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Colorimetry Based Calcium Measurement

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Abstract:- Calcium Analysis is one of the significant parameter for the clinical diagnosis. Calcium controls osmosis and diffusion through the cell membranes, and also the passing of information within the cell. Normal range of Calcium in human serum is around 8.5mg/dl-10.5mg/dl. The paper is focused on the Colorimetric Sensing technique for measurement of calcium. Colorimetry is a useful analytical tool for determining concentration of colored material in the solution. This sensing technique consists of LED as a light source of 650nm and a high speed photodiode BPW34 as a detector. Calcium reacts with ArsenazoIII and forms a colored complex. Concentration of calcium is measured by measuring the absorbance using Beer Lambert's law. The paper describes the test preparation and measurement procedures. The designed colorimetric sensing system is maintenance free and is cost effective.

Keywords:- Absorbance, Calcium, Concentration, Cuvette.

I. INTRODUCTION

Calcium is one of the most essential nutrients needed by the body for healthy functioning. Calcium controls muscle contraction and relaxation, it is also responsible for nerve impulse transmission and the transfer of information between our brain cells. Blood calcium test helps in diagnosing kidney stones, thyroid disorders, intestinal disorders, bone disorders and neurological disorders. Fig.1 shows the calcium regulation in the body. Calcium levels in the blood are regulated by parathyroid hormone. This parathyroid hormone (Pth) is secreted by the parathyroid glands located in the neck. When blood levels of calcium drop, the parathyroid glands release parathyroid hormone. Parathyroid hormone then stimulates the conversion of vitamin D to calcitriol in the kidneys to increase blood levels of calcium. When blood levels of calcium are normal, the parathyroid gland inhibits the production of parathyroid hormone. The kidneys then dispose of excess calcium via urine. Calcium is the main buffer used in the body to neutralize acids and maintain the proper pH.

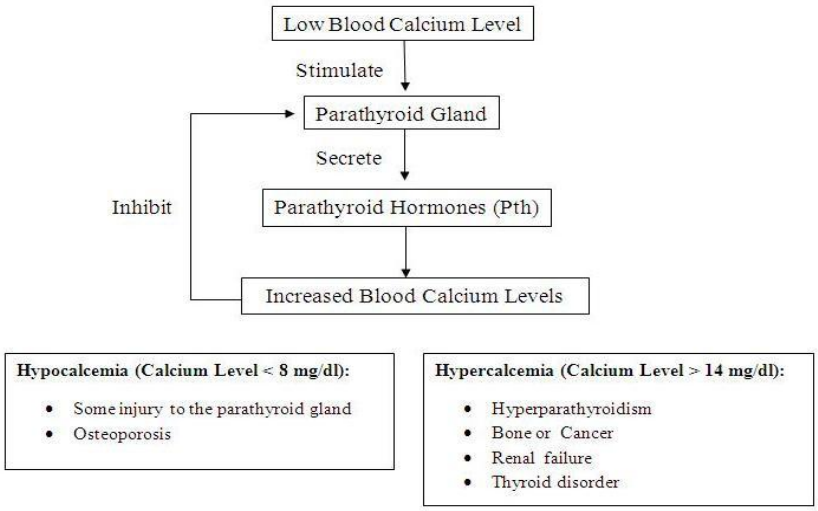

Fig. 1 Calcium Regulation

II. METHODOLOGY

A. Atomic Absorption Spectrophotometry

Atomic Absorption Spectroscopy in analytical chemistry is a technique for determining the concentration of a particular metal element within a sample. It consists of two processes. First is the production of free atoms from the sample and second is absorption of radiation from an external source by these atoms. This method is not used in pathology laboratories as it has a large number of interferences such as formation of non-volatile compounds, smoke formation which will absorb light, contamination of sample large sample quantity required (1ml-2ml) and problems with refractory element.

B. Ion Selective Electrode

An ion selective electrode generates a difference in electrical potential between itself and a reference electrode. The output potential is proportional to the concentration of the selected ion in the solution. The concentration is a measure of the number of ions in a specific volume. The electrode measures the activity rather than the finite concentration This method is most commonly used in the laboratories for précised measurement of all ions. Example Na, K, Ca and Cl. A dedicated electrode and reference electrode is placed in an ISE analyzer. Cleaning of electrodes is done after every test as it causes corrosion. Electrodes are to be replaced regularly which adds to the cost.

C. Colorimetry

Colorimetric technique is a useful analytical tool for determining concentration of coloured material in the solution. Coloured substances absorb light in the visible spectrum and amount of light absorbed is proportional to the concentration of substance in solution. The biochemistry analysers based on colorimetric technique consists of tungsten halogen lamp as the light source. The lamp has to be adjusted with the filters or monochromator to get the desired wavelength. The cost of the instrument increases.

The proposed Colorimetric sensor design consists of LED of 650nm as the light source. As the LED offers the desired wavelength, the need of monochromator or filter is eliminated.

III. SYSTEM OVERVIEW

A. BASIC PRINCIPLE

Colorimetry works on Beer Lambert's Law. It states that the concentration of substance is determined by directing a beam of monochromatic light through a cuvette containing the solution and measuring amount of light absorbed [5]. The formula is given below[5].

Absorbance α path length (l) *concentration

$A = \varepsilon * l * c$

ε = molar extinction coefficient.

B. BLOCK DIAGRAM

The system consists of Light intensity measurement unit and Data acquisition system. Fig2 shows the system block diagram Light Intensity Measurement unit is composed of LED of 650nm wavelength as the light source, a cuvette assembly and a photo detector with the signal conditioning circuitry. The output voltage is amplified and given to the data acquisition system. Data Acquisition System is driven by microcontroller ATmega 644. Atmega 644 is a low power 8 bit microcontroller with 64kbytes in system programmable flash memory, inbuilt A/D converter, and 4kbytes of internal SRAM and 2kbytes of EEPROM. It consists of 8 channels, 10 bit successive approximation A/D converter.

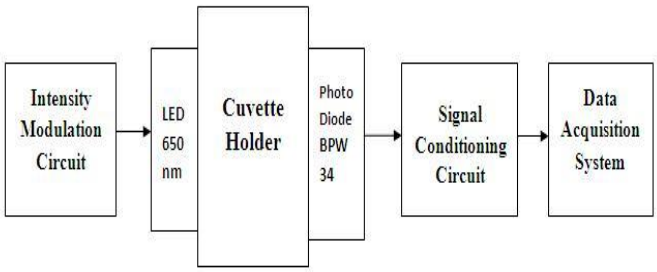

Fig.2 Block Diagram

C. SENSOR DESIGN

The sensor design of the system is shown in the fig.3. It consists of 12v dc power supply regulated to 8v by 7808 regulator. Intensity modulation circuit drives the LED through 1k resistor. The light source used is of 650 nm wavelength. The LED is the best light source used as compared to the incandescent bulbs as they offer enhanced spectral purity, higher brightness, increased lifetime, reduced power consumption[1]. The photodiode BPW34 is used as the detector and driven by the regulator. Photodiodes are semiconductor light sensors that generate a current or voltage when illuminated by light. BPW34 is the silicon PIN photodiode with high speed and radiant sensitivity. It has rapid response and wider range from 430nm-1100nm. It is sensitive to visible and near infrared radiation. The output of photodiode is given to current to voltage converter. The

voltage at the output of signal conditioning circuit is the transmitted voltage which is logarithmically converted to get the absorbance.

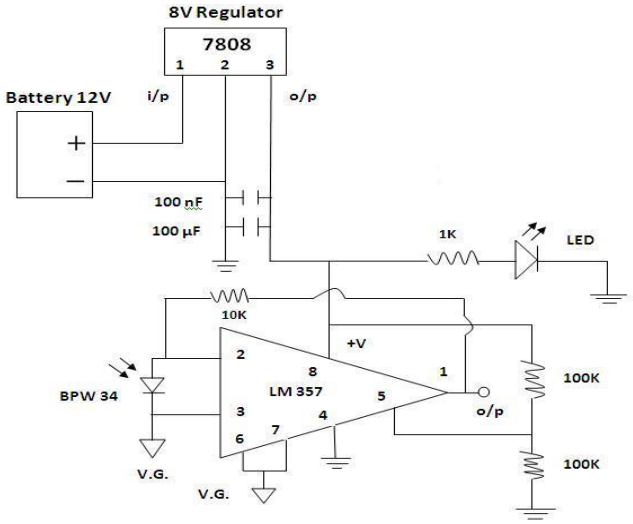

Fig 3. Sensor Design

IV. SAMPLE ANALYSIS

Human Blood is centrifuged and plain serum is used for the calcium test. Calcium is highly sensitive for arsenazoIII reagent. The calcium ions in the serum react with arsenazo to form blue purple colored complex. It generates an absorbance peak at around 600nm- 650nm. To measure the concentration of calcium in human serum, the amount of light transmitted is measured by measuring the voltage at the output of signal conditioning circuit. The measured voltage is logarithmically converted to get the absorbance.

The sample analysis is done in 4 steps.

1. Distilled water Blank
2. Reagent Blank
3. Standard
4. Test

The incident light is measured by placing 1000µl of distilled water in the cuvette and voltage reading is taken as Vblank. Reagent blanking is done by placing 1000µl of arsenazo reagent in the cuvette and reading is taken. The 10µl of standard solution is added to the reagent blank and reading is taken. The Vstd reading is obtained by subtracting the reagent blank from the standard reading. The test sample is run with the same procedure as standard. The 10µl of test sample is added to 1000µl of reagent and the reading is Vtest. Reagent blanking procedure is done for all the test samples and standard solutions, because along with the compound other variables also reduce the intensity of light. The concentration of the sample is calculated using the formula [2].

$$Concentration\ of\ the\ test\ sample = \frac{\log\left(\frac{Vblank}{Vtest}\right)}{\log\left(\frac{Vblank}{Vstd}\right)} \times Concentration\ of\ the\ Standard$$

V. RESULTS

The known concentrations were run on the experimental system and the readings are plotted in fig 4. The graph is linear. The system is linear from 3mg/dl – 18md/dl and requires dilution of the sample at higher concentration of calcium.

Table I: Experimental Readings

Standard Concentration	Acquired Concentration
5	5.7
10	10.3
15	13.8
20	18.2

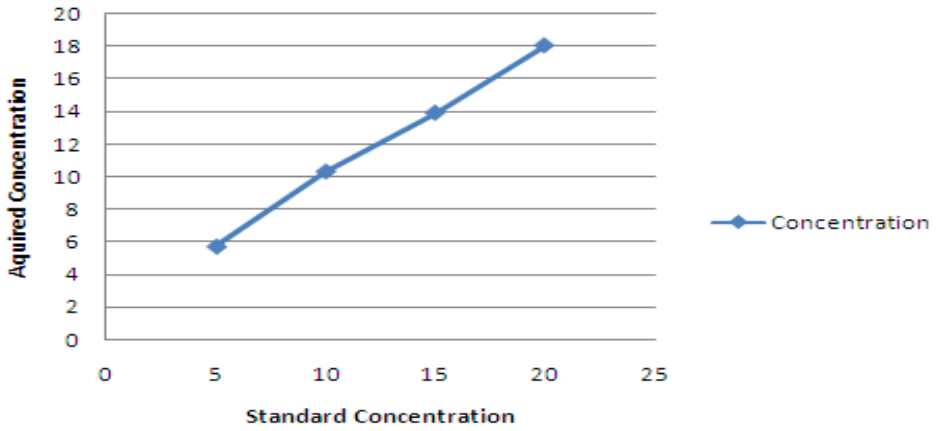

Fig.4 Concentration acquired on experimental system with respect to standard concentration.

VI. CONCLUSION

The colorimetric sensing system is developed and tested with the known standard concentrations of calcium. The graph of standard concentration against acquired concentrations is plotted and found to be linear. At higher concentrations the samples need to be diluted. The cost of the system is less and maintenance cost is negligible.

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