❒   521

A Flexible Optical Sensor for Microalbuminuria Spectroscopy

Suhaila Isaak*, Yusmeeraz Yusof, Khairunnisa Mohd Yusof, Khoo Ming Kwan
Department of Electrical and Computer Engineering, Universiti Teknologi Malaysia, Malaysia

Article Info	ABSTRACT
	A flexible optical sensor unit was developed for the detection of albumin level in human urine. It consists of polydimethylsiloxane thinfilm with the microscopic glass as the substrate. A gradual increase of albumin level may indicate signs of health problem such as diabetes, hypertension, and endothelial dysfunction. The experimental results showed there are a spectral shift and an increase in absorbed light increase in intensity of light with sodium urate at different concentration as a substitute of urine sample. The transmittance and reflection of PDMS was determined in the 400–1400 nm wavelength range using halogen-detrium as the excitation light source to predict the sensor sensitivity based on the Beer–Lambert law. The results exhibited that the light intensity increases as the concentration of the sodium urate increases. This performance of prototype sensor ignites an alternative for albumin detection using flexible structure. **

Corresponding Author:

Suhaila Isaak,
Department of Electrical and Computer Engineering,
Universiti Teknologi Malaysia,
81310, Johor Bahru, Johor.
Email: suhaila@fke.utm.my

1.   INTRODUCTION

The presence of albumin level in urine is commonly known as microalbuminuria has been widely used as renal discharge marker. Microalbumin is used to indicate the sign of kidney damage, which is unrestricted of endanger factors [1]. Patient with high risk of progressive kidney damage shows an increased presence of protein in their urine. The higher the albumin level, the more vulnerable the patient toward kidney function failure [2][3]. Detection of microalbuminuria is commonly associated with the increment of morbidivity and mortality. Patients with high risk of progressive kidney damage and an increased cardiovascular (CV) morbidity show an elevated presence of protein in the urine. Besides being a marker for kidney disease, albumin is also used as a marker for diabetes mellitus, hypertension, and post-streptococcal glomerulonephritis related kidney diseases. It is most important to perform a screening test on any patient at risk for microalbuminuria in whom knowledge of the test will provide information on cardiovascular and renal risk stratification or have therapeutic implications. Confirmation of the presence of microalbuminuria is useful because some conditions cause transient excess albumin in the urine, which is neither a sign of CV nor a reason to treat microalbuminuria [4].

There are multiples method that has been widely used in biomedical field to diagnose microalbumin in human's unrine, such as dipstick and spectrophotometer [4]. Dipstick method uses a strip of 10 different reagents that will react with the urine sample and indicate a positive result with colour changing. The results are positive when the concentration of the albumin is within 20 to 30 mg/L. Diluted or concentrated of urine sample will often give false positive and false negative test results [5]. This method is commonly used by the identification of the color changes on the dipstick due to the chemical properties of the urine chemical composition. The strips will then show the result by changing in the strip color to determine the patient health condition. For normal conditions, daily albumin excretion is in the range of 5–10 mg and the urine albumin: creatinine ratio is in the range of 0–29 mg albumin/g creatinine. Meanwhile, microalbuminuria is defined as

an abnormal increase in albumin excretion rate within the specific range of 30–299 mg of albumin/g of creatinine [6]. These two conditions, which can be identified by using dipstick is not an effective way because the concentration level of albumin can't be accessed.

On the other hand, spectrophotometer is a method to determine the light absorbs by the compound or molecules. The intensity of the light beam that passes through a sample will be calculated to determine the amount of a known chemical substance. The sample will be illuminated with monochromatic light that ia able to transmit the light beam through the sample and will be then detected by a photodetector. This method is applied a Beer-lambert Law in measuring the absorbance level of light in the urine sample, respectively. Spectrophotometer uses albumin/creatine ratio to measure the concentration of the albumin in urine, which is widely used in the hospital during medical assessment. This method is different from dipstick method because the quantity of absorbed light is used to indicate the albumin level. A human body will filter creatine at the same rate, so by comparing the creatine and albumin quantity would help to determine the body albumin excretion rate. If the ratio of albumin/creatine is within 30-300 ig/mg, the patient's urinewill be classified as microalbuumiuria.

In this paper, a proposed method capable of accurately detecting the concentration of the albumin is reported. A microalbumin sensor that made of a Polydimethylsiloxane (PDMS) thin film was fabricated and integrated with glass substrate to detect albumin in human urine. PDMS had been chosen due to optically clear, inert, nontoxic and non-flammable characteristics. Most of the application such as contact lenses, medical devices to elastomers are made from PDMS. In biomedical microelectromechanical systems (bio-MEMS), soft lithography is used frequently in microfluids. It has hydrophobic surfaces making it easy to attract protein molecule. PDMS is a low cost material, easy to fabricate and has good flexibility that make it suitable candidate for prototype of MEMS microalbumin detections [7]. The findings of this study is a breakthrough on microalbuminuria detection as a lowcost and flexible sensing material could determine the albumin level in urine with transmittance light variation and refractive index of light.

2. RESEARCH METHOD

2.1 Fabrication of PDMS thin film

PDMS thin film is the core unit of the device. A spin coater was used to fabricate the thin film with minimum thickness. For the fabrication, a finite volume of the base agent of SYLGARD 184 Silicone Elastomer was transferred into a petri dish using a plastic pipette. After that, the curing agent of SYLGARD 184 was diluted into the petri dish that contained the base. A mixed of base and curing agent was prepared with the ratio of 10:1 (10 parts of base and 1 part of curing agent) the base was 10 part). This mixture was mixed using plastic pipette, and bubbles were seen in the petri dish due to the effect of the curing agent. The mixture was mixed until bubbles visible within all the surface of the mixture. A mixed elastomer was placed in the spin coater and the spin coater was setted at pre-setting mode. After spinning, the mixture was sealed and sitted for the curing process. The process of curing took approximately 24 hour. Detailed information of the volume of base, curing agent, spinning speed is depicted in Table 1.

During fabrication process, the thin film was peeled off from the base as opposed to normal circumstance in the PDMS uses in fabricating and prototyping microfluidic chips. In Table 1, the results were exhibited when the curing time for the PDMS was reached and ready to be peeled off. In overall, the PDMS thin film experience tear as a result of overstressing the thin film during peeling off. Only in one method, the thin film is able to extract out from the petri dish base because in other method, the volume of PDMS after the spinning is usually too low, making the produced thin film become too thin to be peeled off from its base. Any coating speed higher than 1000 rpm with 5ml base volume of PDMS will make the thin film too thin to be peeled off, while any volume lower than 5ml of the base volume will also make the thin film too thin to withstand the peeling force during peeling the film from its base.

2.2 Glass Slide

Optical grade glass slide was selected as the substrate for the thin film due to the transparency criteria. The depth of the trench was made half of the microscopic slide thickness in 0.5 mm. The trench was defined by using a glass drill bit of 5 mm. The problem of using the microscopic glass slide was the small opening size because the trench was created using the drilling technique.

Table 1. The Performance of Fabricated PDMS Thinfilm with Various Base and Curing Agent Ratio

Base Volume (ml)	Curing Agent Volume (ml)	Spin Speed (rpm)	Results
2	0.2	1000	Thin film experience tear when peeling off from petri dish
3	0.3	1000	Thin film experience tear when peeling off from petri dish
4	0.4	1000	Thin film experience tear when peeling off from petri dish
5	0.5	1000	Able to peel off form petri dish
5	0.5	1100	Thin film experience tear when peeling off from petri dish
5	0.5	1200	Thin film experience tear when peeling off from petri dish

As the microscopic glass slide was only 1 mm thick, drilling a trench with a 0.5 mm thick using glass drill bit of 5 mm only yielded a small opening as the length of the drill bit is 0.5 cm, the opening would be as small as 0.1 cm. Another problem of creating a trench in the microscopic glass slide is the transparency of the trench created. During the drilling on the glass, the glass surface will become white, this is because the surface of the glass in the trench is crack and this will greatly affect light going through the trench. In addition, the area surrounding of the trench experience cracking because area surrounding the trench supposed to be the part of the hole. Therefore, a trench with 1 mm thickness of the glass is impossible to be created.

To comprehend the problem cause to create trench on the microscopic glass slide, a drill through method was performed on the microscopic glass slide by using a 5 mm of the glass drill bit to drill 1mm thickness of microscopic glass. This method was able to create a radius of 1.5 mm of hole. Finally, a drilled microscopic glass slide was then stacked with another microscopic glass slide thus creating an air gap for the light to interfere.

For the integration process, firstly the PDMS thin film was slitted into small part. Since the drilled hole was very small, the dimension of the thin film was limited in a dimension of 1cm by 1cm to cover the drilled hole. After that, the thin film was sticked on top of the drilled microscopic glass slide by thermal bonding. The oven was pre-heated at 80 ℃ before l bonded the two pieces together. Some pressure was applied to attach the thin film to a drilled microscopic glass. Then, the integrated device was baked in the oven at 80 ℃ for over 1 hour. Another microscopic glass slide was used to cover the back side of the microscopic glass slide. Two glass slides were then sealed using silicone sealant. Figure 1 illustrates the cross section of the sensor design, meanwhile Figure 2 shows the PDMS thin film on top of the hole on the microscopic glass slide.
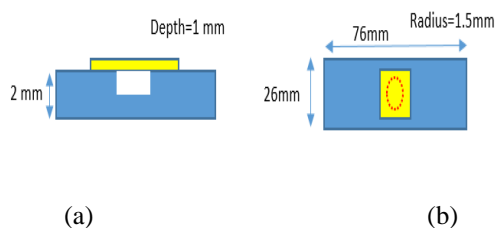

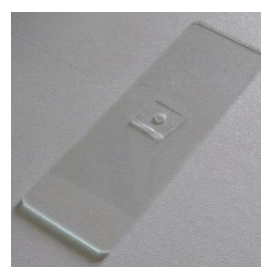Figure 1. The Sensor Cross-Section: (a) Side View (b) Top View

Figure 2. The PDMS Thinfilm on Top of the Hole on the Microscopic Glass Slide

3. RESULTS AND ANALYSIS

In this section, the obtained experimental results are analyzed and discussed. A proposed flexible PDMS thin film on microscopic glass substrate sensor was tested with the spectrometer to validate the capability of the sensor for urine spectroscopy.

3.1   Drilling effects

Figure 3 shows the drilling result on the microscopic glass slide, the first opening was created by drilling through the microscopic slide with minimal damage to the surrounding of the glass slide. For the second opening which was the first idea of creating trench on the microscopic glass slide failed as crack form at the surrounding of the trench which will affect the behaviour of light going through. The trenches created by second and third opening contain scratch within the opening. These opening are tested with illumination of white light on the microscopic glass slide. The light illumination characterization is presented in Figure 4, where only one of the opening allow the light to pass through the microscopic glass slide. However, the other two opening with a trench on the microscopic glass slide do not exhibit the transparency. The opening number one exhibits the desired transparency as the opening one is the result of drilling the hole through the microscopic glass slide.
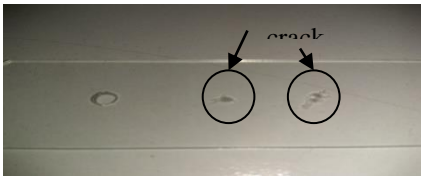

Figure 3. Drilling Result on Microscopic Glass

Figure 4. The Light Illumination Characterization on Three Different Opening Holes

3.2   Spectral Response

The spectral response experimental results are analyzed and discussed in this section. The fabricated sensor made from PDMS was charaterized for optical performance using the spectrometer (HR4000CG-UV-NIR Ocean Optics). A normal microscopic glass was illuminated with light source and the light was then splitted to horizontally direction using a fiber optic cable to identify the performance of visible and near infrared urine substance absorption spectroscopy in the 400–1400 nm wavelength range. The light source illuminated through the glass with 10681 intensity counts at wavelength of 643.41 nm, hence that a high-absorbed light in urine sample on the glass without PDMS was detected at dedicated wavelength of 643.41 nm. Figure 5 shows the spectral range of glass illuminated with various wavelengths.
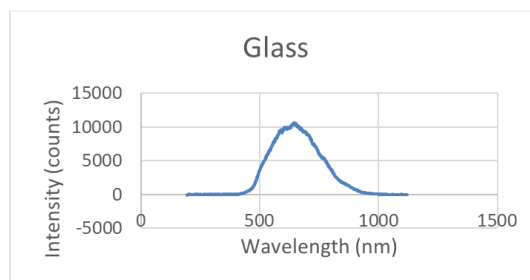

Figure 5. Intensity Counts at Wavelength Range between 200 and 1200 nm on Glass Substrate

In Figure 6, the experimental result using the fabricated flexible sensor shows low intensity counts with 5324 count at wavelength 643.41. This is because the PDMS surface along with the air gap and the microscopic glass surface create a partial transmittance and reflection from low refractive index to high refractive index (PDMS is approximated to 1.4), through air gap and then finally through the bottom part of microscopic glass slide (approximately at 1.52). Based on Fresnel equations [8, 9], the reflectance and transmittance relationship are as follow.

$$T_P = 1 - R \tag{1}$$
$$T_S = 1 - R \tag{2}$$
$$R = \frac{R_S - R_P}{2} \tag{3}$$

where, T_S is the transmittance of s polarised light, R_S is the reflectance of s polarised light, while T_P and R_P represent the transmittance and reflectance of p polarised light. From the equation 1, 2 and 3 respectively, the relationship of transmittance and reflectance is simplified in equation 4. This equation does not take transmittance and absorbance into account as the light travel in the bulk where Beer-Lambert law was applied [8-10].

$$T = 1 - R \tag{4}$$

Optical sensing method in urine is based on the interaction between the incident light and soil surface properties, such that the characterizations of the reflected light vary due to the urine physical and chemical properties. Hence that, Beer-Lambert Law is utilized in determining the microalbuminuria, where there is a relationship between absorbance and concentration of an absorbing molecules in solution and path length [11, 12]. Figure 7 shows the absorption of light by a sample when light passes through it. The measurements are usually made in terms of transmittance (T) and absorbance (A), which can define as

$$T = \frac{I}{I_0} \tag{5}$$

$$A = -\log T = -\log \frac{I}{I_0} = \varepsilon LC \tag{6}$$

Where I_o = initial light intensity, I = light intensity after passing through the sample, ε = molar absorptivity (L mol-1 cm-1), L = path length (cm) and C = concentration of the absorbing chemical species (mol/L) [11, 13, 14]. The significance of Beer-Lambert law is to measure the absorbance of a particular sample and to infer the concentration of the solution. There are two situations need to consider. First, if a light beam of appropriate wavelength passes through a diluted solution in urine, the photons will encounter a small number of absorbing chemical species (chemical molecules will absorb light at a particular wavelength) and the result may have high T and low A [16]. Second, if the beam of light encounter diluted solution for a long period of time, the result might be low T and high A. Thus, Beer-Lambert law stated that absorbance is proportional to the concentration of the sample and proportional to the path light of a beam through the sample [11-14].
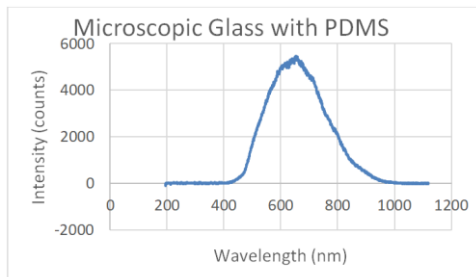

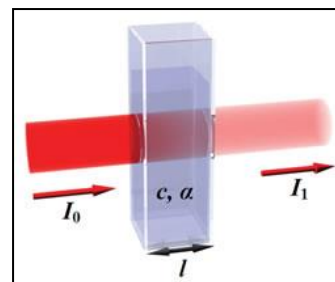Figure 6. Intensity Counts on Microscopic Glass Slide with PDMS

Figure 7. Absorption of Light by a Sample

Figure 8 depicts the spectral range of light with sodium urate concentration of 15mg/dL dropped on top of the fabricated flexible PDMS with microscopic glass slide substrate. There is intensity dropped mesured by the spectrometer reading at t = 0 minute. This is because the solution was applied on the surface by dropping a solution on top of the PDMS thin film and the transmitted light has travelled across molecules of sodium urate, PDMS layer and microscopic glass slide substrate before the absorbed light was detected by the spectrophotometer. The peak intensity for t = 0 minute is 1448 counts at 621 nm. Notice that there is an increase of intensity at the sixth minute, this is because both the solution droplet and PDMS thin film formed a negative meniscus convex lens (converging meniscus) which brings parallel lights to a focus as shown in

Figure 9. The deflection of the PDMS thin film is because of adsorption of uric acid on the PDMS surface, creating a surface tension, thus deflected the thin film.
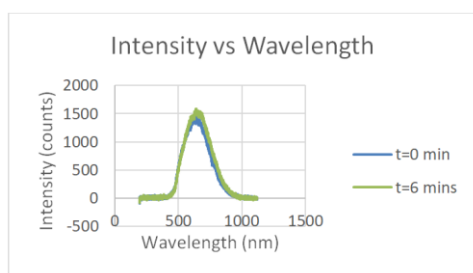

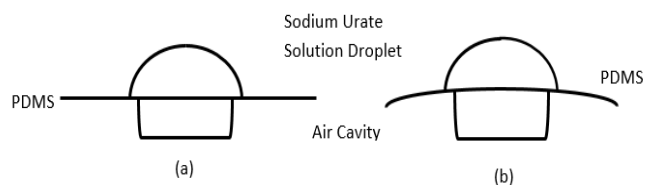Figure 8. Intensity vs Wavelength with Sodium Urate Concentration of 15mg/dL

Figure 9. The Geometry of the Thin Film (a) Before Thin Film Deflect (b) After Thinfilm Experience a Small Degree of Deflection

4. CONCLUSION

In conclusion, the fabrication method for the sensor is able to produce a sensor capable to detect sodium urate, one of the component present in urine. Microalbumin early detection would give doctors more time to cure a patient before the condition get worse. In conventional detection method, dipstick and spectrophotometer enable a person to detect microalbumin, but dipstick only gives qualitative results while spectrophotometer gives quantitative results. However, optical spectroscopy in urine do not grant a doctor to test the urine sample immediately. In this early prototype, an alternative method to detect microalbumin was proposed. Results shows a promising result as the PDMS thin film able to detect sodium urate, a substitute for urine sample. The spectral shift of the spectral range proposed that the PDMS experience adsorption from the reaction of sodium urate. In addition to these criteria, the result of the spectral change will be more obvious as the air cavity and the thickness of PDMS is in the magnitude of hundred nanometer. Further development such as immobilization of microalbumin antibody on PDMS thin film and built in photodiode will enable the full integration of the microscopic glass slide into a back-end detector.

REFERENCES

[1]   A. S. Levey, *et al*., "Definition and classification of chronic kidney disease: A position statement from Kidney Disease: Improving Global Outcomes (KDIGO)", *Journal of Kidney International*, vol. 67, pp.2089-2100, June 2005.
[2]   R.D. Toto, "Microalbuminuria: definition, detection, and clinical significance", *The Journal of Clinical Hypertension*, vol. 6, pp. 2-7, Nov 2004.
[3]   T. Qiu *et al*, "Palmtop spectrophotometer for DNA and protein measurement in micro-nanoliter assays", *Journal of Physics: Conference Series*, vol. 277, pp. 10-21, March 2011.
[4]   A. Takamoto *et al*., "Screening for oral mucosal diseases by a portable spectrophotometer: Comparison between color difference and epithelial thickness", *Journal of Oral and Maxillofacial Surgery Medicine, and Pathology, vol.* 25(4), pp. 314-327, October 2013.
[5]   B. Anjalidevi *et al*., "Prediction of microalbuminuria by using spot urine samples and regression analysis to convert spot microalbumin values to 24 hours microalbuminuria", *Journal of Biochemistry and Bioinformatics*, vol. 1(3), pp. 071-075, April 2011.
[6]   J. Lin *et al.,* "Associations of diet with albuminuria and kidney function decline", *Journal of the American Society of Nephrology*, vol. 5 (5), pp. 836–843, May 2010.
[7]   M. Ahmadi *et al.*, "Flexible Distributed Pressure Sensing Strip for a Urethral Catheter*", Journal of Microelectromechanical Systems*, vol. 24, pp. 1840-1847, July 2015.
[8]   G. Florvall *et al.*, "Measured by three Different Methods, Blood Pressure and Cardiovascular Risk Factors in Elderly Swedish Males", *Journal of Analytical Chemistry Insights*, vol. 3, pp. 69-74, September 2008.

[9]   H. Oyama *et. al*., *"A MEMS Based Fabry-Perot Protein Sensor with Reference Sensor"*, The 14th International Meeting on Chemical Sensors, vol. 1, pp. 353-355, May 2012.
[10] O. Hoffman *et al.*, "Towards microalbuminuria determination on a disposable diagnostic microchip with integrated fluorescence detection based on thin-film organic light emitting diodes", *Journal of Lab on Chip*, vol. 5, pp. 863-868, June 2005.
[11] D. A. Raorane *et al.*, "Quantitative and label-free technique for measuring protease activity and inhibition using a microfluidic cantilever array", *Nano letters*, vol. 8, pp. 2968-2974, August 2008.
[12] S. Grilli *et al.*, "Surface-charge lithography for direct pdms micro-patterning", *Langmuir*, vol. 24 (23), pp. 13262–13265, December 2008.
[13] S. Isaak *et al*., "NPK Detection Spectroscopy on Non-Agriculture Soil", *Jurnal Teknologi*, vol. 78(11), pp. 227-231, November 2016.
[14] M. Tay *et al.,* "Optical path length and absorption cross section optimization for high sensitivity ozone concentration measurement", *Journal of Sensors and Actuators B: Chemical*, vol. 221, pp. 570-575, December 2015.

BIOGRAPHIES OF AUTHORS

Suhaila Isaak received her Bachelor Degree in Electrical and Electronic Engineering from Universiti Teknologi Malaysia in 1998, the Master in Science (Electronic) degree from Universiti Putra Malaysia in 2001, and the Ph.D degree in Electrical and Electronics Engineering from University of Nottingham, England in 2011. Her major research interests are high-speed avalanche photodiodes and InP-GaAs photodetector design, simulation and fabrication. In addition, she is also working on integrated linear array photon camera using FPGA. Currently, she is involved on spectroscopy system for soil analysis.

Yusmeeraz Yusof received the B.S. degree in electronics and the M.S. degree in integrated design engineering from Keio University, Japan, in 2002 and 2004, respectively, and the Ph.D. degree in electrical engineering and computer science from Nagoya University, Japan, in 2011. Before she joined Universiti Teknologi Malaysia (UTM) as a faculty member in 2006, she worked as an R&D engineer with Alps Electric Malaysia for two years. She is currently a Senior Lecturer with the Faculty of Electrical Engineering, UTM. Her current interests include CMOS DNA chip, semiconductor and new material biosensors, and point-of-care biomedical devices.

Khairunnisa Mohd Yusof received her Bachelor degree in Electronics Engineering (Communication) from Universiti Teknologi Malaysia (UTM), Johor Bahru in 2014. She is currently a Master by research student in Faculty of Electrical Engineering at Universiti Teknologi Malaysia (UTM), Johor. Her research interests focus on optical characterization of soil sensor and absorbance spectroscopy using avalanche photodiode.

Khoo Ming Kwan received his Bachelor degree in Electronics Engineering (Communication) from Universiti Teknologi Malaysia (UTM), Johor Bahru in 2017. He is currently working with Opsstar Sdn. Bhd, Penang Malaysia as Integrated Circuit Design Engineer. His research interests focus on microlectronic sensor fabrication and VLSI design.