Development of pH Sensing Devices Based on Optical Fluorescents with Rapid Measurement, Low Cost and Wireless Monitoring

Rahmat Fauzi Siregar
Department of Electrical Engineering
Institut Teknologi Sepuluh Nopember
Surabaya, Indonesia
rfauzisiregar@gmail.com

Totok Mujiono
Department of Electrical Engineering
Institut Teknologi Sepuluh Nopember
Surabaya, Indonesia
totok.m@ee.its.ac.id

Abstract— **The level of acid or base in water based on solution (pH) is a very important measure for living things because about 70% in the body consists of water. Most of the metabolism in the body requires a certain pH level. Having a rapid and accurate pH meter is very demanding, but most of the available pH meters take several minutes to measure the pH of the liquid. The measured water is mixed with fluorescent liquid and then excited with violet light at a wavelength of 405 nm. We have developed a pH meter based on optical fluorescent using pyranine extracted from yellow highlighter using isopropyl alcohol. The pH meter based on optical fluorescent have advantage compared to other methods in terms of measurement time. The intensity of the green fluorescent emitted from the liquid sample is then captured by the AS7262 spectral sensor. A pH sensing device has been developed, tested and verified to be able to measure pH from a range of 4 to 11 with an accuracy of 98.13%, a reading error value of ±0.13 and only takes less than 3 seconds to take measurements.**

Keywords—pH sensor, fluorescent, pyranine

I. INTRODUCTION

Water is a very important and fundamental need for life. Water is a molecular substance that has a unique chemical and physical properties that are related to functions in the human body [1]. The human body tends to maintain a tightly controlled pH range from around 7.35 to 7.45 in extracellular fluid through respiratory carbon dioxide excretion and renal excretion from acidic or basic non-carbonic acids or bases [2]. Because water is the most important requirement for humans, pH measurement is needed to test the quality of water whether the water used is contaminated which can affect changes in pH in water. pH is a measure of the concentration of hydrogen ions or the activity of an aqueous solution and each aqueous solution can be measured to determine its pH value. These values range from pH 0-14 with values below pH 7 indicating acidic properties and values above pH 7 indicating basic or alkaline properties. pH 7 is the center of the measurement scale which shows no acidic or basic nature [3].

Having a fast measurement of acid levels is very important in many cases. One of the methods for measuring pH levels is to use optical fluorescents. The fluorescent dyes are chemical compounds that determine changes in the absorption of light between reactants and reaction processes or measure light with luminescence [4]. Fluorescent dyes are non-protein molecules that absorb light and emit light at longer wavelengths. They are often used in labeling fluorescent biomolecules and can be smaller than

fluorescent proteins but cannot be genetically encoded [5]. Different fluorescent dye variants have different pH sensitivity [6].

In this research, the optical fluorescents method approach is used to measure the pH of liquid water that is directly related to humans or the human environment. This method are now widely used because of their extreme sensitivity, which provides detection limits at picomolar levels and below, and the great variety of sample presentation methods available. Flowing liquids, solid surfaces, concentrated solutions, and suspensions can all be studied in addition to measurements in dilute solution [7]. The fluoerescent dye is extracted and added to the water sample. The type of fluorescent dye used in this research is pyranine fluorescent dye. The development of this pH sensing device consists of a light source which is excited towards a liquid sample mixed with fluorescent liquid and at the same time the intensity of the fluorescent emission is captured using a photodetector. The emission intensity of fluorescent is a function of the pH of the liquid sample. Fig. 1 shows the basic principles of developing pH sensing devices.
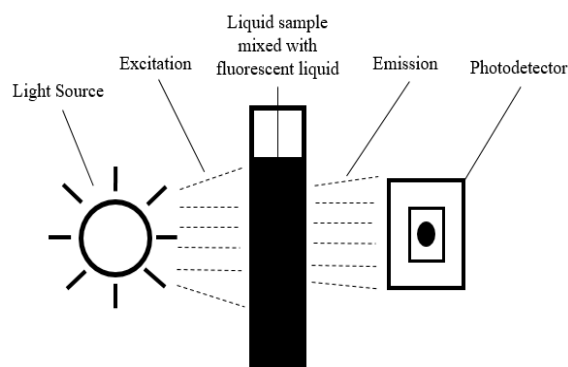

Fig. 1. The basic principles of developing pH sensing devices.

The optical fluorescent method has advantages such as enable simple, fast, and direct acquisition of spectra. Another advantage of this method are nondestructive measurements, performed directly on the untreated samples, avoid time- and labor-consuming chemical treatment steps, eliminate consumption of reagents and production of waste, thus fulfilling the requirements for green analytical chemistry and the availability of fluorescence sensors for certain applications that allow real-time process control measurements. The measurements are fast, and in conjunction with appropriate calibration models determine multiple components or properties from a single

measurement [8]. Therefore, by applying this method to a pH sensing device, the results of measuring the pH of water samples that are closely related to humans such as measuring the pH of the water consumed can be done rapidly and accurately. Rapid measurement is needed to save time. In addition, the measurement only requires very little volume of the liquid sample.

II. RELATED RESEARCH

Sebastian, Alina et al., used the amphiphilic polymer-linked conjugation method (APCNs) modified by nanophase separated by pyranine in making fluorescent ratiometric pH sensors. The thin, freestanding APCN membranes composed of one hydrophilic and one hydrophobic polymer provide an optically transparent, flexible, and stable ideal matrix that enables contact between dye and aqueous environment. An active ester - based conjugation approach results in a highly homogeneous and stable pyranine modification of the APCN's hydrophilic phase. Nanophase separated amphiphilic polymer conetworks provide an ideal matrix system for pyranine. An active ester based functionalization approach ensures stable conjugation to the hydrophilic phase and contact to the aqueous environment, enabling its use as fluorescent ratiometric pH sensors in the range of pH 5–9 [9].

A series of novel optical sensors for determination of broad-range pH based on a single fluorophore and multi-ionophores with different pK(a) values constructed by Qi J, Liu D, Liu X, et al [10]. The pK(a) is a measure of the ability of a compound to release protons into a solvent, under equilibrium conditions or a specific equilibrium constant for an acid and its conjugate base in an aqueous solution. The optical sensors they have constructed use photoinduced electron transfer (PET) as the signal transduction and follow the design concept of "fluorophore-spacer-receptor (ionophore)" which employs 4-amino-1,8-naphthalimide as the single fluorophore, ethyl moiety as the spacer, and a series of phenols and anilines as the receptors. They combine receptors with six different pKa values with a single fluorophore producing true optical properties. This rational design produces a series of optical pH sensors with unique fluorescence properties and accurate pH measurements ranging from 1 to 14 pH units. Due to the covalent immobilization of the indicator, this sensor shows excellent stability, adequate reversibility, and satisfying dynamic range to the full pH range (pH 1-14).

In their research, Yuan, Zhang, Xi dan Tao constructed a novel pH fluorescent probe 2,8- (6H, 12H-5,11-methanodibenzo [b, f] diazocineylene) -di (p-ethenyl-pyridine) (TBPP) incorporating an electron-donating amine moiety and electron-accepting pyridine group through Tröger's base linker was designed and synthesized. As a results, TBPP exhibits an intramolecular charge transfer effect caused by the donor - acceptor interaction between its amine and pyridine units. Its emission can be reversibly switched between blue and dark states by protonation and deprotonation. Such behavior enables it to work as a fluorescent turn-off pH sensor in solution state. 1H NMR spectroscopy analysis suggests that the change in electron affinity of the pyridinyl unit upon protonation and deprotonation is responsible for such sensing processes [11].

Sharma, Graham et al., Has developed a ratiometric fluorescence pH sensing device using FDA-approved dyes and LEDs that aims to evaluate the ability of acid production from plaque deposits in holes and fissures in occlusal and interproximal regions on the surface of dental enamel. Fluorescein spectral profiles were collected using a spectrometer and analyzed with a spectral unmixing algorithm for calibration over the pH range of 4.5 to 7. An in vivo pilot study on human subjects was performed using a sucrose rinse to accelerate bacterial metabolism and to measure the time-dependent drop in pH. The optical system is relatively immune to confounding factors such as photobleaching, dye concentration, and variation in excitation intensity associated with earlier dye-based pH measurement techniques [12].

III. REAGENTS AND MATERIALS

A. Reagents

The reagents used in this research are pyranine, 100 mL of sodium hydroxide (NaOH) with a concentration of 1M, 100 mL of hydrochloric acid (HCl) with a concentration of 1M, 500 mL of 99% isopropyl alcohol, 1 L of distilled water. Pyranine is an organic sodium salt. It has a role as fluorochrome. It contains pyranine (3-) which comes from pyrene hydride. Pyranine or trisodium 8-hydroxypyrene-1,3,6-trisulfonate has a molecular weight of 524.4 g / mol [13]. The peak wavelength of absorption from pyranine is 405 nm and the peak wavelength of emission is 510 nm [14]. Pyranine absorbs light energy from wavelengths of 400 nm to 480 nm and emits light at wavelengths of 487 nm to 550 nm. The wavelength absorbed, the efficiency of energy transfer, and the time before emission depend on the structure of pyranine and its chemical environment, when molecules in an excited state interact with molecules around them. Fig. 2 shows the wavelength of absorption and emission from pyranine [15].
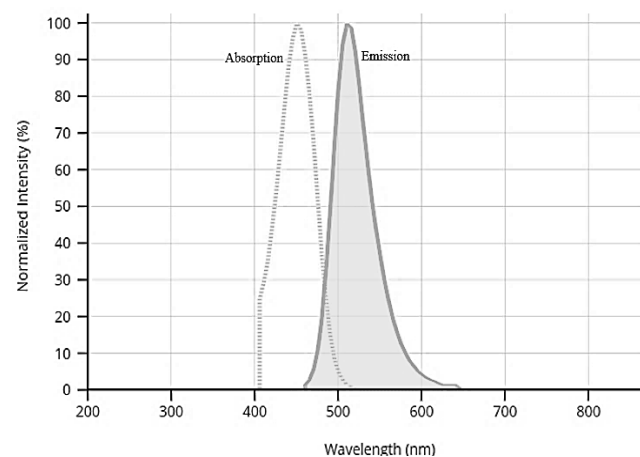
Fig. 2. Principle of pH Sensing Device System

B. Materials

The materials used in this research are dropper, test tube (10 mm x 75 mm), measuring cylinder (10 mL) and beaker.

C. Hardware and Software

The hardware used in this research consisted of an Arduino Nano microcontroller, AS7262 sensor, NRF24L01 wireless module, LED with a wavelength of 405 nm, power supply, 16 x 2 LCD and digital pH meter PH-201 with a pH electrode PE-03 from Lutron Electronic Enterprise CO.,

LTD,. The software used consisted of Arduino IDE and NetBeans IDE. Communication on the ISM Band, ISM (Industrial, Scientific and Medical) band is a radio spectrum that can be used for any purpose without a license in most countries. The 2.4 GHz band is defined in the range of 2.4000 GHz to 2.4835 GHz with a band width of 83.5 MHz. The use of 2.4 GHz ISM for wireless LANs has been defined by the IEEE (Institute of Electrical and Electronics Engineers) in the 802.11-2007 protocol standard. In addition to the use of wireless LAN equipment, the 2.4 GHz ISM band is also intended for microwave ovens, cordless home telephone, monitors and wireless video cameras. Because of its designation on many devices the 2.4 GHz ISM band raises the potential for interference or signal interference [16].

IV. RESEARCH METHOD

A. Making and Determining Fluorescent Dye Solutions

The type of fluorescent dye used in this research is pyranine fluorescent dye. Pyranine dye solution prepared by extracting a yellow highlighter with 25 mL of isopropyl alcohol. This solution is stored under normal ambient temperature conditions of 25 ℃ to 30 ℃.

B. Development of pH Sensing Devices

The pH sensing device consists of a transmitter and receiver. The transmitter section consists of a LED, sample container, reflector, photodetector, microcontroller board and wireless module. The receiver section consists of a wireless module, a microcontroller board and a PC. In the transmitter section, the distance from the sample container (test tube) to the light source is made as close as possible and the distance from the sample container (test tube) to the photodetector is also made as close as possible. Fig. 3 shows the principle of pH sensing device system.
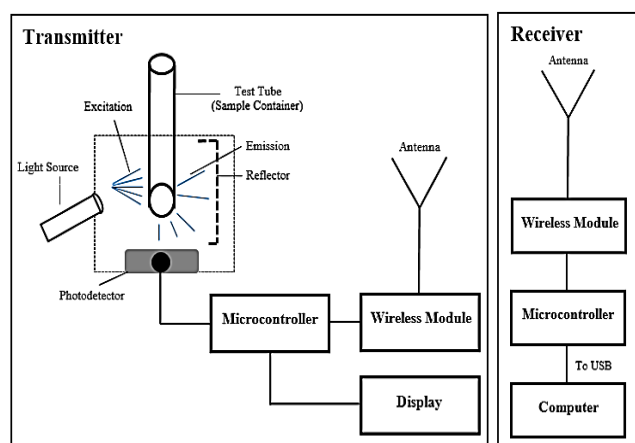

Fig. 3. Principle of pH Sensing Device System

A Light Source with a wavelength of 405 nm is applied to a sample mixed with a fluorescent dye and then at the same time a fluorescent dye emits light where this emission is received by photodetector AS7262. Since the wavelength of the 510 nm emission light is emitted, the response of the wavelength of 510 nm is used or taken. This sensor detects wavelengths in the visible range at 430 to 670 nm with 40nm full half-width detection. The signal from the photodetector is processed by a microcontroller. The processed signal is displayed on the 16 x 2 LCD and sent

via the NRF24L01 wireless module to the data receiver. Fig. 4 shows a block diagram of the transmitter section.
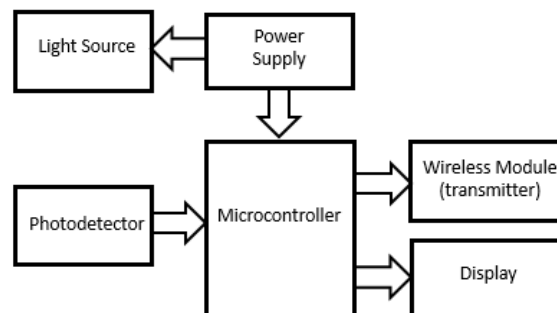

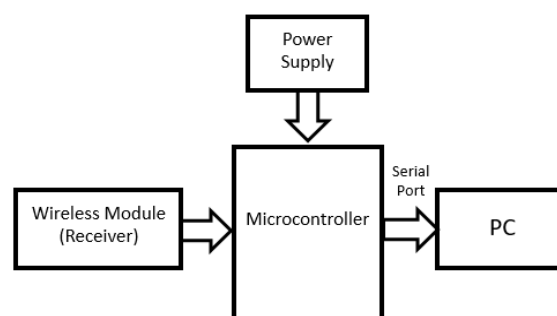Fig. 4. Block diagram of the transmitter section

Fig. 5. Block diagram of the receiver section

Data displayed using applications that have been developed. This application is designed using NetBeans IDE based on the Java programming language. This application was built with a GUI (Graphical User Interface). This application requires several supporting libraries, such as JFreeChart, JSerialComm 2.4.0, and Java Development Kit 1.8 (JDK 1.8). Fig. 6 shows the display interface of the application.
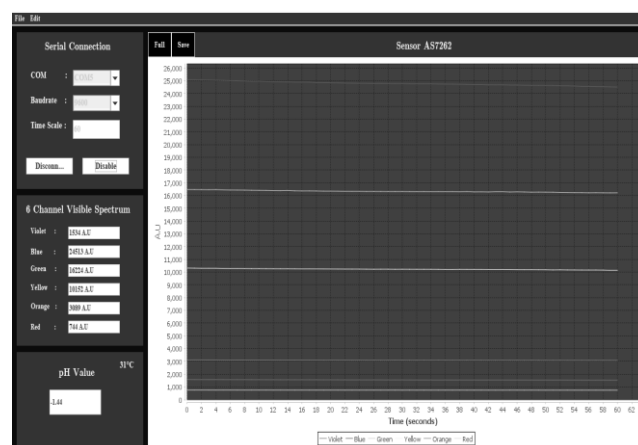

Fig. 6. Display interface of the application

C. Observe The Effect of Fluorescent Dyes

We observed changes in the volume of the fluorescent dye solution using a digital pH meter and a pH sensing device before calibration. Measurements were made with 2 mL of distilled water with different volumes of the fluorescent dye solution. Distilled water was used because is has pH arround 7.0. The middle of the intensity range hass been set for pH 7.0. The volume of the fluorescent dye

solution observed 0.2 mL, 0.4 mL, 0.5 mL, 0.6 mL and 0.8 mL respectively. The sensor output reading is able to read the different values of the pH measured by a digital pH meter. We decided to use 0.5 mL volume of the fluorescent dye solution. Table I shows the measurement results of fluorescent intensity at pH observed using a digital pH meter with 2 mL volume of distilled water.

TABLE I. THE MEASUREMENT RESULTS OF FLUORESCENT INTENSITY AT pH OBSERVED USING A DIGITAL pH METER WITH 2 mL VOLUME OF DISTILLED WATER

Volume of Water	Volume of Fluorescent Dye Solution	Digital pH Meter	Fluorescent intensity (A.U)
2 mL	0 mL	6.27	659.33
	0.2 mL	6.77	5948.17
	0.4 mL	7.28	7543.38
	0.5 mL	7.47	7960.93
	0.6 mL	7.69	11129.94
	0.8 mL	8.11	13099.87

The main reason we use 0.5 mL volume of the fluorescent dye solution is that the value read by the sensor is in the middle of the measurement of some volume of the fluorescent dye solution, so it can be assumed with 0.5 mL volume of the pyranine, the pH sensing device to be calibrated has a wide measurement range. Another reason is because the measurement sample container (test tube) has a volume capacity of only 3 mL. Fig. 7 shows the fluorescent intensity of some different volume of pyranine with a mixture of 2 mL of distilled water.
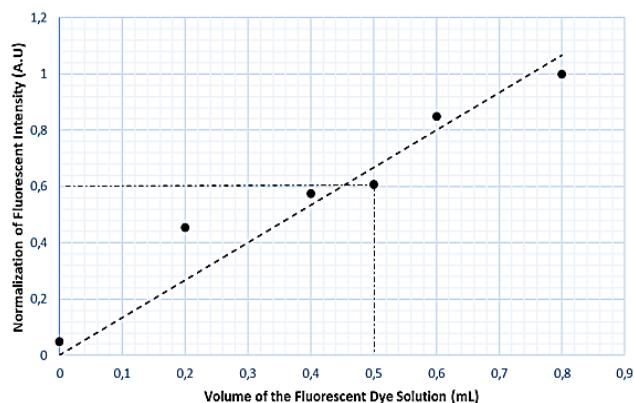

Fig. 7. Fluorescent intensity of some different volume of pyranine with a mixture of 2 mL of distilled water

D. Effect of Photobleaching

In this research, we observed the effect of photobleaching on a fluorescent dye solution. Photobleaching is the phenomenon when a fluorophore loses its fluorescence due to damage induced by light. This leads to loss of fluorescence and signal while imaging a sample. When light of appropriate wavelength is directed on a fluorophore, it transitions from ground state to excited singlet and triplet stages. In its excited state, it may interact with other molecules and undergo permanent covalent modifications [17]. The photobleaching effect was observed with 0.5 mL of a fluorescent dye solution mixed with 2 mL of water. The photobleaching effect was observed with 0.5

mL of a fluorescent dye solution mixed with 2 mL of water into a test tube. A 405 nm light source is excited into the test tube and intensity of fluorescent emission were observed within 30 seconds using an application and a pH sensing device that had been developed. Because of this photobleaching effect, we set the data acquired only at the third second. Fig. 8 shows the effect of photobleaching on fluorescent dye.
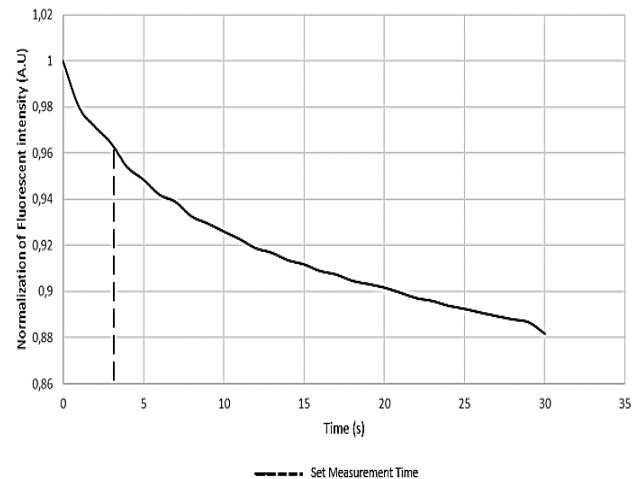

Fig. 8. The effect of photobleaching on fluorescent dye

The reason we set the measurement time at the third second to aim for a rapid measurement time without reducing its accuracy due to the photobleaching effect.

E. Calibration of pH Sensing Devices

The calibration aims to get the right measurements. The data that has been obtained from the measurement results with the pH sensing device that has been developed is processed using the linear regression function. Linear regression is a statistical method that functions to test the extent of the causal relationship between the causal variable (X) against the effect variable. The causative factor is generally denoted by X or also called predictor while the effect variable is denoted by Y or also called response. Linear regression is also one of the statistical methods used in production to make predictions or predictions about the characteristics of quality and quantity [18]. The Linear regression equation is as follows:

$$Y = a + bX \qquad (1)$$

Where, Y is response variable or dependent variable (Dependent), X is predictor variable or causative variable (Independent), a is constant, n is the total amount of data and b is regression coefficient (the magnitude of the response generated by the predictor).

Distilled water is measured using a digital pH meter PH-201 and a pH electrode PE-03 on a scale of 2 to 12 with an interval of 0.5. Distilled water that has been measured using a digital pH meter is then also measured on a pH sensing device, the procedure is by setting 0.5 mL of a fluorescent dye solution mixed with 2 mL of distilled water that has been previously measured using a digital pH meter PH-201 and pH electrode PE-03 for each pH measurement.

we started by measuring the pH of distilled water with pH 2, 2.5, 3, 3.5, 4, 4.5, 5, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5 and 12. The pH is adjusted according to the solution

of sodium hydroxide and hydrochloric acid. At each measurement of distilled water pH that has been measured with a digital pH meter, the same thing is done also measuring the pH of distilled water with a pH sensing device. Measuring the pH of distilled water using a pH sensing device is done by mixing 0.5 mL of a fluorescent dye solution with 2 mL of distilled water into a test tube that has been previously measured using a digital pH meter. It aims to obtain data from each pH measurement with a pH sensing device. This data is used for calibration on the pH sensing device that has been made. Fig. 9 shows a mixture of 0.5 mL of a fluorescent dye solution with 2 mL of distilled water before measurement and Fig. 10 shows a mixture of 0.5 mL of a fluorescent dye solution with 2 mL of distilled water is being measured.

Fig. 9. Mixture of 0.5 mL of a fluorescent dye solution with 2 mL of distilled water before measurement
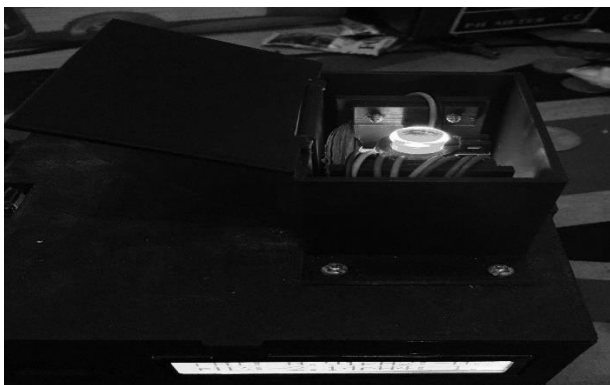

Fig. 10. Mixture of 0.5 mL of a fluorescent dye solution with 2 mL of distilled water is being measured

Measurement tests were carried out four times using the same method using a application developed. Then the results of each measurement that has been done are processed using the linear regression function. We use this function from analyzing data that has been improved. The application works by reading the level of emission intensity emitted from a fluorescent dye solution that has been mixed with distilled pH water samples through the AS7262 sensor. Data is sent to the computer wirelessly using the NRF24L01 module. Of the four tests that have been carried out, the results of each test showed results that were not too significantly different. Fig. 11 shows the curve of the four tests that have been carried out.
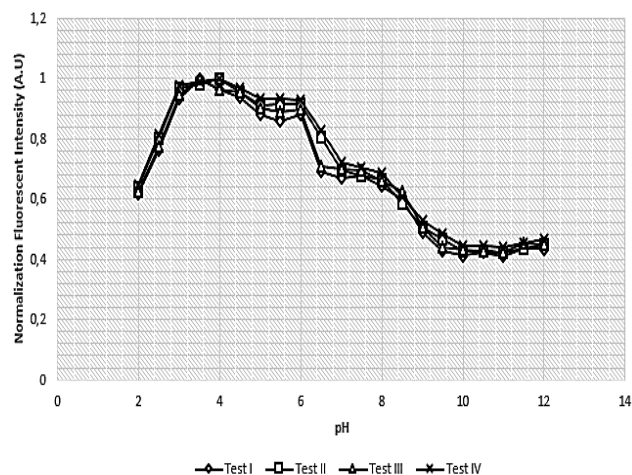

Fig. 11. Fluorescent intensity of the four tests

The data that has been obtained is then processed by applying a linear regression function. We use this function from analyzing data that has been improved. This function can be applied because the test curve that has been done shows a linear pH change in the measurement results. From the four test data that have been obtained, these data are calculated on average. From the average acquisition of the data obtained is processed with a linear regression function to be applied to the pH sensing device. Data processed to obtain the linear regression function is fluorescent intensity data from pH 4.0 to 11.0. This is due to the linearity that occurs at this pH range, as has been shown in Fig. 12. The linear regression function obtained is shown in the following equation.

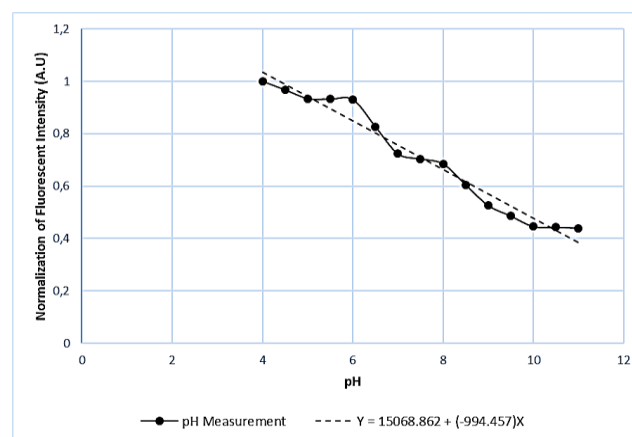$$Y = 15068.862 + (-994.457)X \qquad (2)$$

Fig. 12. The sensitivity curve of the function applied to the pH sensing device

Where, Y is the result of pH measurement (Output), X is the fluorescence intensity of the sensor reading value (Input). This function is applied and used for calibration of 0.5 mL of a fluorescent dye solution mixed with 2 mL of clear water. From the function that has been obtained, the sensitivity of the pH sensing device can be measured from a pH range of 4.0 to 11.0. Fig. 12 shows the sensitivity curve of the function applied to the pH sensing device.

V. EXPERIMENT RESULTS AND DISCUSSION

A. Measuring Errors From Developed pH Sensing Devices

In this test, we compared the measurement capability of a pH sensing device with a digital pH meter from a pH range of 4 to 10 with an interval of 0.5. We used 0.5 mL of a fluorescent dye solution mixed with 2 mL of distilled water with a pH sensing device. We used 0.5 mL of a fluorescent dye solution mixed with 2 mL of distilled water with a pH sensing device. The pH of the distilled water is adjusted with the solution of sodium hydroxide to increase the pH level and the hydrochloric acid solution to decrease the pH level. The linear regression function $Y = 15608.862 + (-994.457)X$ is applied to the pH sensing device. Table II shows the results of comparison of measurements of pH sensing devices with digital pH meters in the pH range of 4.0 to 10.0 with an interval of 0.5.

TABLE II.   COMPARISON OF MEASUREMENTS OF PH SENSING DEVICES WITH DIGITAL PH METERS IN THE PH RANGE OF 4.0 TO 10.0 WITH AN INTERVAL OF 0.5

Digital pH Meter	pH Sensing Device	ΔError
4.01	4.11	0.1
4.50	4.67	0.17
5.01	5.04	0.03
5.52	5.61	0.09
6.03	5.96	0.07
6.53	6.46	0.07
7.03	6.89	0.14
7.53	7.39	0.14
8.02	7.88	0.14
8.52	8.40	0.12
9.01	9.33	0.32
9.52	9.76	0.24
10.03	9.94	0.09

From the comparison test results above, the pH sensing device has a pretty good accuracy. The average accuracy of the pH sensing device is 98.13% with an average reading error value of ±0.13. The pH sensing device has the best sensitivity at the pH range of 5.0 to 8.5.

B. Measurement of pH in Random Sampless

In the final step, we tested pH sensing devices on four samples with unknown pH levels. We compared the test results with a digital pH meter. The samples must be clear water. We used 0.5 mL of a fluorescent dye solution mixed with 2 mL of water samples with a pH sensing device. Table III shows the measurement results from the pH sensing device and the measurement results from the digital pH meter with unknown pH levels of the samples.

TABLE III.   THE MEASUREMENT RESULTS FROM THE PH SENSING DEVICE AND THE MEASUREMENT RESULTS FROM THE DIGITAL PH METER WITH UNKNOWN PH LEVELS OF THE SAMPLES

Unknown pH	pH Sensing Device	Digital pH Meter
Sample I	7.51	7.50
Sample II	9.15	9.35
Sample III	5.79	5.71
Sample IV	3.69	3.77

The samples obtained from several places. For the first sample obtained from PDAM water, the second sample was obtained from community wastewater located on the Kedung Pengkol Street, Surabaya, the third sample was obtained from rain water and the fourth sample was obtained from water mixed with hydrochloric acid. Fig. 13 shows the results of comparison of measurements with digital pH and pH meters in random samples.

Where pH3 in Fig. 13 is the result of pH measurement using a linear regression function $Y = 15608.862 + (-994.457)X$ applied to the pH sensing device.

All pH measurements on the pH sensing device at each test were carried out under normal ambient temperature conditions from 25ºC to 30ºC. The time needed for the pH sensing device to measure pH for each test is 3 seconds.

Fig. 13. The results of comparison of measurements with digital pH and pH meters in random samples

VI. CONCLUSION

The development of pH sensing devices using a fluorescent dye solution was successfully applied to measure the pH level in liquid water. However, there are limitations in measurement, such as high measurement accuracy can only be measured in the pH range of 5.0 to 8.5 because pyranine only has a high sensitivity at that pH. The pH sensing device that has been developed has an accuracy of 98.13% and a reading error value of ±0.13 from the tests that have been done. The pH sensing device only takes less than 3 seconds to measure the pH level. This device is only calibrated for 0.5 mL of pyranine mixed with 2 mL of the solution to be tested. Pyranine solution was prepared by extracting a yellow highlighter with 25 mL of isoropil alcohol. In the sample container there are 20% by volume of pyranine and 80% by volume of liquid samples. To take measurements with other samples, this device must be calibrated first, calibration is carried out using the method described in this research. This device is not able to measure pH < 4 and pH > 10 due to changes in the color of the solution in the pyranine so that it results in an error reading of the measurement by the AS7262 sensor due to significant changes in light intensity. This pH sensing device was developed at an affordable cost and easily available materials. The development of this device only costs half

the cost of a digital pH meter. This pH sensing device is also connected to applications that were developed wirelessly, but this application only runs on Windows operating systems.

REFERENCES

[1] K. Kanokvalai, N. Piyavadee, and V. Supenya, "The pH of water from various sources: an overview for recommendation for patients with atopic dermatitis," Asia Pac Allergy, vol. 3, pp. 155-160, 2013.

[2] A. L. M. Haider, "Health effects of alkaline diet and water, reduction of digestive-tract bacterial load, and earthing," Alternative Therapies in Health and Medicine, April 2016.

[3] Ben Chioma, A. E. Jack, A. S. Philipokere, G. K., "A comparative study on the measurement of ph of water, using ph metre and water testing kit [testube method] in port harcourt," IIARD., vol. 1, no. 3, 2015.

[4] Xu Yu-Ling, Li Feng-Ye, Ndikuryayo Ferdinand, Yang Wen-Chao, and Wang Hong-Mei, "Cholinesterases and engineered mutants for the detection of organophosphorus pesticide residues," vol. 18, pp. 4281, 2018.

[5] "Fluorescent Dyes" 25-Des-2016 [Online]. Available: https://www.nature.com/subjects/fluorescent-dyes. [Accessed: 28-Mei-2020].

[6] Campbell Tessa N. and Choy Francis Y.M., "The effect of pH on Green Fluorescent Protein: a Brief Review," vol. 2, pp. 1-4, 2001.

[7] J.N. Miller., "FLUORESCENCE | Quantitative Analysis," Encyclopedia of Analytical Science (Second Edition), Elsevier, pp. 163-170, 2005.

[8] Ewa Sikorska, Igor Khmelinskii, Marek Sikorski, "Fluorescence spectroscopy and imaging instruments for food quality evaluation," Woodhead Publishing Series in Food Science, Technology and Nutrition, pp. 491-533, 2019.

[9] U. Sebastian, O. Alina, P. Guido, R. René, B. Nico and B. Luciano., "Pyranine‐Modified Amphiphilic Polymer Conetworks as Fluorescent Ratiometric pH Sensors," Macromolecular Rapid Communications., vol. 40, pp. 1900360, 2019.

[10] Qi J, Liu D, Liu X, et al., "Fluorescent pH Sensors for Broad-Range pH Measurement Based on a Single Fluorophore," Anal Chem., 2015;87(12):5897‐5904.

[11] C. Yuan, Y. Zhang, H. Xi and X. Tao., "An acidic pH fluorescent probe based on Tröger's base," RSC Adv., 2017, 7, 55577.

[12] Manuja Sharma, Jasmine Y. Graham, Philip A. Walczak, Ryan M. Nguyen, Lauren K. Lee, Matthew D. Carson, Leonard Y. Nelson, Shwetak N. Patel, Zheng Xu, Eric J. Seibel, "Optical pH measurement system using a single fluorescent dye for assessing susceptibility to dental caries," J. Biomed. Opt. 24(1) 017001 (8 January 2019).

[13] National Center for Biotechnology Information. PubChem Database. Pyranine,CID=61388,https://pubchem.ncbi.nlm.nih.gov/compound/Pyranine (accessed on May 28, 2020).

[14] Manuja Sharma, Jasmine Y. Graham, Philip A. Walczak, Ryan M. Nguyen, Lauren K. Lee, Matthew D. Carson, Leonard Y. Nelson, Shwetak N. Patel, Zheng Xu, Eric J. Seibel, "Optical pH measurement system using a single fluorescent dye for assessing susceptibility to dental caries," J. Biomed. Opt. 24(1) 017001 (8 January 2019).

[15] "Fluorescence Spectrum Viewer" [Online]. Available: https://www.aatbio.com/fluorescence-excitation-emission-spectrum-graph-viewer. [Accessed: 09-June-2020].

[16] Minhas, Neha and Kumar, Dr., "Performance Analysis of ISM Antennas Band: A Survey," International Journal of Advanced Computer Research., 2018.

[17] P, Letter. "Photobleaching in Fluorescence Microscopy". AZoLifeSciences.https://www.azolifesciences.com/article/Photobleaching-in Fluorescence-Microscopy.aspx. (accessed June 06, 2020).

[18] Chatterjee, S. and Hadi, A.S. (2006). Simple Linear Regression. In Regression Analysis by Example (eds S. Chatterjee and A.S. Hadi).