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Urea Biosensor Development using Immobilized Urease and Light Dependent Resistor

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Abstract. The existence of urea in human's body becomes an important biological parameter for kidney disease identification. Development of urea analysis methods are widely studied, including the using a biosensor. This study was developed a urea biosensor using immobilized urease in alginate matrix and a light dependent (LDR) as continuous color change detector. The color detection was based in the reaction of urea with urease to produce ammonia which finally identified using bromothymol blue as color indicator. The LDR, a cheap electronic component was controlled by Arduino microcontroller to monitor the color change related to urea concentration. The result showed the optimum reaction time was 4 minutes with the best linearity was obtained by red color change with urea concentration ($y = 0.42x + 39.34$, $R^2 = 0.991$). Furthermore, the limit of detection of this developed urea biosensor was 0.35 mM and the limit of quantification was 1.17 mM. The linear range of the urea biosensor was 1.17 mM to 42.65 mM. The immobilized urease in alginate bead could be used up to 11 times. Urea detection in blood samples showed no significant differences between the results of the clinical analysis with the results of developed urea biosensor with a correction value of 1.3.

INTRODUCTION

Urea is an organic compound that can be formed biologically in the living body as an final product of the nitrogen cycle in the liver [1]. The presence of urea in the body commonly used as an important biological parameters for identification of kidney disease [2]. Kidney disease characterized by increasing the levels of urea in serum above

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normal levels. The normal limit of urea levels in the blood ranges from 5-25 mg/dL. When the urea level in the body exceeds the normal limit it will indicate an imbalance in the body that is not normal such as having kidney failure [3].

Determination of urea in the blood or urine as an indicator in the diagnosis of a disease in the hospital usually use standard method including the use of expensive equipment such as UV-Vis spectrophotometer. Recently, may studies have been developed to measure levels of urea with a simple and low-cost equipment such as the biosensor. Biosensor is an analytical method that uses of biological components (e.g. microbial cells, tissues, bacteria, proteins, enzymes, antibodies) integrated with an electronic equipment to convert the analyte-sensing interaction into measurable signal [4]. The biological sensing element widely used in the biosensor fabrication is the enzyme. Various enzymes have been applied in biosensor development such as the use of glucose oxidase for glucose biosensor [5], urease for urea biosensor [6], n-acetylneuraminidase for sialic acid biosensor [7] and cholesterol oxidase for cholesterol biosensor [8].

Urea biosensor could be developed by the uses of urease enzyme as a biological sensing element to convert the urea into measurable product. Urease is an enzyme catalyzing the hydrolysis of urea to form ammonia and carbon dioxide. This enzyme can be found in bacteria, fungi, and plants plays an important role in the circulation of nitrogen in nature [9]. The complexity of enzyme isolation and purification tend to increase the cost of the use of enzyme and their derivative methods. The high cost of enzymes could be reduced by reuse of enzyme through immobilization. The enzyme immobilization was also widely use in the biosensor development to improve the efficient method by repeatedly use. Immobilized urease showed a high stability, can be used repeatedly and can retain its activity [10].

Urea biosensors have been widely developed recently using electrochemical detectors. However, the use of colorimetric methods in urea biosensor still interesting, especially the use of digital image processing for easy operation and reduce the biosensor cost. The digital image processing for analytical method could be performed using several daily equipment such as smartphone [11], document scanner [12] and pocket camera [13].

This study reported the development of urea biosensors using immobilized urease on calcium alginate (Ca-alginate) and a detector of LDR (Light Dependent Resistor. The LDR is a light sensor or an electronic component that can change the resistant according to changes in light intensity which could be related to the color of sample. The data recording used was Arduino microcontroller to convert the color change of the sample to digital signal. Determination of urea by the enzymatic reaction of urea with a urease selectively produces ions NH_4^+ and CO_3^{2-}. The NH_4^+ ion formed change the color of an indicator bromothymol blue (BTB). The analytical performance of the biosensor was determined. The use of cheap and easy build detector could be applied in future color based analytical technique.

MATERIALS AND METHODS

Materials

Urease from *Canavalia ensiformis* (34310 U/g) was purchased from Sigma-Aldrich (US). Ammonium hydroxide, sodium alginate, calcium dichloride, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium hydroxide, bromothymol blue and ascorbic acid were form Merck (Germany). Blood samples was collected from local hospital.

Apparatus and measurements

LDR-based color detector was built to measure the intensity of the color solution. The detector was made by arranging LDR and red-green-blue LED lamp as light sources. This detector was connected to Arduino microcontroller to control the LED light and to record the LDR reading. The microcontroller also converts the LDR resistant reading to red, green and blue color intensity and send to USB port. The data was show and record using developed computer software to easy analyze (Figure 1).

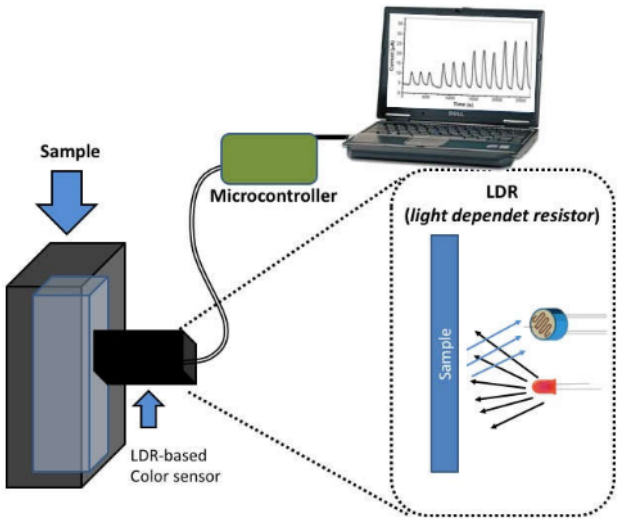


FIGURE 1. Design of LDR-based color detector for urea biosensor

Color change measurement study

Ammonium color change measurement using the UV-Vis Spectrophotometer

Standard solution of NH_4OH was used as simulation of enzymatic reaction of urea with a urease enzyme solution which produces ammonium ion (NH_4^+). The color change was first measure using UV-Vis Spectrophotometer to determine the maximum wavelength of sample solution with the addition of bromothymol blue. The NH_4OH standard solution used were 0.1; 0.2; 0.3; 0.4; and 0.5 mM of 5 ml and 250 mL of 0.04% (b/w) bromothymol blue. The standard curve was then prepared by plotting the concentration of NH_4OH and absorbance.

Ammonium color change measurement using the LDR-based color detector

The sample preparation and color measurement were similar to the measurement using UV-Vis spectrophotometer. Sample of NH_4OH standard solution at various concentration of 0.1; 0.2; 0.3; 0.4; and 0.5 mM and bromothymol blue were mix and kept in 2.5 ml cuvette. The sample was then insert in the LDR color sensor connected to computer. Color intensity were recorded and save for ten times measurement of each concentration. The obtained data of color intensity and concentration were then analyzed to make the calibration curve between the color intensity (Red, Green, Blue) and Ammonium concentrations. The three color of RGB calibration curve was the compare to get the best color intensity (highest slope and highest coefficient of determination). The calibration curve was also compared to the calibration curve by the UV-Vis Spectrophotometer.

Urease immobilization in calcium alginate

Urease immobilization was performed by trapping in calcium alginate. Sodium alginate solution was prepared by dissolving 2.5 g sodium alginate in 100 ml of 0.1 M phosphate buffer (pH of 7.0). Enzyme solution of 500 uL was then mixed with Na-alginate solution of 7500 uL and stir carefully. The mixture was then drooped on the crosslinking solution of 0.2 M $CaCl_2$ and allowed for 30 minutes to complete the beads formation.

Linearity study

The study was carried out by reacting urea solution with urease-alginate to produces NH_4^+. Urea solution used were 1, 2, 3, 4, and 5 mM, using similar procedure of ammonium measurement using spectrophotometer above. The highest sensitivity results were selected from the color intensity obtained (Red, Green or Blue) from the highest slope of calibration curve with the best coefficient of determination. The best color change was the used for further procedures.

Optimization of the enzymatic reaction time

The enzymatic reaction duration required for the urease catalyze urea breakdown effectively. The urea solution used was 5 mM reacted with alginate-urease bead at incubation time of 2, 4, 6, 8, 10, and 12 minutes. The highest color intensity change in combination with the shortest reaction duration was then selected as optimum reaction time.

Linear range, limit of detection and limit of quantification study

The linear range was studied from the lowest concentration to the highest concentration of standard urea solution. The series urea solution of 0.01; 0.05; 0.1; 0.5; 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mM was reacted with alginate-urease beads for optimized reaction time. The bromothymol blue was added to the resulted ammonium and measured using LDR-based color detector. Linear range was determined by plotting the urea concentration and color change with the coefficient of determination larger than 0.9. The limit of detection and limit of quantification were then calculated from the linear equation obtained.

The stability of the immobilized Urease test

The immobilized urease in alginate beads was studied the recycle use to catalyze the 3 mM in the optimum reaction time. The color change was measured using LDR-based color sensor up to the responses decrease to 50% of the first use.

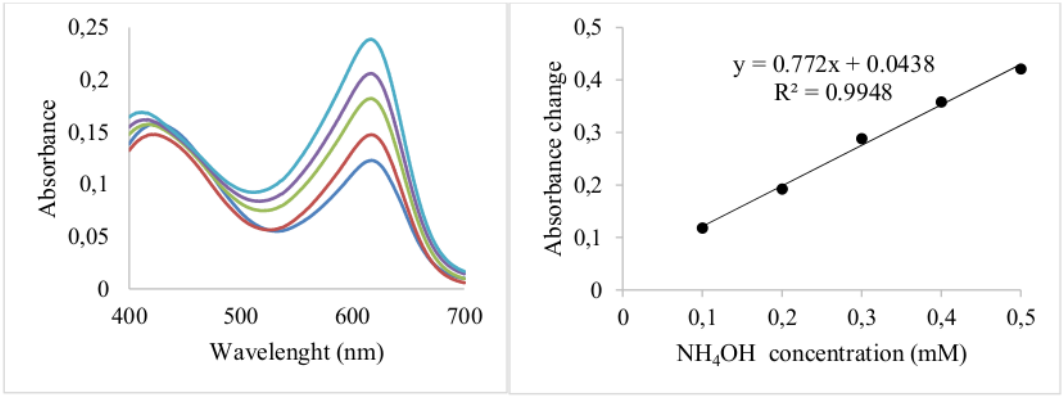
Determination of urea in blood serum samples

Urea determination was performed using blood serum samples previously measured by the standard method in the hospitals. A series urea solution of 1, 2, 3, 4, and 5 mg/dL was used the standard to create a calibration curve. The blood serum samples of 30 µL was diluted to 3 mL with phosphate buffer, and continued reacted with the alginate-urease bead as described in the previous urea determination at the optimum time. Sample urea concentrations were calculated and analyzed compare to the urea concentration by the hospital method.

RESULTS AND DISCUSSION

Color change measurement

The color change was first studied using ammonium hydroxide (NH_4OH) as simulated urease reaction product with indicator of bromothymol blue. The maximum wavelength was obtained at about 620 nm using spectrophotometer (Figure 2a). Furthermore, the color change showed a linear relationship with the concentration of ammonium hydroxide for 0.1 to 0.5 mM (Figure 2b).



a **b**

FIGURE 2. Ammonium hydroxide and BTB measurement using spectrophotometer

Color change measurement using LDR-based color detector

LDR color changes using the detector as well as it did in testing the changes by using UV-Vis Spectrophotometry. The output signals were Red, Green and Blue value corresponding to the resistant change of LDR when the reflected of the three LED light (red, green and blue) turned on alternately. The sequence of LED light on and off were set for 150 milliseconds. for 0.5 The result showed a linear response between increasing of ammonium concertation and color intensity change with the increasing of ammonium hydroxide concentration for all Red, Green and Blue color. However, the best sensitivity (slope) was the red color intensity (Figure 3).

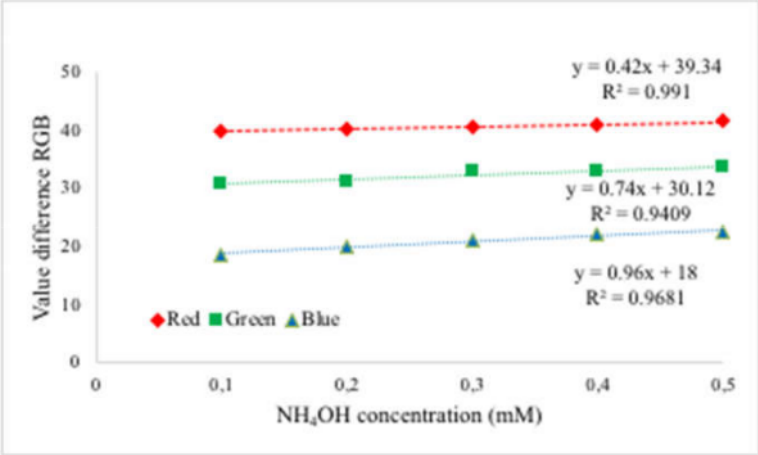


FIGURE 3. Ammonium hydroxide and BTB measurement using LDR based color detector.

Linearity study of urea detection using immobilized urease-alginate beads

Urea standard solution of 1.0 to 5.0 were reacted with urease-alginate beads for 15 minutes. The reaction results were then separated from the beads and added the bromothymol blue indicator. The color changes were measured using LDR-based color detector and Color Sensor Reader Software. The result showed linear responses of color

change with the increasing of urea concentrations (Figure 4). The best sensitivity was obtained of the red color intensity change, similar to those the detection of ammonium hydroxide on the previous procedure. The change of red color intensity was then used for further procedures.

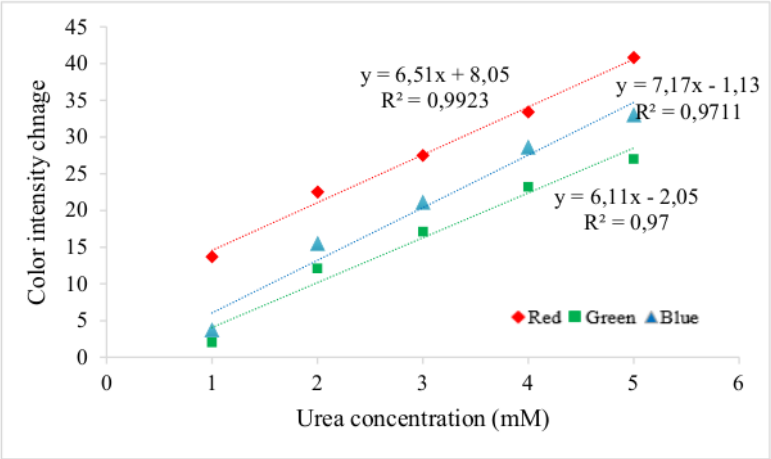


FIGURE 4. Urea linearity study using developed urea biosensor

Optimization of enzymatic reaction time

The optimum time was studied to determine the enzyme-substrate reaction to get the combination of highest product with the lowest duration. The urea and alginate-urease beads were studied for 2, 4, 6, 8, 10, and 12 minutes reaction. The red color changes were increase from 2 to 4 minutes reaction and afterward no significant increasing found (Figure 5). Therefore, the optimum reaction time of 4 minutes was the used for further study.

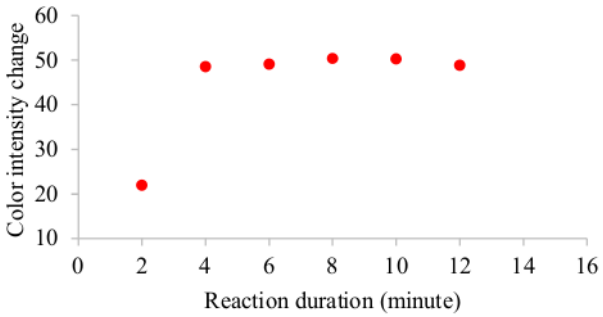


FIGURE 5. Effect of reaction duration on color intensity change

Linear range, LOD and LOQ determination

Under the optimum reaction time, linear range was studied from the lowest concentration could be detected to the highest concentration of urea which show the linear responses. The results showed the developed urea biosensor linear to detect urea from 0.5 mM to 6 mM, with the regression equation of $y = 6.14x + 7.03$ and coefficient of correlation coefficient (r) of 0.998. Furthermore, the calculated LOD and LOQ were 0.35 mM and 1.18 mM respectively.

The reusability of alginate-urease beads

The main purpose of the use of immobilized urease was to reduce to enzyme cost by the recycle use them. The study of reusability cycles were performed using urea standard solution of 3 mM under the optimum condition. The red color change was then measured using LDR-based color detector. The result showed recycle use of immobilized urease decrease with the cycle used, up to 50% after 11 using cycle (Fig).

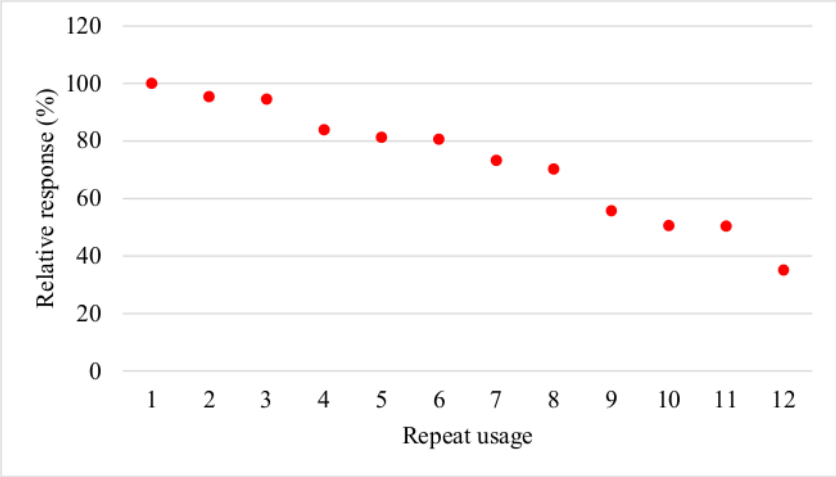


FIGURE 6. Reusability cycle of fabricated urea biosensor using alginat-urease beads

Urea determination in blood samples

The urea determination in blood samples was performed to verify the analytical performance of developed urea biosensor. Standard curve of urea was first prepared using urea solution of 1, 2, 3, 4, and 5 mg/dL. Furthermore, six blood serum sample was the analyze using urea biosensor in the same way of standard curve, with three repetition. The result showed that urea concentration in the samples obtained by biosensor method showed higher values than urea concentration from standard method use in the hospital. However, there were a similar ratio value of biosensor method to standard hospital method with the average of 1.3 (Table 1). Therefore, for real sample application, the biosensor results should be divided by 1.3 to get the equal value as standard method results. By using the constant value of 1.3 to divide the biosensor results, the calculated urea level in the samples were then no significant difference between biosensor and standard hospital method (Wilcoxon Signed-rank Test, $p < 0.05$).

TABLE 1. Blood urea determination using urea biosensor compared to standard hospital method.

	Urea concentration (mg/dL)		constant
	Hospital method	Biosensor	
1	44	56.56	1.29
2	27	33.90	1.26
3	24	28.69	1.20
4	22	27.95	1.27
5	21	27.02	1.29
6	19	24.51	1.29
			Constant everage = 1.3

CONCLUSION

Urea biosensor has been developed using alginate-urease beads combined with an LDR-based color detector. The biosensor saw good linearity and sensitivity, with a linear range of 1.18 to 42.64 mm and calculated LOD and LOQ 0.35 mM and 1.18 mM respectively. The immobilized urea in alginate bead showed a recycle used up to 11 times (remained activity > 50%). Furthermore, the fabricated urea biosensor has been applied to detect urea in blood serum sample with no significant different compare to standard method use in the hospital.

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REFERENCES

1. C.-P. Huang, Y.-K. Li, and T.-M. Chen, *Biosens. Bioelectron.*, **22**, 1835–1838 (2007).
2. A. Joshi, R. Chaudhari, and R. Srivastava, FITC-tagged macromolecule-based alginate microspheres for urea sensoring, in: Nanosensors, Biosensors, Info-Tech Sensors Syst. 2014, International Society for Optics and Photonics, (2014), p. 90600P.
3. R.K. Murray and J.C. Davis, "Harper's Illustrated Biochemistry", (2003).
4. A. Koyun, E. Ahlatcolu, and Y. Koca, Biosensors and Their Principles, in: A Roadmap Biomed. Eng. Milestones, (2012).
5. A. Fatoni, M.D. Anggraeni, and D.W. Dwiasi, Simple colorimetric glucose biosensor using chitosan cryogel supporting material, in: AIP Conf. Proc., (2016), pp. 53–66.
6. M. Tyagi, M. Tomar, and V. Gupta, *Biosens. Bioelectron.*, (2013).
7. A. Fatoni, A. Numnuam, P. Kanatharana, W. Limbut, and P. Thavarungkul, *Electrochim. Acta*, **130**, (2014).
8. R.S. Dey and C.R. Raj, *J. Phys. Chem. C*, (2010).
9. K. Panpae, N. Wiyok, and N. Kanthiwivorn, *J. Chem. Chem. Eng.*, **6**, 726 (2012).
10. C. Selvamurugan, A. Lavanya, and B. Sivasankar, *J. Sci. Ind. Res. (India).*, (2007).
11. M.D. Anggraeni and A. Fatoni, Non-invasive Self-Care Anemia Detection during Pregnancy Using a Smartphone Camera, in: IOP Conf. Ser. Mater. Sci. Eng., (2017).
12. M. Kompany-Zareh, M. Mansourian, and F. Ravaee, *Anal. Chim. Acta*, (2002).
13. A. Lapresta-Fernández and L.F. Capitán-Vallvey, *Analyst*, (2011).

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