# STRESS DETERMINATION VIA AMYLASE ENZYME USING PHOTOMETER

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# Stress Determination via Amylase Enzyme using Photometer

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## Abstract

Stress is both a physical response that protects us and a natural defence mechanism that allows us to survive. However, overstressing may lead to serious illness. This paper emphasizes on the stress detection via amylase enzyme in one's person saliva. Since salivary amylase is a digestive enzyme, changes are expected in its activity and be associated to the levels of stress. The analysis of amylase enzyme on one's saliva has been done using a photometer; to measure the light intensity of the chemical reaction when Lugol's iodine, starch solution and salivary amylase are added together. The outcome indicates that this Amylase Enzyme Detection System has proved to be the simple, portable and cheap device using GUI and stopwatch designed in MATLAB. Further investigation will look onto the behaviour and characteristics of the salivary amylase using other promising technique.

## 1 Introduction

Stress is both a physical response that protects us and a natural defence mechanism that allows us to survive. Stress can be motivating, energizing, exciting and sometimes fun since it can challenge one to greater achievements in life. However, at the same time, it is known as the 80 percent factor of illness that plagues modern society today [1].

The infamous illness of stress; physical or emotional, has an effect on the amylase level in one's body. The amylase level is also affected by other conditions such as strenuous activity, infection, or injury [1]. For example, when one does not enjoy and fears riding the roller coaster, one's stress level is higher compared to one who thinks that the roller coaster gives him or her enjoyment. Amylase containing in saliva can be collected and plays the role of an indicator of the level of stress. Higher content of amylase in one's saliva shows that the person has a higher stress level.

The concentration of glucocorticoid, a type of steroid hormone, and catecholamine, a neurotransmitter, are often used as an index to evaluate the level of human stress. In particular, cortisol (a type of glucocorticoid) has been variously reported to have been used because it is considered as a salient index [2]. However, since cortisol's concentration in blood or saliva is very low, the analysis requires not only specific large-scale equipment, such as HLPC (high performance liquid chromatography) or EIA (enzyme immunoassay) but the analysis also takes quite some time to complete [2]. Therefore, a simple quantitative measurement technique to monitor human stress has been developed. The technique is based on salivary amylase due to its innervated secretion by the sympathetic nervous system since salivary amylase is a digestive enzyme. Changes are then expected in its activity and be associated to the levels of stress [2]. Based on the concept, a system has been designed that puts one's saliva sample on the test using a simple, portable and cheap device.

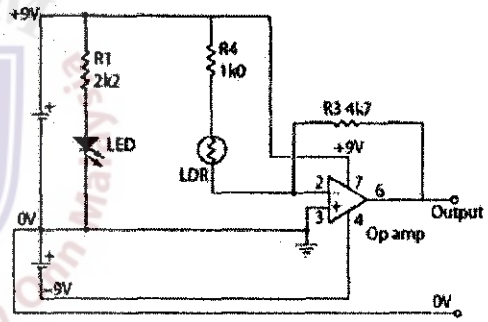


**Figure 1: Photometer circuit diagram.** The sample is placed between the LED and LDR. The output is connected to the voltmeter. The batteries are connected to a switch to save power.

## 2 The System Process

The Amylase Enzyme Detection System consists of five stages:

a. Stage 1 involves the collection of saliva. The saliva can be collected by chewing a clean rubber band and drooling into a small container.

b. Stage 2 can only be performed after stage 1 is completed. The Amylase-Starch Test is carried out by firstly dropping 800 µL of 1% starch solution in the cuvette followed by 800 µL of Lugol's iodine. Afterwards, 800 µL of collected saliva is dropped in the solution. The cuvette is already placed in the box at this stage while all solution is dropped using a micro-pipette.

c. Stage 3 is the heart of the whole system where portable device running on two 9V batteries is fabricated. The photometer circuit (as in figure 1) acts as the electronic sensor to the chemical reaction in stage 2. Cuvette containing sample is placed between the light sensor (LDR) and the light source (LED). The photometer is used to measure the light intensity of the chemical reaction when Lugol's iodine is added to starch solution (deep-purple) and afterwards, when salivary amylase is added (clear). Rather than measuring absorbance directly, the photometer gives information as a voltage.

d. Stage 4 measures the output voltage obtained from stage 3. Voltage is measured when iodine is dropped into starch solution. The change of voltage is expected when salivary amylase is dropped in afterwards and slowly matches the voltage before Lugol's iodine is dropped into the starch solution.

e. Stage 5 is the software part and the final stage of the system. A GUI using MATLAB 7.7 containing a stopwatch was designed. The time elapsed starting from when the output voltage when salivary amylase has completely reacted with the starch solution until the voltage matches that of the voltage before Lugol's iodine is added to the starch solution is. Based on the time elapsed, one's level of stress (either 'no problem', 'moderate', 'stress' or 'need help') can also be determined.

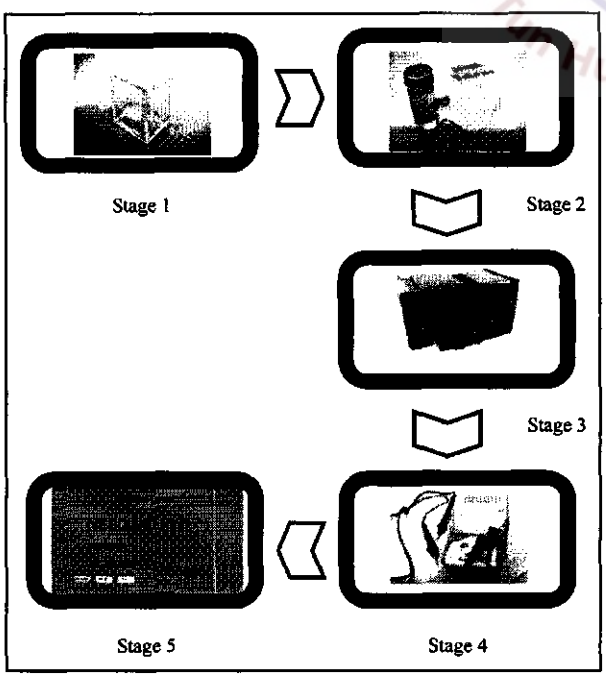


**Figure 2: Block diagram of Amylase Enzyme Detection System**

## 3 Results and Analysis

*A. The Photometer Circuit*

The photometer circuit has been tested on breadboard. When the LED placed parallel to LDR, a voltage value can be observed (as in figure 3) while there is no voltage recorded when the light sensor is blocked (as in figure 4). After confirming that the circuit is successful, the same arrangement of circuit is transferred on strip board. The transferred circuit also succeeded and placed in a 'black box' (as in figure 5) so that the voltage measured would not be affected by unwanted strays of light. The portable box has been developed by using prospect plastic with dimensions of 12.8cm long, 7.3cm wide and 6cm high. The material used is light with the thickness of 1mm.

**Figure 3: An output voltage is recorded when LDR is positioned adjacent to LED**

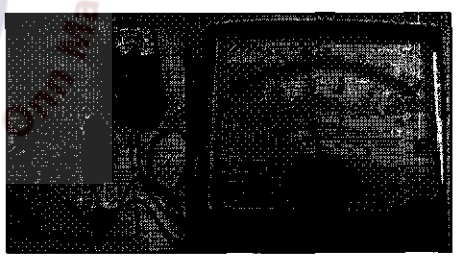

**Figure 4: A decreased output voltage is recorded when LDR is blocked**

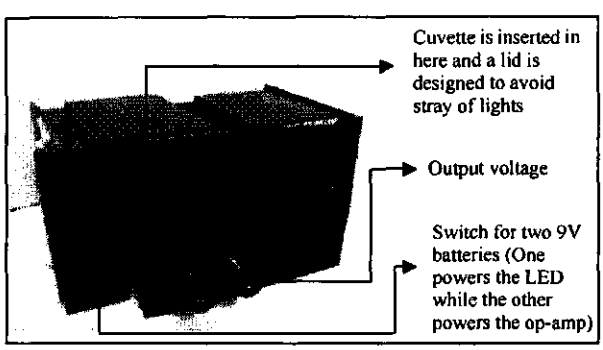


**Figure 5: A portable 'black box' designed to easily measure the output voltage of salivary amylase activity**

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*B. Salivary Amylase Characteristics*

A test has been performed on a 23 years old healthy female to observe the activity of salivary amylase. The test has been performed in a closed laboratory where source of light that might affect the lighting of the 'black box' was only those turned on in the laboratory. The digital multimeter is used to measure the output voltages in order to obtain more accurate results due to the small changes of voltage that might be recorded. The voltage measured when the cuvette was empty is 0.9463V. When 800 µL of 1% clear starch solution is dropped in the cuvette using a micro-pipette, the voltage recorded was 0.9432V.

After 800 µL of Lugol's iodine was added, the voltage changed to 0.4639V due to the change of the solution colour to deep purple which increased the light intensity. Lugol's iodine which was formely light brown in colour transformed to deep purple due to its function as an indicator of starch. Afterwards, 800 µL of saliva is dropped in. Table 1 and figure 6 records and shows the change in voltage recorded in 11 minutes. Note that after 11 minutes, the voltage recorded matches the voltage recorded before Lugol's iodine is added to starch solution. An increment in voltage can also be observed from 0 minute to minute 11. This increment of voltage is due to the salivary amylase activity of breaking down the starch to sugars, thus changing the deep purple solution back to a clear solution.

**Table 1: Output voltage measured during the reaction of salivary amylase with starch solution (after lugols's iodine is added)**

| Time, minutes | Output voltage measured |
|---|---|
| 0 | 0.642 |
| 1 | 0.756 |
| 2 | 0.7956 |
| 3 | 0.8272 |
| 4 | 0.8518 |
| 5 | 0.8609 |
| 6 | 0.8864 |
| 7 | 0.9011 |
| 8 | 0.9251 |
| 9 | 0.9359 |
| 10 | 0.9372 |
| 11 | 0.9394 |

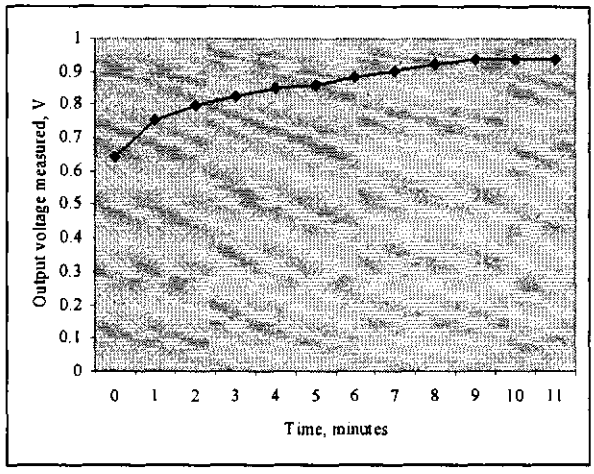


**Figure 6: Output voltage measured during the reaction of salivary amylase with starch solution (after Lugols's iodine is added). Note that the voltage increases.**

*C. Determining the Time Taken for Salivary Amylase Activity and Analyzing It's Relation with Stress Levels*

The faster it takes for the voltage of the reaction between salivary amylase and starch solution (after Lugol's is added) to match the initial voltage; before Lugol's iodine is added to starch solution, shows that there is more amylase in one's saliva to break down the starch in the starch solution. More amylase indicates that the person is more stressed. Figure 7 shows the GUI designed using MATLAB 7.7.

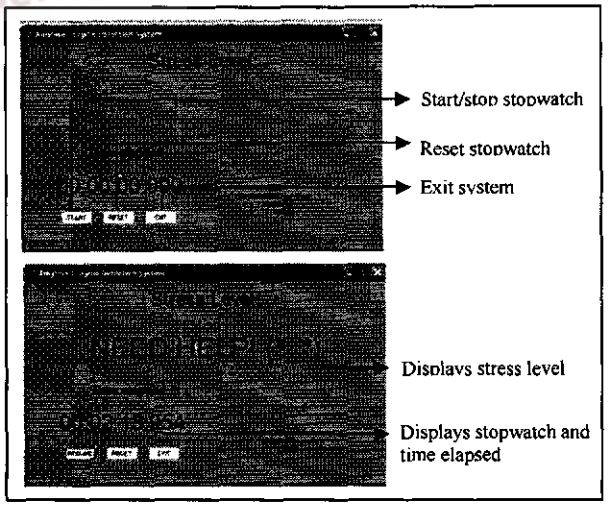


**Figure 7 The GUI designed to determine levels of stress based on voltage measured from photometer circuit on salivary amylase activity**

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## 4 Conclusions

The Amylase Enzyme Detection System using Photometer has been developed. This system allows medical practitioners to conveniently monitor their patients' stress level without dealing with the current fuss of complex machinery. The result shows an accurate detection of stress level according to level of amylase enzyme where salivary amylase could replace cortisol as an indicator of stress changes. This system can be improved by using a higher quality type of cuvette and be able to increase the sensitivity of the LDR by giving better results of voltage readings. In addition, more accurate levels of stress would be determined.

## Acknowledgement

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