The effect of uric acid in the detection of calcium stones in urine through turbidimetry method

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ABSTRACT
An increase of 10-12 percent in the number of Filipinos afflicted with kidney or renal disease is recorded per year according to the National Kidney Transplant Institute. Thus, the diagnosis of calcium stone formation, or urinalysis, in urine is a significant contribution to the medical industry. This study presents a diagnostic LED photoresistor sensor that can selectively determine calcium stones in the presence of uric acid. The LED photoresistor sensor, with Hantek data logger, was used to determine the concentration of Ca (II) in the urine sample. The features of the sensor system have a signal stability of 0.0596 % relative standard deviation (RSD) and repeatability of 10.35% RSD when 80 ppm of standard Ca (II) is compared to a blank in the presence of 60 ppm of uric acid. The calcium sensor photoresistor shows a calibration curve of y=0.0001x-0.0023 with a limit of detection of 46.84 ppm. The linear coefficient of the calibration curve, R^2 is 0.9902.

Keywords: diagnosis, calcium stones, urinalysis, photoresistor sensor, uric acid

1. Introduction
Biomedical Engineering is a field in engineering which merges engineering and medicine that designs, installs, sustains, secures, and helps to safely operate medical devices, systems, and networks. This field gives importance to systems design work and engineering problem solving. These concepts are applied in developing healthcare devices, such as sensors, for the safe treatment of patients [1].

Under the development of biomedical engineering, there are already traditional methods used in the medical field to aid humans in determining their health condition by means of testing body fluids, but some of these methods are costly and requires much fluid samples. The traditional means of analyzing urine is divided into physical, chemical, and microscopic test. Physical analysis of urine normally involves the testing of the urine’s color, clarity, and specific gravity. The information obtained in the physical examination could be used to diagnose preliminary information and in predicting if a person has a disorder. In addition to the preliminary information obtained, any information gathered during the examination can be used to explain and relate the findings in chemical and microscopic tests. Chemical analysis however, are tests that commonly use other reagent strips to analyze the pH, protein, glucose, and many more components of urine that can only be identified chemically. Microscopic tests are done to detect or identify insoluble material present in urine. One common insoluble material that can be detected microscopically are crystals. Some of the common crystals detected are uric acid, amorphous urates, calcium oxalates, and many other stones that depend on the acidity or basicity of the urine. The said crystals are normally formed due to precipitation that
occurs in the urine. Among all the other normal urinary crystal that precipitates in the urine, calcium oxalate is the only urinary stone that can precipitate in both acidic and neutral urine condition. Uric Acid is a white odorless crystalline product of protein metabolism, found in the blood and urine, as well as trace amounts found in the various organs of the body. It can build up and form stones or crystals in various disease states [2].

By means of biomedical engineering, engineers could improve the traditional methods used to analyze urine samples. These augmented methods are studies about kidney stone sensors that would improve the traditional methods. Grases et al. (2007) the mechanism by which uric acid affects calcium oxalate crystallization and the role of crystallization inhibitors in this process [3]. Uric acid crystals can clearly induce the development of Calcium Oxalate Monohydrate (COM) crystals on them through a heterogeneous nucleation process. A study claims that whenever crystals are found in urines, the person that contains urinary crystals undergo urolithiasis wherein stone originates from the urinary tract, bladder, and particularly kidneys. These studies conducted show that diagnostic sensors can effectively form calcium oxalate. The formation of the precipitate on the solution means there is a presence of kidney stones [2].

However, diagnostic test results can cause confusion if there is a huge amount of uric acid present. Since CT scans have replaced the common abdominal flat plate, uric acid stones cannot be differentiated from calcium stones unless an astute radiologist, urologist, or medical specialist measures radiological density. Little attention is given in studying the relationship of uric acid in calcium oxalate stone formation in urine. It cannot be determined whether a number of precipitates formed was induced due to calcium which would indicate the presence of kidney stones, or merely from high uric acid content [4]. This study is an attempt to confirm if the calcium sensor developed from the previous study will be able to selectively determine calcium stones in the presence of uric acid [5]. This study will determine the effect of uric acid present in the standard solutions of calcium ions.

2. Material and Methods

2.1 Materials and Reagents

The analytical reagents used were ammonium oxalate monohydrate ((NH₄)₂C₂O₄•H₂O), Univar USA, calcium nitrate (Ca(NO₃)₂•4H₂O), Univar USA and uric acid (C₅H₄N₄O₃) Merck, Darmstadt and ultra-pure water from Millipore Elix Advantage 10 without preliminary preparations.

A stock solution of 1000 ppm calcium nitrate was prepared by dissolving 3.6672 g Ca(NO₃)₂•4H₂O in ultrapure water to make 1 L of solution. Afterwards, it was serially diluted to obtain concentrations of 110 ppm, 100 ppm, 90 ppm, 80 ppm, 70 ppm, 60 ppm, and 50 ppm. On the other hand, a 0.1 M ammonium oxalate solution was prepared by diluting 14.211 g of ammonium oxalate in 1 L ultrapure water.

A stock solution of 1000 ppm of uric acid solution was prepared by dissolving 1.0008 g of uric acid in ultrapure water to make 1 L of solution. It was diluted in ultrapure water and was filtered twice to obtain a concentration of 60 ppm [2].

The urine samples used for this study were obtained both from the researchers and from a stone former volunteer. The samples are stored in low-density PE plastic containers with lid. The samples were tested within 2-hour collection to avoid significant change in quality of substances present in the sample.
2.2 Instrumentation

A photoresistor sensor device is composed of 3V white LED, a 5mm photoresistor, a digital multimeter, a DC power supply, a black earth acetal system holder and copper connecting wires was used to detect Ca in urine [6].

Hantek 365-B Version 1.0.0.2 data logger is connected to the photoresistor sensor. This installed logger is used in transferring and recording the data into the computer.

The DC power supply, Model MCH-305A, is the power source of 3V for the LED light in the sensor. It is connected to the other end of the photoresistor sensor.

2.3 Signal Stability Test

A 0.1M oxalate solution at 4 mL was tested to determine the change in voltage reading of the system if any, it is then placed in the sample cell which is located between the photoresistor and LED in the sensor. The voltage reading was monitored for an hour to verify the stability of the signal readings. The percent relative standard deviation (RSD) of voltage reading was the basis in determining the stability. An RSD greater than 10% will be considered unstable [1].

2.4 Repeatability Test

One milliliter of 0.1 M of oxalate solution, and 1 mL of 60 ppm uric acid were pipetted in the cuvette and were read in the photoresistor for 3 minutes. Two (2) mL of 80 ppm calcium solution was then added to the same solution in the cuvette and was read in this case for 3 minutes. For every run, the cuvette was cleaned using a cotton bud in order to acquire repeatable results. The delta voltage for each run was determined by getting the difference of the last voltage reading of the oxalate solution and the last constant voltage reading after adding calcium solution. Six runs were done, and six delta voltage readings were taken, and their percent relative standard deviation was calculated.

2.5 Sensitivity Test

Seven samples of ammonium oxalate and 60 ppm uric acid solution, with calcium nitrate solution of 50, 60, 70, 80, 90, 100, and 110 ppm incremental concentrations, were prepared to test the sensor. One milliliter of oxalate solution was pipetted in the cuvette with 1 mL of 60 ppm uric acid solution and was read for 5 minutes. Two mL of 50 ppm calcium solution was then added to the same solution in the cuvette. The procedure was repeated for the remaining concentrations of 60, 70, 80, 90, 100, and 110 ppm calcium using the same cuvette. It was washed after every run before placing the new solution in the cuvette. These solutions were read for 5 minutes or until constant reading was obtained. Each batch of solution with varying concentrations were done in three trials.

A graph of ΔV vs. Concentration was plotted for each trial. The difference of the last voltage reading of the uric acid-oxalate solution and the last constant voltage reading of the added calcium solution was acquired to determine the delta voltage for each run. Taking into consideration that each voltage reading obtained were from the same time interval for every run.

The average of the ΔV readings at each predetermined concentration was plotted to check the linearity of the graph. Each delta voltage reading for the different concentrations resulted to a calibration curve showing a linear relationship. One set of data is one trial of the samples to establish a
calibration curve. The delta voltage readings for each concentration were compared by getting the error bars for each set. The linear correlation can be known by plotting the calibration curve and getting its equivalent linear equation. It shows how the change in voltage readings from the calcium concentrations are closely related. It is desired to achieve a linear correlation near to 1. The smallest measurement detected by the method is called Limit of Detection. The limit of detection of the photoresistor sensor was computed using Eq. 1 where SD is the standard deviation and m is the slope determined in the calibration curve,

\[ LOD = \frac{3SD}{m} \]  

(1)

2.6 Determination of Calcium Concentration in Urine

The photoresistor sensor was used to read the urine samples. Two milliliters of oxalate solution were pipetted in the cuvette and read for half minute. Afterwards, 2 mL urine sample was added to the same cuvette containing the oxalate solution. The solution was read for 2 minutes until a constant reading is obtained. The delta voltage reading for each trial was obtained by subtracting the last voltage reading of the oxalate solution to the last constant voltage reading of the urine sample. Each voltage reading that will be obtained are from the same time interval for every run. The average of the voltage readings was then determined. The voltage readings were substituted in the linear equation from the calibration curve of each trial to determine its corresponding concentrations. The result was then verified by comparing it with the result of the clinical test.

3. Results and Discussion

3.1 Stock Solutions

A cloudy solution was formed after adding oxalate solution in a calcium solution. This can be expressed in a chemical reaction (R1),

\[ \text{Ca (NO}_3\text{)}_2 + (\text{NH}_4)_2\text{C}_2\text{O}_4 \rightarrow \text{CaC}_2\text{O}_4 \ (s) + 2\text{NH}_4\text{NO}_3 \]  

(R1)

The solubility of calcium oxalate, CaC₂O₄, in urine is 9 x 10⁻⁵ M. The dissociation of this compounds can be expressed as CaC₂O₄ → Ca²⁺ (aq) + C₂O₄²⁻ (aq). For this reaction, the solubility product constant, ksp, is obtained to be 8.1 x 10⁻⁹. A slightly turbid solution occurred after adding the calcium solution in an oxalate solution, the same result when the urine was added to the oxalate solution. However, the pale-yellow color was more noticeable when the urine sample was used.

3.2 Signal Stability

The voltage reading should remain unchanged for a long period of time for a system to be considered stable. The results are shown in Figure 1.
The graph shows that the data recorded from the 1-hour voltage reading (V) of the oxalate solution in the photoresistor sensor has almost negligible fluctuations. The percent relative standard deviation (% RSD) was computed by using the mean and the standard deviation of the data, which are 2.87 V and 0.0017, respectively. The computed % RSD was 0.0603%, which is acceptable, since it is lower than 10% RSD [7]. Therefore, having observed that the data recorded are precise, the sensor system is stable.

3.3 Repeatability Test

The repeatability test measures the capacity of the sensor to give a replication of output readings in every experimental run. The results recorded by the data logger for the repeatability test of the sensor are graphically presented in Figure 2. Six samples of a mixture of calcium nitrate and ammonium oxalate with uric acid solution were read consecutively. The standard deviation for the Ca-Ox with Uric Acid solution is 0.000488 and the average voltage reading is 0.004714 V, resulting to 10.35% for the relative standard deviation.

3.4 Sensitivity Test and Limit of Detection

The sensitivity test was done in order to determine how the sensor responds to increasing calcium concentration. In this test, the difference of the final and initial voltage readings of the sensor was determined with varying concentration of calcium nitrate solutions added, as shown in Figure 3. Seven samples of ammonium oxalate containing 2 mL each were mixed with increasing concentrations (50, 60, 70, 80, 90, 100, and 110 ppm) of 2 mL calcium nitrate solution and obtained the voltage reading using the sensor. At comparatively low concentrations we expect to produce a slightly turbid solution, yet not to the extent of forming settling precipitation. An increase in concentration of calcium solution shows a direct relationship to the turbidity of the solution in the cuvette. It was evident that lesser light was able to pass through as the solution become more turbid. Therefore, the voltage reading decreases.
Figure 3. Voltage reading vs. time for seven varied concentrations in sensitivity test for Trial 1 (n=3).

Figure 4. shows the direct relationship between the change in voltage reading of the calcium oxalate solution and the concentration of calcium. A plot of the change in voltage between the reading of the oxalate and the readings when the calcium is added is shown in Figure 4. The coefficient of determination is obtained to be 0.9824.

The limit of detection gives the smallest amount of calcium that can be reliably detected by the sensor. The LOD can be computed using the equation (1) which results to 46.36 ppm.

3.5 Urine Sample

For the real sample testing in this study, two different types of urine samples were used. One sample is from a non-stone former and the other one is from a stone-former. These samples were examined within the 24-hr collection of the urine kept in a low-density PE plastic container with lid and separately analyzed through the device LED photoresistor sensor.
Two milliliter of 0.1 M ammonium oxalate mixed with 2 mL of urine sample as the source of calcium, and uric acid were read using the sensor. The mixture was read in the sensor for two minutes. Three trials were made, and the change in voltage reading per time is shown in Figure 5.

The resulting average of the delta voltage reading of the three trials was 0.005 V. Using the equation acquired in the sensitivity test,

\[ y = 0.0001x + 0.0023 \]  \hspace{1cm} (2)

Where \( x \) = amount of calcium in parts per million, while \( y \) = delta voltage reading, the calcium concentration obtained is 66.36 ppm.

The same procedure was done on the second sample which in this case a sample of a stone-former. Two milliliter of 0.1 M ammonium oxalate mixed with 2 mL of urine sample, and uric acid were read using the sensor. The mixture was read in the sensor for two minutes. Three trials were made, and the change in voltage reading per time is presented in Figure 6.

The average delta voltage from the readings is found to be 0.01525 V on substitution to Eq. 2. The Ca concentration is 159.54 ppm.

The following feature of the calcium sensor characterizes our device, as shown in Table 1.
Table 1. Summary of Results

| Units                     | Value          |
|---------------------------|----------------|
| Stability                 | % RSD          |
|                           | 0.0603         |
| Repeatability             | % RSD          |
|                           | 10.35          |
| Sensitivity Calibration   | y = 0.0001x - 0.0023 |
| Limit of Detection        | ppm            |
| Non-stone former sample   | 46.36 ppm      |
| Stone former sample       | 66.36 ppm      |

4. Conclusion And Recommendation

In line with the primary objective of this study, the effect of the presence of uric acid in the oxalate solution in the detection of calcium stones using the photoresistor was determined. The signal stability, repeatability, sensitivity, and limit of detection of the Ca sensor were determined. The graph of the stability test has almost negligible fluctuations. It yielded a low percent relative standard deviation, which is 0.0603%. This proves that the sensor system is stable. The repeatability test also presented a standard deviation of 0.00049, and relative standard deviation of 10.35%. The calcium sensor photoresistor shows a calibration curve of $y = 0.0001x - 0.0023$ with a dynamic range of 50 to 110 ppm concentration of Ca$^{2+}$ at 10 ppm intervals, with a limit of detection of 46.36 ppm. The linear coefficient of the calibration curve, $R^2$ is 0.9824.

Previous study revealed that the relationship between the voltage reading and the Ca concentration presents an indirect or inversely proportional behavior [8]. The solution becomes turbid as the calcium solution is added to the oxalate solution. Turbidity is the cloudiness or haziness of a fluid caused by suspended solids. This is an optical property that expresses the interaction between light and the suspended particles in the solution. This study proves that the presence of uric acid in the oxalate solution has no significant effect on the detection of calcium stones using the calcium sensor. The study interpreted the same relationship between the Ca concentration and the voltage reading, even with the presence of uric acid in the oxalate solution.

This study also presents the amount of calcium present in a urine sample. The Ca concentration obtained from the non-stone former and stone former were 66.36 ppm and 159.54 ppm, respectively. This method provides an improvement of the conventional way of diagnosis, which only reports the presence or absence of calcium stones through microscopic analysis. The Ca sensor may monitor and detect pre-forming stones to consider its prevention.

Future researchers may opt to consider testing other components of urine aside from calcium and uric acid to improve the validity of the Ca sensor. The use of a wider range of uric acid concentrations is also recommended for a more varying result. Different chemical analysis can be used as well such as Atomic Absorption Spectroscopy (AAS) to validate the results in the study by checking its actual calcium content. To further improve the methodology, pre-treatment of the urine sample can be done in order to remove other interfering substances that may precipitate together with Ca or contribute to the turbidity of the solution, thus, decreasing the reading errors. The concept of selective precipitation may also be used to remove the substances that interfere to the production of more valid results. To make the experimental set up and the solutions used more realistic in resembling urine, varying the pH of the solutions may also be considered. In testing the real samples for stone formers and non-stone formers, getting samples from other people of controlled age range or gender is recommended in order
to see if there are any significant changes or differences.

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