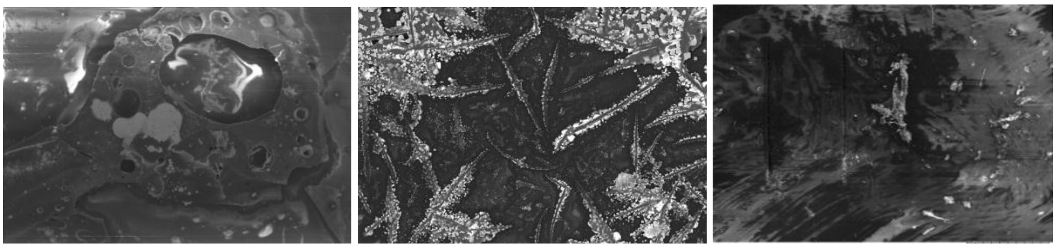
Reagent	%PMMA	Mix solvent EA: Reagent	Homogeneous polymer	Result	Reaction time
$FeCl_3$	5%	6:4	Non-homogeneous	+	1 s
		7:3	Non-homogeneous	+	1 s
		8:2	Non-homogeneous, fragile	+	1 s
	7,5%	6:4	Homogeneous, fragile	+	1 s
		7:3	Homogeneous	+	1 s
		8:2	Homogeneous	+	1 s
	10%	6:4	Non-homogeneous	+	1 s
		7:3	Homogeneous	+	1 s
		8:2	Non-homogeneous	+	1 s
Folin-ciocalteu	5%	6:4	Homogeneous	+	5 s
		7:3	Homogeneous	+	11 s
		8:2	Homogeneous	+	10 s
	7,5%	6:4	Homogeneous	+	7 s
		7:3	Homogeneous	+	10 s
		8:2	Homogeneous	+	41 s
	10%	6:4	Homogeneous	+	45 s
		7:3	Homogeneous	+	50 s
		8:2	Homogeneous	+	1 m 31 s
$AgNO_3$	5%	6:4	Homogeneous	+	1 s

s= second m= minute

Table 3
Color test of optical sensor membrane in the presence of methampyrone

Optical sensor membrane	Membrane	Added methampyrone
$FeCl_3$-PMMA 7.5% 7:3		
Folin-PMMA 5% 6:4		
$AgNO_3$-PMMA 5% 6:4		

Characterization of optical sensor membrane using Scanning Electron Microscope-Energy Dispersive X-Ray (SEM-EDX): The best performance of optical sensor membrane was characterized by Scanning Electron Microscope-Energy Dispersive X-Ray (SEM-EDX) to observe the surface topography of membrane and to confirm the element on the membrane. Visually, all of this membrane is homogeneous (table 2). However as shown in figure 1, the optical sensor membrane is non-homogenous and pore size is not uniform. This may be due to the difference polarity between PMMA solvent and the reagent, so the reagents cannot be mixed well with the polymer. Table 4 shows that the elements present in the membrane are the same as the constituent element. This result confirms that the reagent is absorbed into the membrane.



(a) (b) (c)

Fig. 1: Surface topography of the optical sensor membrane by SEM-EDX (a) $FeCl_3$-PMMA 7.5% 7:3 (b) Folin-PMMA 5% 6:4 and (c) $AgNO_3$-PMMA 5% 6:4

Table 4
The elemental content of the membrane

Membrane	Element	Element concentration (%)
$FeCl_3$-PMMA 7.5% 7:3	Carbon	70.944
	Oxygen	26.268
	Chlorine	1.846
	Iron	0.942
Folin-PMMA 5% 6:4	Carbon	59.462
	Oxygen	36.219
	Sodium	1.233
	Phosphor	0.674
	Sulphur	1.432
	Chlorine	0.651
	Tungsten	0.330
$AgNO_3$-PMMA 5% 6:4	Carbon	70.494
	Oxygen	29.290
	Silver	0.123
	Alumunium	0.094

Performance of optical sensor membrane

Stability test: The stability test was performed by stored the optical sensor membrane in a ziplock plastic at room temperature for several days until the color of membrane did not give the appropriate color when methampyrone was added. The results are summarized in table 5.

Table 5
The stability time of optical sensor membrane

Optical sensor membrane	Stability time (day)
$FeCl_3$-PMMA 7.5% 7:3	122
Folin-PMMA 5% 6:4	125
$AgNO_3$-PMMA 5% 6:4	125

Sensitivity test: In this experiment, the sensitivity of optical sensors membrane was analyzed by determining the lowest measurable concentration of methampyrone that can be detected. The methampyrone was added into the membrane in different concentration (0 – 10 mg/ml). The result shown in table 6 describes that the lowest measurable concentration of methampyrone is in accordance with the minimum dosage form of methampyrone usually added to *jamu* at 500 mg/3000 mg. The different lowest concentration of the membrane was influenced by a particular reagent and sensitivity of reagent. The intensity of the color change increased as concentration of methampyrone increased.

Table 6
The lowest measurable concentration of methampyrone on the membrane

Optical sensor membrane	Concentration mg/ml
$FeCl_3$-PMMA 7.5% 7:3	0.98
Folin-PMMA 5% 6:4	1.25
$AgNO_3$-PMMA 5% 6:4	0.99

Selectivity test: Chemical substances usually added into *jamu* who have similar effect with methampyrone were fenylbutazon, sodium diclofenac, mefenamic acid, paracetamol, acetosal and ibuprofen. To investigate the selectivity of this membrane, these chemical drug were tested on the membrane. All of these chemical drug were dissolved in ethanol (100 mg/ml) and then added to the membrane respectively.

Table 7 shows the color of optical sensor membrane changed when another chemical drug was added. This result does not affect the methampyrone detection since the color change of the membrane is not same in the presence of methampyrone. In addition, the design of this optical sensor membrane will contain three strip of $FeCl_3$-PMMA

7,5% 7:3; Folin-PMMA 5% 6:4 and $AgNO_3$-PMMA 5% 6:4 where all of these strip must give positive result for methampyron. Generally, this membrane is applicable for methampyrone detection since the regulation states that *jamu* should not contain the chemical drug.

Application for real sample *jamu*: The applicability of optical sensor membrane for methampyrone detection was demonstrated by determining 15 *jamu* using spectrophotometry UV and optical sensor membrane. Methampyrone was not detectable in these samples by using spectrophotometry UV and the developed optical sensor membrane. The samples were then spiked with 1 mg/ml of methampyrone and the results show good agreement between optical sensor membrane method and spectrophotometry data. Both methods confirm that the sample contains methampyrone. The reaction time of the membrane to detect paracetamol is summarized in table 8.

Table 7
The selectivity test of optical sensor membrane

Membrane	Fenylbutazon		Sodium Diclofenac		Mefenamic acid		Paracetamol		Acetosal		Ibuprofen	
	Color change	Reaction time	Color change	Reaction time	Color change	Reaction time	Color change	Reaction time	Color change	Reaction time	Color change	Reaction time
$FeCl_3$-PMMA 7.5% 7:3	Brown	1 s	-	-	Brown	1 s	Green-blue	1 s	Purple	1 s	-	-
Folin-PMMA 5% 6:4	-	-	Dark blue	3 m 23 s	-	-	Dark blue	2 m 36 s	-	-	-	-
$AgNO_3$-PMMA 5% 6:4	Dark purple	50 s	-	-	-	-	-	-	-	-	-	-

Table 8
Reaction time of optical sensor membrane on methampyrone detection in *jamu* sample

Sample	Reaction time		
	$FeCl_3$-PMMA 7.5% 7:3	Folin-PMMA 5% 6:4	$AgNO_3$-PMMA 5% 6:4
1	3 m 15 s	12 m 4 s	5 m 35 s
2	2 m 45 s	12 m 13 s	4 m 15 s
3	2 m 32 s	13 m 25 s	4 m 23 s
4	3 m 10 s	13 m 34 s	5 m 38 s
5	2 m 55 s	12 m 47 s	4 m 28 s
6	2 m 42 s	12 m 15 s	4 m 40 s
7	3 m 24 s	13 m 37 s	5 m 20 s
8	2 m 46 s	12 m 44 s	4 m 40 s
9	2 m 35 s	13 m 28 s	4 m 47 s
10	3 m 12 s	12 m 49 s	5 m 19 s
11	2 m 47 s	13 m 46 s	5 m 26 s
12	3 m 12 s	12 m 14 s	4 m 34 s
13	2 m 40 s	13 m 26 s	4 m 47 s
14	3 m 14 s	12 m 16 s	5 m 15 s
15	2 m 38 s	12 m 38 s	4 m 48 s

Conclusion

The optical sensor membrane based on PMMA polymer can be developed for metampyrone detection in *jamu*. This membrane can absorb and react with colorimetric reagent of methampyrone. The characteristic of this membrane depends on the concentration of PMMA and the reagent. The best results of optical sensor membrane in this experiment are $FeCl_3$-PMMA 7.5% (7:3), Folin-PMMA 5% (6:4) and $AgNO_3$-PMMA 5% (6:4). In conclusion, this optical sensor membrane is applicable and simple for methampyrone detection in *jamu*.

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