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Device Diagnosing Health of Bovine

Sumi Kankana Dewan

Abstract

The research problem taken into consideration for study dealt with the design of a low cost hand-held ZnO based sensing device for testing blood serum of bovine (cow), to diagnose their health of liver and kidney by detecting four biological parameters in-situ. Zinc oxide nanoparticles were synthesised by chemical bath deposition method. Using transmission electron microscopy (TEM) and X-ray diffraction (XRD), the size of ZnO nanoparticles were determined. It shows a hexagonal wurtzite structure with an orientation along the direction (101). TEM images show various morphological changes of nanostructured ZnO. The average crystallite sizes of ZnO molecule is found to be 0.004 nm from XRD. The constituents of nano sized ZnO are found to be of Zn (57.27%), Cl (33.01%), C (8.04%) and O (1.68%) as obtained from EDS. The samples of blood serum of bovine, avian and caprine are characterised by transmission electron microscopy (TEM) and Benesphera Avantor Performance (Biochemistry Analyser). ZnO based sensing device is designed with the help of Arduino and Microsoft visual basic 6.0 version software. The resistance of blood serum is taken into consideration for carrying out the experiment. It has been measured after adding ZnO (1 μ l) to blood serum of (1 ml) to detect four biological parameters – Serum glutamate pyruvate transaminase (SGPT), Serum glutamic-oxaloacetic transaminase (SGOT), Blood urea nitrogen (BUN) and creatinine of bovine more precisely. The device can indicate whether the blood serum of bovine have normal/diseased parameters. This device will also help the veterinarians in the field.

Keywords: SGPT, SGOT, BUN, creatinine, bovine, ZnO

1. Introduction

Many recent studies demonstrate that most nanoparticles (NPs) show an adverse or toxic effect on blood cells. Researchers have found that administration of ZnO nanoparticles ($\text{pH} < 7$) to whole blood samples and blood serums of animal and bird cause damage to the blood cells and tissues [1]. But NaOH are added to the acidic solution of ZnO to make the solution alkaline ($\text{pH}=7.2$) [2]. This alkaline nanostructured ZnO solution when added to whole blood and blood serum does not deteriorate the blood cells due to which biological parameters such as – Serum glutamic pyruvic transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT), Blood Urea Nitrogen (BUN) and creatinine can be more efficiently observed for different animals such as –cow (Class-Bovine).

Now a days nanoparticles based biosensors are introduced for obtaining the desired results more efficiently [3]. There are different types of biosensors which are incorporated with nanoparticles for diagnosing different problems in biological field [4].

2. General concept of biological parameters of animals

On Earth, there is no such living creatures which are free from diseases whether in case of human being or in case of animals, birds, insects or any other living organisms. It is true that cause of disease might be different. The reasons might be due to bad environmental conditons, due to lack of proper diets, due to hormonal imbalance, due to malfunctioning of organs, or it may be congenital or due to many more different conditions. Out of all diseases some are curable while few are beyond the control of doctors. Researchers are still doing researches to find out the solution of the remaining unsolved problems i.e. trying their best to make all the diseases curable. Some of the diseases can be controlled by the patient themselves by maintaining the biological parameters within the range which is safe for living a healthy life. But this will be possible only for human beings, not for the animals, birds and other living organisms. And it is the human beings who can also save the animals by taking care of the animals. Biological parameters such as creatinine, Serum glutamic pyruvic transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT) and Blood Urea Nitrogen are the parameters which gives an indication about proper functioning of liver and kidney.

Creatinine gives an accurate estimation for keeping a track on proper working of filtration processes of kidney. Formation of creatinine is shown in **Figure 1**:

Aspartate aminotransferase (AST) or SGOT and alanine aminotransferase (ALT) or SGPT are the enzymes that are present not only in the liver cells in large number but also in the muscle cells to a smaller number. If the liver gets injured or damaged, these enzymes are spilled into the blood by the liver cells, thereby raising the SGPT and SGOT enzyme blood levels and hence, indicating liver disease.

Figure 1.
Formation of creatinine.

BUN and creatinine levels gives a very accurate estimation of proper functioning of the kidneys. BUN measures urea level in the blood. To maintain a normal level of urea in the blood, both the liver and kidneys must function properly.

3. Motivation and layout of the research work

In India, cow, goat and poultry are tamed by human beings for fulfilling different needs. For milk production from cow, meat production from both goat and poultry and egg production from poultry. It should be our first priority to save our tamed animals from different diseases. After being consulted with Veterinary doctor, Dr. Jitendra Nath Dewan, Ex-Deputy Director, North Eastern Disease Diagnostic Laboratory, Khanapara, Guwahati, Assam, it has been found that once these tamed animals are affected with diseases affecting liver and kidney, then it becomes very tough to save them. So, focussed has been on those parameters whose values will give us the exact information about the proper functioning of liver and kidney.

In this chapter how the four different biological parameters of blood serum of bovine, i.e. SGPT, SGOT, BUN and creatinine are measured using nanoparticle ZnO based biosensing device has been discussed. This sensing device will be designed using ARDUINO board which will measure the resistance values of the serum samples of animal and bird. Corresponding to this resistance values, a look up table of above mentioned four biological parameters for the same serum samples will be maintained using Clinical Chemistry Analyzer. The microcontroller ATMEGA328P will be used to control the device in such a way that whenever this device will measure the average resistance value of any random sample of blood serum of bovine then it will immediately point to the nearest value of all the four parameters of the corresponding category of animal which will be stored in the look up table. Finally the result will be displayed on the device in-situ within seconds showing whether the health of the animal is in NORMAL /NOT NORMAL condition.

4. Sample preparation

All chemicals were purchased from the Emsure, are of analytical purity and used without further purification. All experiments were carried out in atmospheric pressure.

4.1 Synthesis of ZnO nanoparticles

Zinc Chloride is dissolved in 200ml deionised water in beaker of 250ml and placed on the magnetic stirrer and heated up to 70°C and at a speed rate of 450rpm as shown in **Figure 2**. Similarly, Polyvinyl alcohol is dissolved in 200ml deionised water in beaker of 250ml and placed on the magnetic stirrer and heated up to 70°C respectively. After this 180ml of Zinc Chloride solution is mixed with 180ml of Sodium Hydroxide solution [5] and to this mixture about 10ml of PVA solution is added in 500ml beaker, which is then placed on the magnetic stirrer and is heated upto 70°C for one hour. This solution is then kept inside a black box for one whole night for proper mixing and for cooling. After this solution is filtered using filter paper [6]. This will give a solution and powder of ZnO nanoparticles as shown in (**Figure 3**).

Figure 2.
Zinc chloride solution is heated on a magnetic stirrer at speed 450 rpm upto 70°C.

Figure 3.
Beaker containing ZnO solution.

4.2 Preparation of slides

Slides were divided equally so as to make it fit for the size required for XRD test. After that slides are washed properly with distilled water and dried. Those slides were dipped into a beaker containing the solution of concentrated Nitric acid (conc. HNO_3) at an angle of 45° . After few hours slides were taken out and were again dried and are also covered by paper so as to avoid the accumulation of dust particles on the slides. Using dropper, few drops of the solution of ZnO is then dropped over

Figure 4.
Preparation of slides.

all the slides at the center properly in such a way that solution remains stagnant as shown in the **Figure 4**. Again this slides are kept covered for few days for drying.

4.3 Mixing of blood of animals and birds with ZnO solution

From recent studies it is found that most nanoparticles (NPs) show an adverse or toxic effect on blood cells. Studies show that administration of ZnO nanoparticles to whole blood samples and blood serums of animal and bird cause damage to the blood cells and tissues [7–9]. Researchers have investigated that microscopic ultrastructural changes occur in mice when ZnO nanoparticles is incorporated in its body [10].

Zinc oxide solution so prepared is best for use upto one month from the date of preparation but can be used for more number of days if it is kept in colder

Figure 5.
Left side: Blood sample of cattle: right side: ZnO mixed with blood sample.

Figure 6.
Left side: Blood serum of cattle: right side: ZnO mixed with blood serum.

temperature. As the solution so prepared is highly acidic ($\text{pH} = 4.6$), so after mixing it with sample of whole blood and serum respectively, it has been observed that colour of the blood and serum changes immediately, it changes from red to pink and colour of serum becomes milky which indicates that haemoglobin of that blood sample decreases as shown in **Figures 5 and 6**.

As pH of blood is 7.2, sodium hydroxide solution is again added drop by drop using micropipette of range 2-20 microlitre to the Zinc oxide solution to make pH value of that solution equal to or greater than the pH value of blood [11]. pH is recorded after every dropwise addition of sodium hydroxide solution to zinc chloride solution which is found as 7.5. After this the solution having pH greater than 7.5 is added to 1ml of blood of cattle. 0.5ml of Zinc oxide solution is added to the same proportion of blood kept for one night. No haemolysis is observed immediately. Again 0.5ml of Zinc oxide solution is added to the same proportion of blood serum of the same cattle and is kept for one night. No haemolysis is observed. But in the next morning again haemolysis is observed in both blood and blood serum.

Due to the occurrence of haemolysis, pH of ZnO solution has been made exactly equal to the pH value of blood sample (i.e.7.2). 20 μl of NaOH solution is added to 70 μl prepared ZnO solution dropwise and after addition it has been observed that pH of ZnO solution becomes 7.2. Again 20 μl of the ZnO solution ($\text{pH}=7.2$) is added to 1 ml of blood of cattle as well to blood serum and immediately after addition as no haemolysis is observed as shown in **Figures 7 and 8** respectively, so, that sample has been used for manual test of blood to observe the parameters whether any changes have occurred.

And according to the blood test report of bovine given by the Central Instruments Laboratory, College of Veterinary Science, Khanapara, it has been found that RBC(red blood cells) counts shrinks and WBC(white blood cells) are vanished.

Again a new set of experiment is performed with the same ZnO solution. Initially only four parameters of Bovine has been tested. After that, ZnO solution ($\text{pH}=7.2$) of 2 μl is added to the 1.5ml of the same blood serum of bovine and is

Figure 7.
Left side: Blood sample of cattle: Right side: 20 µl ZnO mixed with blood sample.

Figure 8.
Left side: Blood serum of cattle: Right side: 20 µl ZnO mixed with blood serum.

Figure 9.
Clinical chemistry Analyser (Benesphera).

tested using Clinical Chemistry Analyzer (Model C-61, Make Benesphera) in the Clinical Laboratory, Khanapara as shown in the **Figure 9**.

4.4 Measurement of resistance of blood serum of bovine

In the next set of experiment 1µl ZnO is mixed with 1ml of blood serum of 5 different blood serums of bovine collected from different specimen. The five different samples of blood serum of the three different species were then undergone clinical test for testing four biological parameters - Bun, creatinine, SGPT and SGOT. Also the above mentioned five samples of each three species were then mixed with ZnO and then again clinical test has been performed. It has been found that there is an increase in the values of the parameters after mixing all the 5 serums with ZnO. **Table 1** shows the comparison chart of biological parameters (after mixing with ZnO) of three species- bovine, avian and caprine respectively [12]. **Table 2** shows the normal range of biological parameters of bovine as obtained from clinical reference interval values given by Central Instrument Laboratory, College of Veterinary Science, Khanapara, Guwahati.

Samples	SGPT (U/L)		SGOT (U/L)		BUN (mg/dl)		CREATININE (mg/dl)	
	Serum	Serum + ZnO	Serum	Serum + ZnO	Serum	Serum + ZnO	Serum	Serum + ZnO
B1	46.2	51.3	37.4	40.2	64.3	73.6	1.8	2.1
B2	31.5	33.9	40.6	48.8	41.3	45.7	1.0	1.3
B3	32.3	36.8	54.6	59.2	67.3	71.4	2.1	2.5
B4	71.2	77.3	45.6	49.2	71.3	78.3	1.7	1.9
B5	17.4	21.3	26.3	33.5	44.6	48.2	1.1	1.4

Table 1.
Comparison of biological parameters of Bovine.

Normal range	Bovine
SGPT(U/L)	8–57
SGOT(U/L)	9–49
BUN(mg/dl)	18.8–55.4
Creatinine(mg/dl)	0.5–1.6

Table 2.
Normal range of biological parameters of Bovine.

Figure 10.
Vial containing serum with steel electrode.

The resistance of 5 samples of blood serums and same 5 samples mixed with ZnO are measured using multimeter and also using Arduino as shown in **Figure 10** so as to check how resistance changes after mixing serum with ZnO. Arduino is an open source electronics board which is easy to use for programming. **Figure 11** shows the circuit diagram to measure the resistance of blood serum using multimeter and Arduino. **Tables 3** and **4** shows the values of resistances. It has been observed that resistance increases when serum is mixed with ZnO when measured in both the cases, i.e. using multimeter and using Arduino [13].

The experiment is carried out by inserting two electrodes of stainless steel at a distance of 0.81 cm into a vial consisting of serum. This electrodes are dipped into the vial of 1.5ml (as shown in **Figure 10**) in such a way that both the electrodes only touch the surface of serum. This particular set of electrodes are used for all type of samples after cleaning. The measurements of vial are taken using vernier calliper of zero least count are as follows:

- Outer diameter = 1.07 cm
- Inner diameter = 0.92 cm
- Inner diameter with clip (stainless steel) as electrode = 0.81 cm

The circuit shown in the **Figure 11(a)** has been used to observe the variation in the value of resistance of blood serum in the multimeter when current flows

Figure 11.
(a):Circuit diagram of rmeasuring the resistance of blood serum using multimeter; (b): Circuit diagram for measuring the resistance of blood serum using Arduino.

Samples	Resistance (k)	
	Serum	Serum + ZnO
Bovine		
B1	7.24	10.35
B2	10.46	15.00
B3	12.07	13.44
B4	1.90	11.60
B5	17.00	18.00

Table 3.
Measurement of Resistance of 5 different samples of bovine using multimeter.

through the serum between the electrodes. These electrodes are connected to 5V dc supply so that when current flows through the serum there occurs a change in resistance of blood serum which is then further get stable at certain value. Again when nanoparticle ZnO solution is added to the same serum sample, then it has been observed that value of resistance measured by multimeter is greater than that

Samples	Resistance (Ω)	
	Serum	Serum + ZnO
BOVINE		
B1	27741.9	30497.76
B2	29106.5	30023.35
B3	25700.0	26204.20
B4	26204.2	27088.70
B5	28663.8	29106.50

Table 4.
Measurement of Resistance of 5 different samples of bovine using Arduino.

value of resistance measured without adding ZnO. We have used same set up for measurement of all the samples. Using circuit shown in **Figure 11(b)**, resistance of same samples are measured using Arduino [14]. Here, ADC gives voltage and current value and in this model we have taken into consideration the resistance mode, so using voltage divider rule the unknown value of resistance of blood serums has been found out as shown in the Eqs. (2) and (3):

$$V_{out} = V_{in} \times \frac{R_2}{R_1 + R_2} \tag{2}$$

$$R_1 = \frac{V_{in} - V_{out}}{V_{out}} \times R_2 \tag{3}$$

where, R_1 = resistance of blood serums, R_2 = known value of resistance i.e. 10 K, V_{in} = Supply voltage (5 V), V_{out} = Output Voltage (4335.9 mV).
It has been observed that resistance increases after addition of ZnO to 5 different samples of blood serum of three species [15].

4.5 TEM study of blood serum

The necessity to carry out this TEM study of blood serum sample (with and without adding ZnO) is that the device which we were going to design is ZnO based biosensor in which we have to check the compatibility of ZnO with serum sample. Already it has been analysed that ZnO can be added to serum sample but the proportion must be maintained otherwise ZnO may damage the serum sample. It has been concluded that if 1 microlitre of ZnO is added to 1ml of serum sample then it does not alter the biological parameters of serum. In fact addition of ZnO will be more effective in designing a ZnO based biosensor as discussed in the previous section.

As per suggestion given by expert technician of NEHU Shillong performing TEM experiments, serum sample mixed with ZnO should be tested under biological sample category although ZnO comes under material sample category because we need to compare the TEM images of serum sample (with and without ZnO) basically to observe the existence of protein structure even after addition of ZnO so that we can proceed one more step to design our proposed ZnO based device of high efficiency.

Figure 12.
TEM images of blood serum of bovine.

Figure 13.
TEM images of blood serum of bovine mixed with ZnO.

4.5.1 TEM study of blood serum of bovine (cattle)

To obtain TEM image of blood serum of bovine, 1ml serum sample is taken. The images showing protein structure, cell membrane are observed using TEM (JEOL-100CX) as shown in the **Figure 12**:

4.5.2 TEM study of blood serum of bovine (cattle) mixed with ZnO

TEM images are observed using TEM (JEOL-100CX) as shown in the **Figure 13** after mixing 1ml blood serum of bovine with 1 μ l of ZnO using micropipette. The irregular black spots of serum proteins are observed [16].

In TEM study, the irregular black spots of about 30-40nm are serum proteins. The average size of serum albumin is 36 nm. Bubble like structure are the aggregated lipoproteins. It has been observed that mixing of 1 μ l ZnO with 1ml blood serum cause an increase in order of the biological parameters viz. BUN, creatinine, SGPT and SGOT and electrical parameter (resistance) which will help to analyse the variation of biological parameters of blood serum, after the incorporation of ZnO nanoparticle in it [17].

This study also tells us that the protein structure still exist in the images observed from TEM of blood serum of bovine mixed with ZnO same as that of the images observed from TEM of only blood serum of animal .

5. Experimental set-up

Blood serum is obtained from the blood which has been collected with the help of the veterinary doctor Dr. Jitendra Nath Dewan, Former Deputy Director, North Eastern Regional Disease Diagnostic Laboratory. Blood serum of cow, goat, poultry are mixed with nanostructured ZnO solution for measuring biological parameters SGPT, SGOT, BUN and creatinine. As it has been observed that there is an increase in the order of the biological parameters alongwith the increasing value of resistance of blood serum, when the blood serum is mixed with nano-structured solution. And based on this principle, circuit has been developed to design the sensor.

5.1 Circuit diagram

The circuit diagram has been shown below in the **Figure 14**. The basic components of the device are as follows:

- a. Microcontroller–ATMEGA328P
- b. LCD Module –20 x 4 display
- c. I2CDriver
- d. USB to TTL converter
- e. PC/Laptop
- f. Steel sensor probes
- g. Resistance of known value-10 K
- h. Five Push down tactile switches
- i. Voltage regulator-7805

5.2 Operation of ZnO based sensor

The heart of the circuit is an 8-bit AVR series microcontroller ATMEGA328P. This particular microcontroller has been selected as it has an inbuilt ADC. The power supply can be provided, either by using 9-12V battery or by connecting through USB port, it can be driven or by using adapter. A very popular voltage

Figure 14.
Circuit diagram of ZnO-NP based biosensor.

regulator 7805 has been used to provide regulated +5V supply which gives regulated 5V with 1 Ampere current tracking. Two 100 nF ceramic capacitor are used across pin no.1 and 2 and across pin no.2 and 3 of 7805 to reduce harmonic noise or ripple.

A 20 x 4 LCD module has been used to display the calculated value and to display the result. Instead of using parallel communication, serial LCD (I2C protocol) has been used to simplify the circuit, which helps to display information from microcontroller to LCD.

Only two wires are enough for the communication, i.e., SDA (Serial Data) and SCL (Serial Clock) in case of I2C protocol. ATMEGA328P is also having inbuilt I2C support. Pin no.27 (PC4) of ATMEGA328P is SDA which is connected to the SDA pin of the LCD module for transferring the data as well as command from microcontroller to LCD serially. In I2C protocol, synchronous communication is implemented where both the device require a common clock source. In this circuit, the microcontroller is working as master and the I2C driver for LCD is working as slave. SCL pin is used for the clock pulse. Pin 28(PC5) of ATMEGA328P is connected to SCL pin of the I2C driver. Pin no.1 is assigned as "RESET" pin. For normal operation pin no.1 must be pulled up and then the device will restart if a low pulse is provided at pin no.1. Hence, a 10K resistance is connected from pin no 1 to Vcc.

In this device five push to on tactile switches are used for different purpose. To START or STOP the ADC reading, a switch is connected to pin no. 14 (PB0). To SEND the data from microcontroller to a computer system through a USB cable, as with is connected to pin no.15 (PB1).

The program executing in the computer is responsible for collecting the data from the microcontroller. This data are processed further and compared with the data already stored in database. The stored data are collected from the real sample. And data of 5 samples of bovine, avian and caprine are entered into the database. Whenever the computer receive a data from the microcontroller, it start searching for the nearest match in the sample table. To find out the nearest data, a simple mathematical formula has been derived. After retrieving the data, the application

Figure 15.
Measurement using steel electrode.

program display the resultant value on the computer's screen and at the same time the data has been transferred to the microcontroller through serial communication.

The program executing in the microcontroller is responsible for retrieving the information from PC and to display the received data. USB to TTL converter has been used to transfer or communicate between PC and the hardware section. Steel probe has been used to get the resistive value of the sample. One probe is connected to ground and the other probe is connected to the ADC channel '0' of ATMEGA328P. To keep the ADC pin high at no load, 10K resistance has been used. Whenever the probe are placed on/in the sample, it returns different resistance value which is processed to predict the resultant.

Due to different resistive characteristics of different material, the probe material will affect on the result. Measurement can be taken by using electrode of any material (e.g. steel, lead) as shown in the **Figures 15** and **16** but the experiment is performed using steel electrode.

5.3 Arduino board

Arduino UNO board is the most popular board in the Arduino board family. In fact, it is the best board to start with electronics and coding. Some boards are

Figure 16.
Measurement using carbon electrode.

Figure 17.
Arduino board.

different from one another, but most Arduinos have almost same components in common as shown in **Figure 17**:

5.4 Datasheet of ATMEGA328

Figure 18 shows the datasheet of ATMEGA328P and also shows the input output mapped with Arduino UNO.

5.5 Microsoft visual basic

Visual Basic, is a basic programming language. Both simple and complex GUI applications can be created by programmers. **Figure 19** shows GUI created using Microsoft visual basic 6.0 version and **Figure 20** shows an example.

Figure 18.
Datasheet of ATMEGA328P.

Figure 19.
GUI showing blood sample analysis system.

Figure 20.
GUI showing blood sample analysis system of caprine (goat).

5.6 Database management system

Database management system such as Microsoft access binds the Microsoft Jet Database Engine with a graphical user interface (GUI) and software-development tools.

Table 5 show the look up table of five different samples of blood serum of bovine, alongwith their normal ranges of all the four different biological parameters i.e. SGPT, SGOT, BUN and creatinine.

The resistance of five samples of bovine are measured respectively using the Arduino and their corresponding values of four biological parameters so measured by clinical test are maintained in Microsoft access as look up tables so that any serum sample of bovinewhen taken for test using the sensing device, it will read the analog value of resistance and will immediately compare that value of resistance with the look up table and if both the values of resistance get matched then corresponding four biological parameters will be displayed indicating whether the parameters are in normal range or not.

Cow	SGPT (U/L)	SGOT (U/L)	BUN (mg/dl)	Creatinine (mg/dl)	Resistance (K)
Normal range	8–57	9–49	18.8–55.4	0.5–1.6	—
1	51.3	40.2	73.6	2.1	30.5
2	33.9	48.8	45.7	1.3	30.02
3	36.8	59.2	71.4	2.5	26.20
4	77.3	49.2	78.3	1.9	27.08
5	21.3	33.5	48.2	1.4	29.10

Table 5.
Look up table of five different samples of cow (bovine).

Figure 21.
Front view of ZnO based sensing device.

Figure 22.
Left hand side view of ZnO based sensor; right hand side view of ZnO based sensor.

5.7 ZnO based biosensor

ZnO based biosensor is designed as shown below. Front view, side view of the biosensor is shown in the **Figures 21** and **22**.

5.8 Flow chart showing the steps to take the readings of biological parameters

Flow chart below shows the steps to take the readings of the parameters of bovine, avian and caprine:

6. Measurement of biological parameters for bovine

Samples of bovine mixed with 1 µl of ZnO is tested with the device i.e. ZnO based sensing device which gives the reading of SGPT, SGOT, BUN and creatinine and also it tells whether it comes under the normal range or not. If the values does not lie within the normal range then owner of the patient should immediately consult a doctor. **Figures 23 and 24** show measurement of biological parameters of bovine.

6.1 Comparison chart

Samples which has been tested earlier in Clinical Laboratory one year ago are again used for clinical test (Clinic) and compared with the ZnO based

Figure 23.
Measurement of biological parameters of bovine.

Figure 24.
Biosensor displaying the biological parameters.

sensing device (Device). Serum samples belongs to the same bovine which has been collected in mass amount to carry out the test successfully to make conclusion.

6.1.1 Biological parameters of bovine (cow)

Samples of bovine (cow) are used for clinical test and compared with the ZnO based sensing device. Five blood serum sample are used which are denoted by B1, B2, B3, B4 and B5.

6.2 Statistical analysis and conclusion

Statistical analysis is done using IBM SPSS version 20 for the data of **Table 6**.

Standard deviation and standard error are evaluated using paired sample test as shown in **Table 7**. Also to check the efficiency of the device, correlation and t-test are carried out as shown in **Table 8**. Correlation test tells us about the similarity in the observations of both CLINIC values and DEVICE values and t-test tells us the difference in the paired samples i.e. CLINIC values and DEVICE values [13].

SAMP LES	SGPT(U/L)		SGOT (U/L)		BUN (mg/dl)		CREATININE (mg/dl)	
	Clinic	Device	Clinic	Device	Clinic	Device	Clinic	Device
B1	37.1	36.8	59.8	59.2	71.7	71.4	2.6	2.5
B2	77.8	77.3	50.4	49.2	78.6	78.3	1.9	1.9
B3	34.2	33.9	49.5	48.8	45.9	45.7	1.4	1.3
B4	36.9	36.8	59.5	59.2	71.6	71.4	2.5	2.5
B5	35.4	36.8	61.8	59.2	69.4	71.4	2.5	2.5

Table 6.
Comparison chart of biological parameters of bovine between clinical test and sensing device reading.

Paired Samples	Mean	No. of Samples	Std. Deviation	Std. Error Mean
BOVINE				
Pair 1 SGPT_Clinic SGPT_Device	44.28	5	7.59506	3.39663
	44.32	5	7.29872	3.26410
Pair 2 SGOT_Clinic SGOT_Device	56.18	5	5.76689	2.57903
	55.12	5	5.58856	2.49928
Pair 3 BUN_Clinic	67.44	5	12.52809	5.60273
BUN_Device	67.64	5	12.62351	5.64541
Pair 4 Creatinine_Clinic	2.18	5	0.51672	0.23108
Creatinine_Device	2.14	5	0.53666	0.24000

Table 7.
Paired sample test of bovine.

Paired Samples	No. of Samples	Correlation Test		t-test	
		Correlation	Sig	t	Sig
Bovine					
SGPT_Clinic & SGPT_Device	5	0.999	0	−0.116	0.913
SGOT_Clinic & SGOT_Device	5	0.987	0.002	2.566	0.062
BUN_Clinic & BUN_Device	5	0.997	0	−0.444	0.680
Creatinine_Clinic & Creatinine_Device	5	0.995	0	1.633	0.178

Table 8.
Paired sample Correlation test and t-test of bovine.

From correlation test it has been observed that CLINIC values and DEVICE values are highly correlated as the significant values are less than 0.05 and from t-test it has been found that CLINIC and DEVICE values are almost similar as the significant values are greater than 0.05. Standard deviation and standard error mean value is also low which reveals that each sample is closer to the mean value. Correlation test and t-test helped us to conclude that the developed low cost handheld ZnO based sensing device is highly efficient for measuring the biological parameters- SGPT, SGOT, BUN and creatinine of bovine to diagnose the health of liver and kidney. This device is user friendly.

7. Future scope

A handheld low cost ZnO based biosensor can be developed using Raspberry Pi so as to make the device more efficient and portable. Raspberry Pi is a low cost small sized device that are good for software applications. Using Raspberry Pi, task of maintenance of database will be reduced as it has its own storage capacity. Hence, look up tables of bovine, avian and caprine of more than five samples can also stored in Raspberry Pi. Besides this I also intend to design a same design for human being so as to save peoples from being affected by liver and kidney related diseases.

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