Creatinine and Albumin Electronic Reader

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ABSTRACT
Creatinine, an easily measured byproduct of muscle metabolism, is excreted unchanged by the kidneys. So it is an important indicator of renal health. A rise in blood creatinine level is observed only with marked damage to functioning nephrons. A decrease in muscle mass will cause a lower reading of creatinine, as well as pregnancy.

Albumin is a main protein of human plasma. Low albumin may be caused by liver diseases nephrotic syndrome etc. High albumin is mostly caused by dehydration.

A portable ACR reader is designed which is a POCT (Point Of Care Testing) device and very easy to use. It is essential that the test be carried out with a small volume of urine. POCT for creatinine and albumin concentrations offers advantage of providing a result within minutes outside the hospital which potentially enables faster diagnosis of conditions threatening kidney function. The instrument developed is a great boon for small pathological labs and clinics.

Keywords
Electronic reader, medical device, creatinine and albumin.

1. INTRODUCTION
Albumin, the protein found in plasma, binds water, cations, fatty acids, hormones, bilirubin, thyroxin (T4) and pharmaceuticals (including barbiturates). [4] Its main function is to regulate the colloidal osmotic pressure of blood. [4] The normal range of urine albumin is 0-8mg/dl. [5] Liver disease, nephrotic syndrome, protein-losing enteropathy, genetic variations and malignancy cause low levels of albumin. [4] High albumin is usually caused by dehydration. In some cases of retinol deficiency the albumin level can be elevated to high-normal values. This is because retinol causes cells to swell with water [4]. A healthy kidney does not let albumin pass into the urine. A damaged kidney lets some albumin pass into the urine. [1]

Creatinine concentration is also checked during standard urine drug tests. The normal urine creatinine levels range from 500 to 2000 mg/day depending on age and the amount of lean body mass. [6]Low amount of creatinine in the urine indicate low baseline levels. Random urine creatinine levels have no standard reference ranges. To reference levels of other substances measured in the urine, they are usually used with other tests. Creatinine levels potently decrease due to diuretics such as tea and coffee as they cause frequent urination.. The normal urine creatinine levels range from 500 to 2000 mg/day depending on age and the amount of lean body mass. [6]

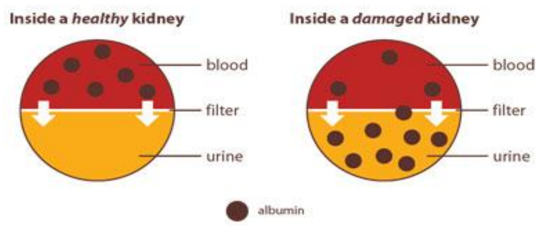

Fig.1. Filtration in healthy and damaged kidney [6]

2. BIOSENSOR
The most important technology used in the field of developing micro sized devices and portable devices such as biosensors is BioMEMS. BioMEMS is an abbreviation for Biological Microelectromechanical Systems, which involves developing sensors for analyzing biological components and biological activity by means of micro technology. The driving force behind Bio-MEMS includes Diagnostics, Health Screening, individualized treatment, Drug delivery Systems, Tissue Engineering and minimally invasive Procedures. [10]

Bio-MEMS is also employed in devices designed for personalized medicine. Personalized medicine includes technologies such as molecular diagnostics, integrating diagnostics with therapy, pharmacology and point of care devices. A major area of application of BioMEMs is Point of Care Devices which have been developed in order to render service to patients in a portable manner and to produce fast as well as accurate results so as to monitor the patient health. The main component in a point of care system is the biosensor. A biosensor can be defined as an analytical device for the detection of an analyte that reacts with a biological component by means of a physicochemical detector. The analyte and analyte are sensitive biological elements (e.g. tissue, micro-organisms, organelles, cell receptors, nucleic acids, enzymes, antibodies, etc.) they interact (bind or recognizes) to produce analysis of the analyte. The biosensor consists of a transducer element or detector element, which converts the resultant signal from the reaction of analyte with the biological element into a measurable output. The system also requires a biosensor reader, which contains the associated electronics, and signal processors that are designed to display the results in a user-friendly format.

There are few types of biosensor principles, which can be listed as follows: Calorimetric, Potentiometric, Amperometric and Colorimetric biosensors. At present there are various biosensor strips available in the market such as Home Pregnancy Strip and Glucose Biosensors. The biosensor strip used for the determination of the creatinine and albumin ratio values uses the colorimetric principle. This sensor strip was developed in the IIT Bombay laboratory.

3. LITERATURE SURVEY

Bromophenol blue was been found to be the optimum reagent to be used for colour development of Albumin in the sample. DNB stock solution was used to develop colour of creatinine. These colour developing reagents were found by the IIT lab as they have a fast reaction time. This develops the colour quicker and reduces the testing time. Also the chemical reactions produced variable different colours for different concentrations of albumin and creatinine.

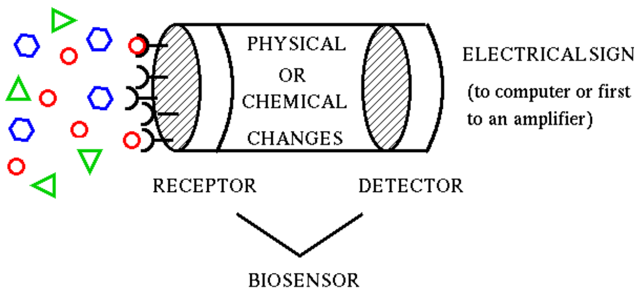

Fig.2. Generalized diagram of a biosensor

4. ELECTRONIC READER

A biosensor reader is basically a device which detects the biological signal of the biosensor and converts this analog value in into digital signal. This value is then displayed on the output device. The output device can be a seven-segment display, a LCD screen, TFT LCD touch panel. The reader guarantees its fast processing speed along with exact results.

In this project a reader is developed to determine the Albumin to Creatinine Ratio (ACR). A 3.2" TFT touch panel integrated with an Arduino mega 2560 has been incorporated along with color sensor. As the method developed to acquire the reading is based on the colorimetric principles, color is detected using a color light to frequency converter. Frequencies are recorded and then they are processed to get ACR and then displayed on the LCD screen. Everything is done automatically and the final readings are displayed in less than a minute.

5. PROCEDURE

The development of color on the bio compatible strip will lead to the readings of ACR. The biocompatible strip was developed in the IIT Bombay laboratory. The biocompatible strip developed is shown in fig 3. This included the reaction of urine sample with bromophenol blue for the detection of the albumin. The detection of creatinine followed benedict beher's reaction which involved DNB (3, 5- Dintrobenzoate). These reactions initiated a color change on the strip which is detected by the sensor. The strength of color change will be proportional to the concentration of the albumin and creatinine present in the urine sample which are the key markers of kidney disorders

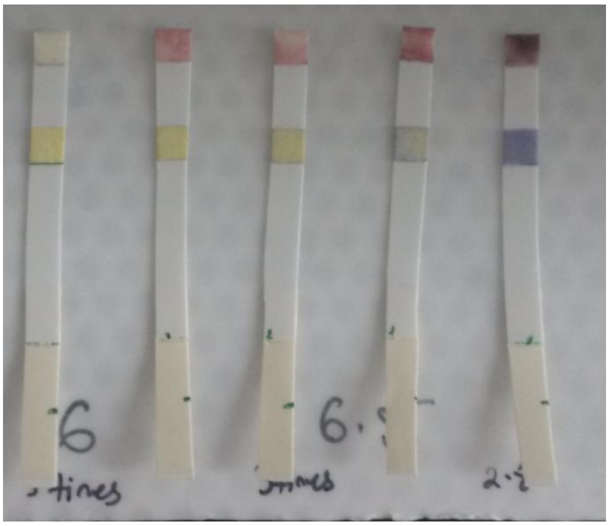

Fig. 3. Design of the strip

6. WORKING

Urine sample is taken in a container and the biocompatible strip is dipped in it. The strip is then dabbed on the tissue paper so that the excess urine is absorbed by it. The strip is then inserted in the device. A timer is run where the colour development takes place and the computation of the ACR is done. It is then displayed on the screen. Fig 4 shows the actual flow of the working of the device.

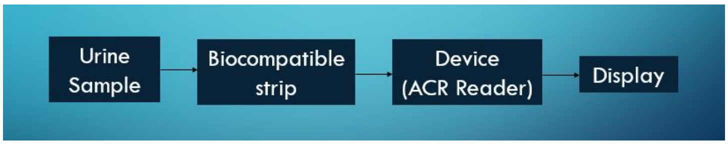

Fig. 4. Flowchart of the working of the device

6.1. LCD Working

The TFT LCD screen displays the various options available on the device.

Screen 1: Home screen-The home screen has two options: ACR and URS. ACR is Albumin to Creatinine Ratio. On pressing this block, a new screen opens. Home screen is shown in fig 5.

Screen 2: This screen contains precise instructions regarding the procedure to get ACR. There are 2 options available here. The user can use BACK to go back to the home screen or can press BEGIN to start the test. Fig 6 shows the instruction screen.

Screen 3: On starting the test a 45 seconds timer starts off immediately and a new screen opens. This time of 45 seconds is required for the colour of reagents to develop on the strip after it is dipped in urine. There is a STOP button to stop the timer and then to return to the home screen. The timer runs off and then the ACR value is displayed on new screen.

Screen 4: ACR is displayed on this screen. There is a BACK button to return to the home screen and continue testing.

The USR block is for displaying other parameters which will be the part of the advanced version of the device. The other parameters include glucose, protein, pH, specific gravity, bilirubin etc. Five RGB values will be taken and their average will be used to calculate the final value to increase accuracy.

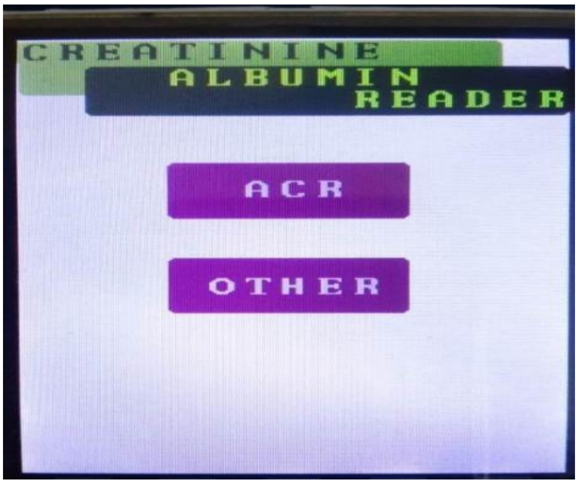

Fig. 5. Home screen

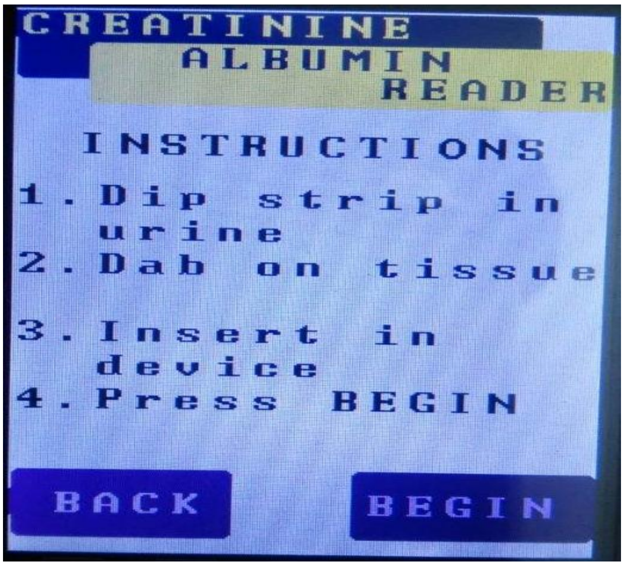

Fig. 6. Instruction Screen

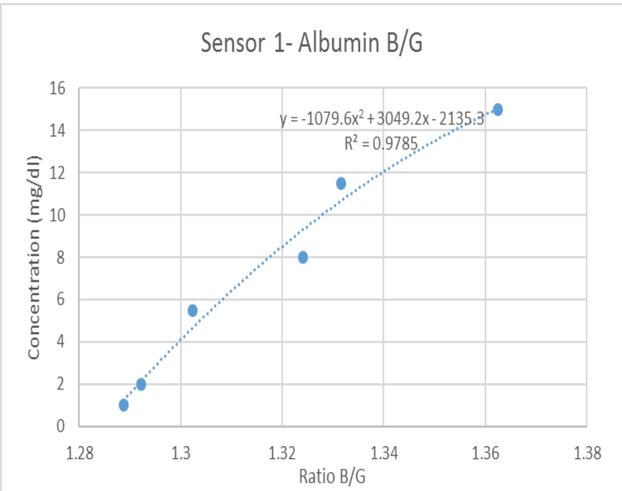

Fig. 7. Instruction screen

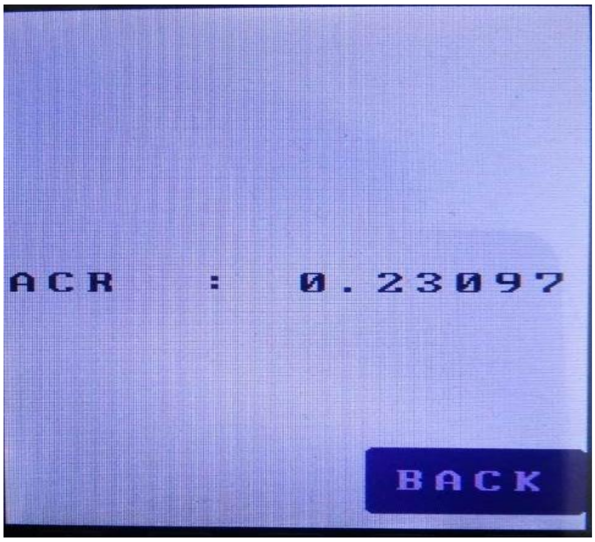

Fig. 8. Final ACR screen

6.2 Sensor Working

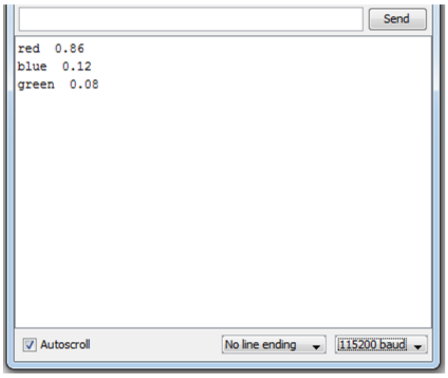

Fig. 9. Graph of B/G ratio for Albumin

The color sensor is used to detect the RGB values. The sensor is so programmed that it first calculates the amount of red, blue and green content in the sample. A ratio of these readings with the 'clear' or white content value is taken.

7. CALIBRATION OF DEVICE

7.1 Design of Strip

The strip is 10 cm x 0.5cm. The pad dimension on the strip is 0.5 cm x 0.5 cm in length and breadth. The material which is used for the strip is PVC and opaque plastic sheet. The opaque plastic sheet was preferred more over PVC sheet because it absorbs less light. If the strip material absorbs more amount of light then wrong readings of the RGB values of the colour which is developed on the strip are obtained

7.2 Calculation of ACR

The final calibration of the sensor was done with solutions of albumin and creatinine of known concentrations of in the IIT laboratory. The concentration of albumin solutions used were 1mg/dl, 3mg/dl, 8mg/dl and 15mg/dl. The concentration of creatinine in these solutions was 50mg/dl, 100mg/dl, 200mg/dl and 300mg/dl.

Solutions of intermediate concentration were obtained by mixing 2 solutions at a time to get more number of readings. The ratio of B/G was then taken of the RGB value readings obtained to get a trend between the concentration and the output. This also compensates for the instantaneous changes in the light intensity.

The readings were obtained from sensor 1 for the above mentioned concentrations of albumin. The value of R^2 obtained for B/R, B/G and B/W were obtained. The lowest value of R^2 was B/R with 0.2671. The best value of R^2 was represented by B/G which was 0.9785. Fig. 9 shows the graph of B/G ratio to concentration of albumin. As the value of 0.9785 is larger than the other values of R^2, the ratio B/G was specifically chosen. It gives the best relation between concentration of albumin and ratio.

The readings were then obtained from sensor 2 for the above mentioned concentrations of creatinine. Again B/G ratio showed the best R^2 ratio. Fig. 10 shows the graph of B/G ratio to concentration of albumin and the value of R^2 to be 0.9295. As the value of 0.9295 is larger than the other values of R^2, the ratio B/G was specifically chosen.
The equation obtained from Fig. 28 is

$$Y1 = -1079.6x^2 + 3049.2x - 2135.3 \qquad \ldots\text{eqn (1)}$$

The equation obtained from Fig. 33 is

$$Y2 = -11004x^2 + 26797x - 15910 \qquad \ldots\text{eqn (2)}$$

The two equations eqn (1) and eqn (2) have been coded into the microprocessor. So when the sensor takes a reading, the processor calculates the B/G ratio for both the readings. This becomes the value x that is obtained. Then this x is substituted into the respective equation to get the value of Y1 and Y2 which is the concentration of albumin or creatinine. The ratio of these concentrations of albumin and creatinine is taken and then the ACR value is finally displayed on the screen.

8. DESIGN OF THE DEVICE

8.1 Inventor AutoCAD Design

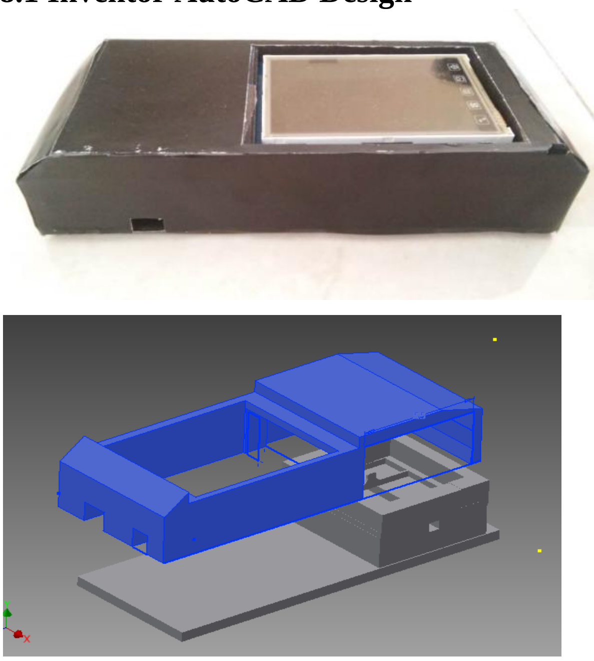

Fig. 10. Design of the device in Inventor Autocad

As this is a portable device its casing is developed on a special software known as INVENTOR AUTOCAD. The device has three parts i.e. the body covering, the base plate and the Sensor assembly. Fig 11 shows that designs in software and fig 12 shows actual device.

8.2 Makerbot Software

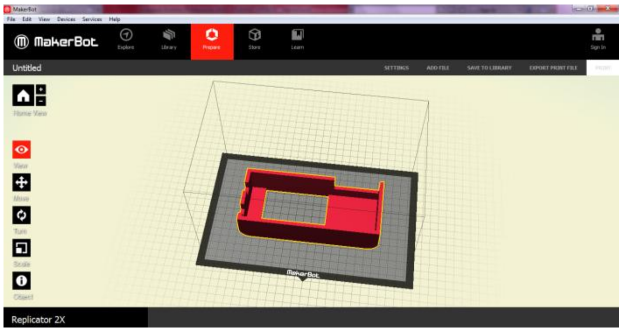

Fig. 12. Outer casing on MakerBot Software

It is an interface which connects the inventor autocad design with the 3d printer. The MakerWare interface is that of a simple STL/OBJ positioning tool, used to move, rotate, scale, and otherwise transform imported models. It also connects to either Skeinforge or MakerBot Slicer to perform the toolpathing required to produce machine code out of objects, and can save. Fig 13 shows the design of the outer casing when uploaded in Makerbot.

9. CONCLUSION

The two key markers for chronic kidney diseases are urine albumin and estimated glomerular filtration rate. Excess urine albumin and creatinine excretion are used to diagnose and monitor kidney damage in patients. Measuring the albumin to creatinine Ratio (ACR) will therefore give a more accurate evaluation of the albumin excretion rate.The literature survey gave us the result that bromophenol blue (BPB) is the most suitable dye for the detection of albumin. The creatinine detection was done using DNB. Various tests were carried out to see the colour change on the strip with different concentrations of albumin and creatinine. At the end albumin and creatinine were mixed together and the strip was tested with this solution to get noticeable colour change. The instrumentation part consists of the reader which detects the colour and then displays the ACR. The biosensor strip that is made detects albumin and creatinine levels. There are further developments to be done for the strip to include other regular urine parameters such as glucose, pH, bilirubin, specific gravity, protein. The effect of various parameters like change in temperature has to be done.

The future scope of the device is making the device capable of computing other urine parameters such as glucose, hemoglobin, pH, bilirubin, specific gravity, protein. Mini printer for printing results and SD card for storing patient data will enhance the device.

10. ACKNOWLEDGMENT

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