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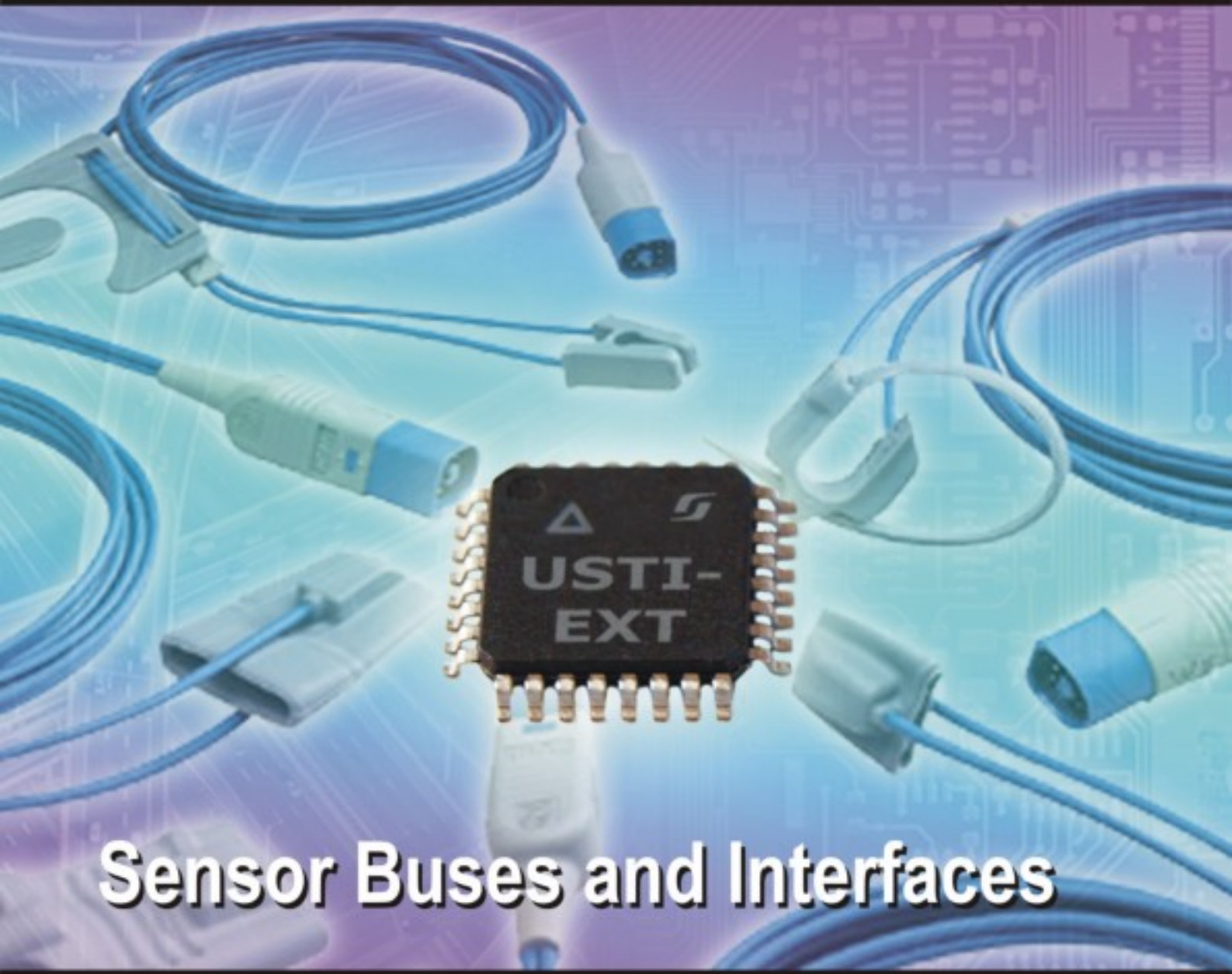

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Design of Optoelectronic System Using Multi Wavelength Illuminator for the Analysis of Sodium Ion in Blood Serum

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Abstract: This paper explains a portable and inexpensive Multi Wavelength Illuminator based optoelectronic system for the measurement of concentration of sodium ion in human blood serum. The Measurement system is developed using a Multi wavelength Illuminator LED lamp (OTLA-0100) as the light source, photodiode as the detector and the LPC2136 microcontroller for data acquisition and the processing unit. Algorithm is developed to monitor, to control the process sequences and transmit data to PC via serial communication module using RS232 protocol. Statistical analyses are carried out to evaluate the performance of the developed instrument and results are compared with that of a conventional instrument (ELDEX-3.8). Linear regression analysis reveals the excellent correlation with correlation coefficient(r) values of 0.99889. No precise differences are observed between the methods by the t-test and *F*-test results. The t-test and F-test values are 0.436619 and 0.998097 respectively. The chi-square test is also performed to check the significance of obtained results against the conventional method. The developed system shows the good performance and the results are in good agreement with the current clinical method at 98% confidence level. *Copyright © 2012 IFSA.*

Keywords: Spectrophotometry, LPC2136, Sodium, OTLA-0100LED, Clinical sample.

1. Introduction

Sodium is the major extracellular cation, plays a vital role in fluid distribution among body compartments. It helps to keep the water (the amount of fluid inside and outside the body's cells) and

electrolyte balance of the body [1]. Sodium is also important in nerves and muscles to work. Most of the sodium in the body (about 85 %) is found in blood and lymph fluid. Sodium levels in the body are partly controlled by a hormone called aldosterone, which is made by the adrenal glands. Aldosterone levels tell the kidneys, when to hold sodium in the body instead of passing it through the urine. Small amounts of sodium are also lost through the skin during sweat. The ingested Sodium is filtered in the renal glomerulus and approximately 70 % is reabsorbed in the proximal tubule. Further re-absorption occurs in the loop of Henle and <5 % is reabsorbed distally under the influence of aldosterone. The minimum and maximum requirement of Sodium is about 1,500 mg (<70 mMol) and 2,300 mg (<100 mMol) per day respectively [2]. The normal concentrations range of Sodium ion in blood serum is 135–150 mMol/L [3, 4]. A high plasma sodium concentration is more than 150 mMol/L is referred to as Hypernatremia [5, 6]. This can occur due to simple dehydration, excess sodium intake and steroid therapy as well as in diabetic insipidus. Hyponatremia, with plasma sodium concentration less than 135 mMol/L [7-9]. Low sodium levels are not uncommon and most often seen the eldest persons. It occurs as a side effect of taking medicines (diuretics medication). Severe diarrhoea, or vomit or heavy sweating and gastrointestinal disorder may also cause low sodium levels in human body.

Many methods are available to measure the Sodium concentration in blood serum samples. Traditional EDTA titration method involves a presample preparation and lengthy chemical procedure. ISE method is accurate but membrane life time is less. Chromatography is an efficient separation technique, which needs huge volume of chemical solvent. AAS and ICP-MS use complex instruments setup makes these methods too expensive [10]. Injection analysis is an automatic procedure, but optimizations of manifold blocks are difficult. Conventional spectrophotometer uses tungsten filament lamp as light source for visible radiation, grating or film is used to produce monochromatic wavelength and photocell is used as light detector. Even though the measurement procedure is straight forward, the instrumental and human errors make this whole measurement less reliable and inaccurate. Hence, an attempt is made to design a portable, inexpensive spectrophotometer with rich variety of features based on microcontroller to perform the easy sodium assay. The LPC2136FBD64 microcontroller is based on a 16/32-bit ARM7TDMI-S CPU with real-time emulation and embedded trace support, that combine the microcontroller with 256 KB and 32 KB of embedded high-speed flash memory and RAM respectively. A 128-bit wide memory interface and unique accelerator architecture enable 32-bit code execution at maximum clock rate. The advantages offered by the microcontroller in processing the Sodium analysis contribute to the improved measurement accuracy and robustness of device in its ability to reject noise and identify false measurements. The microcontroller also provides the ability to store test results for further reference [11]. For performance and clinical applications, the proposed system is calibrated with Sodium standard solutions.

2. Experimental

2.1. Design Scheme

The design scheme for Multi Wavelength Illuminator based optoelectronic system for the measurement of sodium ion is shown in the Fig. 1. Block A contain multi wavelengths Illuminator light source (OTLA-0100). The OTLA-0100 light source contain nine LED chips packaged onto a compact, thermally conductive ceramic substrate providing wavelengths ranging from Ultra Violet through Visible region is shown in Fig. 2. It emits discrete radiations is 383 nm, 405 nm, 470 nm, 505 nm, 530 nm, 570 nm, 594 nm, 660 nm, and 695 nm. The effective illumination diameter is only 0.125 inch, which provides easier coupling of secondary focusing optics. This module can also be customized to incorporate additional wavelengths and integrate secondary optics. The Opto Technology OTLA-0100 is designed to illuminate a target sample with multiple wavelengths of light along the same optical path. The OTLA-0100 offers the inherent long-life, low voltage, and reliability advantages of LED technology compared to traditional lamps or lasers [12].

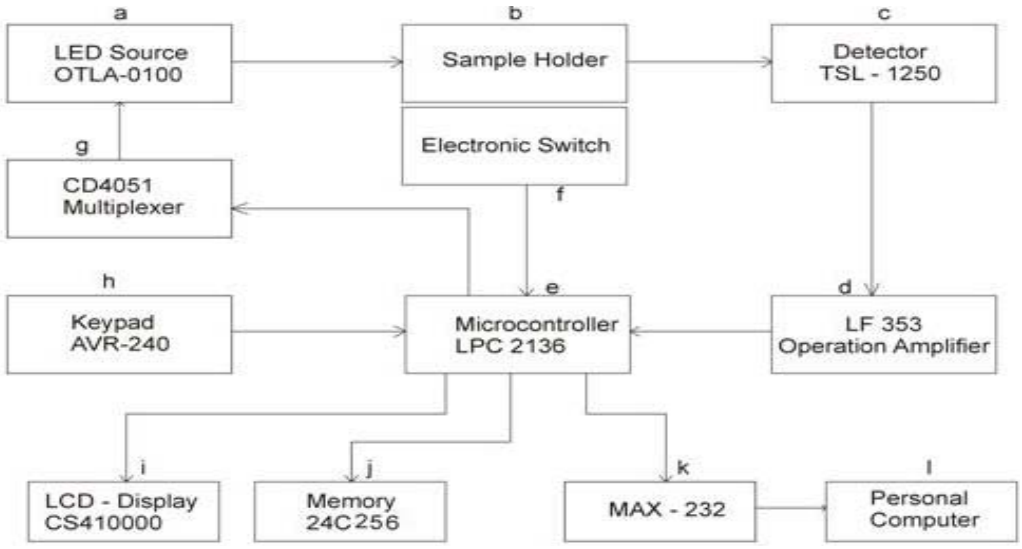

Fig. 1. Design scheme of LPC2136 microcontroller based optoelectronic system for Sodium analyzer.

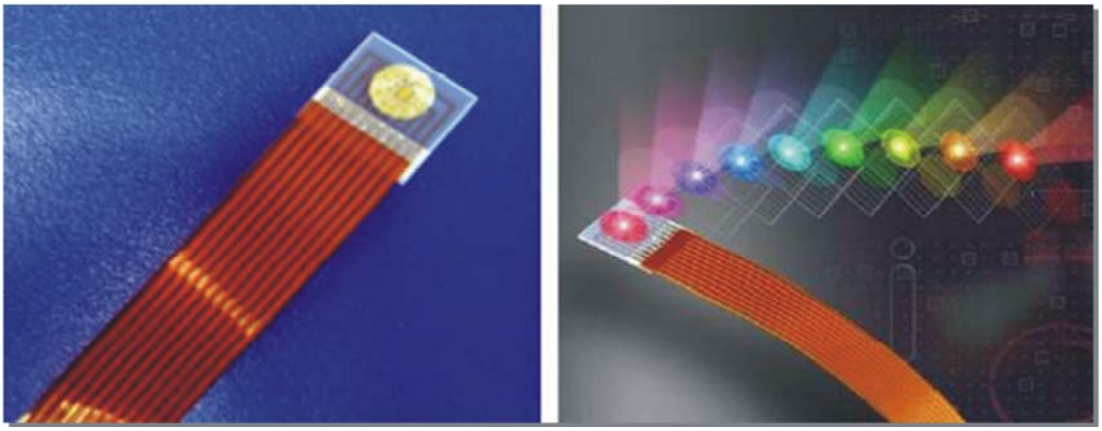

Fig. 2. Multi wavelength illuminator LED (OTLA-0100).

Block B is sample holder which is used to hold the Blank, **S**tandard and **S**ample solution. Texas Instrument's TSL1250 light to voltage optical sensor is used to detect the amount of light absorbed by the Blank, Standard and Sample solution. The output voltage is directly proportional to the light intensity on the photodiode, is kept in the Block C**.** The output of photodiode is connected to the inverting and non-inverting input (Pin No 2 and 3) of the LF353 Operational Amplifier. LF353 is low cost, high speed, fast slew rate, dual JFET input operational amplifiers with an internally trimmed input offset voltage [13]. The Op-Amp convert the current in to voltage and it is output connected to the Port 0.27(ADC0) of the microcontroller, which is kept in the Block D**.** LPC2136 microcontroller, the heart of the measurement system is kept in the Block E. It is a 64 pin microcontroller and it has 2 Port (Port 0.0-Port 0.31 and Port 1.16-Port 1.31), Flexible On-Chip Memory for Flash Program storage and SRAM for data storage. The LPC microcontroller work with 1MHz to 30 MHz speed and it functioning at low power (3 V-3.3 V). It consists of two 8-channel 10 bit ADCs provide a total of up to 16 analog inputs, with conversion times as low as 2.44 μs per channel and single channel 10-bit DAC provides variable analog output[14].The microcontroller is used for the processing the data.

Block F contain switch, which is used to sense the presence of test tube in sample holder. The switching circuit output is connected to Port 0.1 of the microcontroller. An 8-channel analogue multiplexer IC CD4051 from Philips, it having three address selection inputs are used to select multi

wavelength illuminator by channel selection command from microcontroller. Selection of channel inputs lines are connected to Port 0.11, Port 0.12 Port 0.13 respectively, which is kept in the Block G. The user enters experimental variables, modes of operation and selection of menu into the system through a keypad, which is kept in Block H. The 4×4 keypad is interfaced with the microcontroller through Port 0.4 to Port 0.7 and Port 0.20 and Port 0.23. The Four rows and twenty characters (4 X 20) CS410000 dot matrix LCD display from Hitachi, which can show 160 different numeric and alphanumeric characters is interfaced with microcontroller to display the absorbance, concentration of Sodium in blood serum, selection of wavelength and the incubation temperature. Port 0.16 to Port 0.19 lines are connected to MSB nibble of LCD connector (D4 – D7), Port 0.14 is connected to Register select and Port 0.15 Enable pin of the LCD, which is kept in the Block I. IC 24C256 is used to store the patient information, test details, absorbance and concentration value for further calculation, which is kept in Block J**.** The SCL and SDA signal lines of 24C256 are connected to Port 0.2 and Port 0.3 respectively. An RS-232 serial interface is implemented by configuration of Digital Block with the USART module. The serial block is set up using a MAX232 (Maxim Integrated Products, Sunnyvale, CA) to achieve the necessary level shifting for communication between a PC [15] and microcontroller. These blocks are kept in K and L respectively.

2.2. Reagent

All reagents are analytical grade (AR) and used without any further purification. Sodium is precipitated by the addition of Magnesium Uranyl Acetate as Uranyl Magnesium Sodium Acetate. Then the excess of Uranyl salt reacts with potassium Ferro-cyanide. The prepared solution is centrifuged. The clear supernatant solution is allowed to reacts with sodium ion in serum sample and forms a brownish colored complex which gives maximum absorption at 530 nm of wavelength. Precipitating reagent, Sodium standard and Colour reagent are purchased from +Med Source Ozone biomedical Pvt. Ltd, Delhi [16].

2.3. Sample Preparation

Blood samples are collected from various patients in Hospital (VB Diagnostics & Diabetic care centre, Thanjavur, Tamilnadu, South India). The collected blood samples are stored until their analysis, which is accomplished within one week. Samples are kept at their natural pH. The measurement of Na^+ by the designed instrument and conventional method are performed on the same day in order to avoid any possible bias. Blood samples are centrifuged at 3000 RPM for 2 minutes, to get a clear blood serum. For Standard solution preparation, 1ml of precipitation reagent added with 0.01 ml of Sodium standard. From the mixture, 0.02 ml is taken in the test tube and adds 1ml of colour reagent, to find the absorbance of standard. 1 ml of precipitation reagent added with 0.01 ml of blood serum. From the mixture of sample solution 0.02 ml are taken in the test tube and add 1ml of colour reagent, mixed well and allowed it to stand at room temperature for 5 minutes. This prepared solution is used to determine the absorbance of sample. Same procedure is followed for the preparation of Blank solution. Blank is prepared using the 1ml colour reagent and 0.02 precipitating reagent.

2.4. Measurement

Blank, Standard and Sample solution are taken in separate test tube and is placed in to sample holder one by one. The voltages $V_{blank}(V_b)$, $V_{std}(V_s)$ and $V_{sample}(V_t)$ for the blank, standard and sample solution are measured at 530 nm wavelength by the microcontroller and the unknown concentration of the sample is computed using the relation,

$$\text{Concentration of Sodium ion in blood serum} = \frac{\log 10 (Vb / Vt)}{\log 10 (Vb / Vs)} XCs, \quad (1)$$

where $\log_{10} (V_b / V_s)$ is the absorbance of standard; $\log_{10} (V_b / V_t)$ is the absorbance of sample and Cs is the concentration of the standard solution (150 mMol/L).

2.5. Software

Software has been developed using C language to configure the ports, to initialize LCD, to send start of conversion to ADC, to poll the end of conversion (EOC), to read light intensity data from. the photodiode, and to store in the internal RAM in sequence, to process the acquired data, to display the absorbance and concentration, to read user information and command from keyboard, to set serial communication with 9600 baud rate and COM port in PC to transfer/ receive data with microcontroller. The compiled code is downloaded into the flash memory of LPC2136 using the programmer. All the events are executed automatically.

3. Results and Discussion

The performance of the LPC2136 microcontroller based optoelectronic system in measuring the concentration of Sodium is investigated. The optical characteristic of green LED of Multi Wavelength Illuminator is studied under different source current to achieve similar emission band related to absorption spectra of Sodium. The LEDs are typically operated under nominal, recommended conditions, in order to maintain the lifetime stability. Initial experiments are carried out to establish the sample processing conditions to allow analyzing samples containing different Sodium standard concentrations (every rise in 25 mmol/l). The test samples are diluted and adjusted to a proper concentration for application in the system. Standard stack solutions are prepared to check the linearity of developed system. A calibration curve is obtained using different concentrations of Sodium standard solutions ranging from 0 to 200 mMol/l to determine the sensitivity of Sodium ion concentration using the developed instrument. At wavelength of 530 nm, a calibration curve is drawn between concentration (0 to 200 mMol/L) and absorbance is shown in Fig. 3.

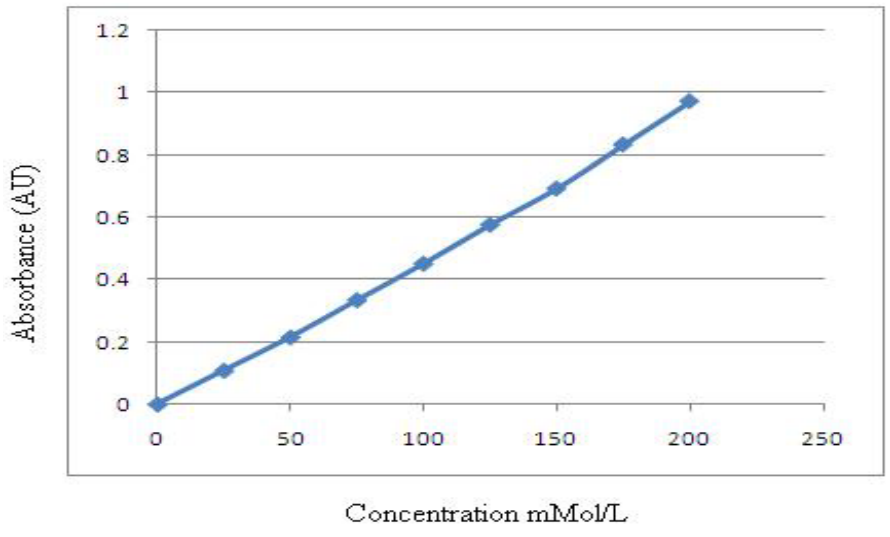

Fig. 3 Calibration curves for the standard solutions of Sodium (Concentration versus Absorbance).

The accuracy and reproducibility of the instrument is checked by repeating the measurement in 20 times consecutively to determine the within-run variations. The resultant 98 % of reproducibility is achieved with good sensitivity level. The samples are again run randomly ten times each day for 10 days to determine the between-run variation. Good reproducibility narrowing down to about 98 % in the results was also reflected by the instrument with different samples. Table 1 shows the concentration of Sodium ion in blood serum for various patients measured using the developed instrument as well as ELDEX-3.8. From the Table 1 it is found that the sodium ion in blood serum is high for patient no 6 which shows that the patient is affected by Hypernatremia, having symptoms like Kidney damage, water depletion, Nausea, Headache, Lethargy (central, nephrogenic), Hyperaldosterronism and Cushing diseases. The Sodium concentration is very low for patient no 1 and 2 which indicates that the patient is affected by Hyponatremia and they are suffering from Severe diarrhoes, Heavy sweating, Emesis and severe. Hyponatremia can cause confusion, heavy disorientation, seizures, coma and death.

Table 1. Concentration of Sodium ion using Designed Instrument and ELDEX-3.8 for blood serum.

Patient No.	Concentration of Sodium using designed instrument (mMol/L)	Concentration of Sodium using conventional method (mMol/L)	State	Diseases
1	126.3	126.4	Low	Severe diarrhoes, confusion
2	128.7	128.7	Low	Heavysweating, heavy disorientation
3	142.7	142.5	Normal	-------
4	143.3	143.3	Normal	-------
5	144.5	144.5	Normal	-------
6	154.1	154.0	High	Kidney damage, Headache
7	142.1	142.1	Normal	--------
8	136.6	135.8	Normal	--------
9	139.2	140.0	Normal	--------
10	146.3	146.3	Normal	--------

4. Statistical Analysis

4.1. Optimization of Incubation Period

The effect of incubation period is studied by measuring the absorbance for a period of time. For better complex formation between reagent and solution, the mixed final solution is left for incubation for 5 minutes. The optimization of incubation period is carried out and it is shown in Fig. 4. It is observed that for 6 minutes, the absorbance keep on increasing and it is constant after 6 minutes. To get quick results, readings are taken after 6 minutes of incubation period, even though the color remains stable for one hour.

4.2. Linear Regression

Linear regression analysis is performed and tested for lack of fit to the linear model. The linear regression curve drawn between concentration of sodium in blood serum (for various patients) for the developed system and conventional system (ELDEX-3.8) is shown in Fig. 5. Linear regression attempts to model the relationship between two variables by fitting a linear equation that comes closest to fitting a collection of data points. The most common method for fitting a regression line is the method of least-squares. In simple linear regression, the model function represents a straight line. The

strength of the linear association between two variables is quantified by the correlation coefficient. Table 1 shows the concentration values determined for the samples using the developed instrument and the conventional system. All the samples values fall into the linear range, and there is sufficient precision in the data to continue with the linearity study. The equation for linear regression analysis is follows,

$$Y = a + bX \tag{2}$$

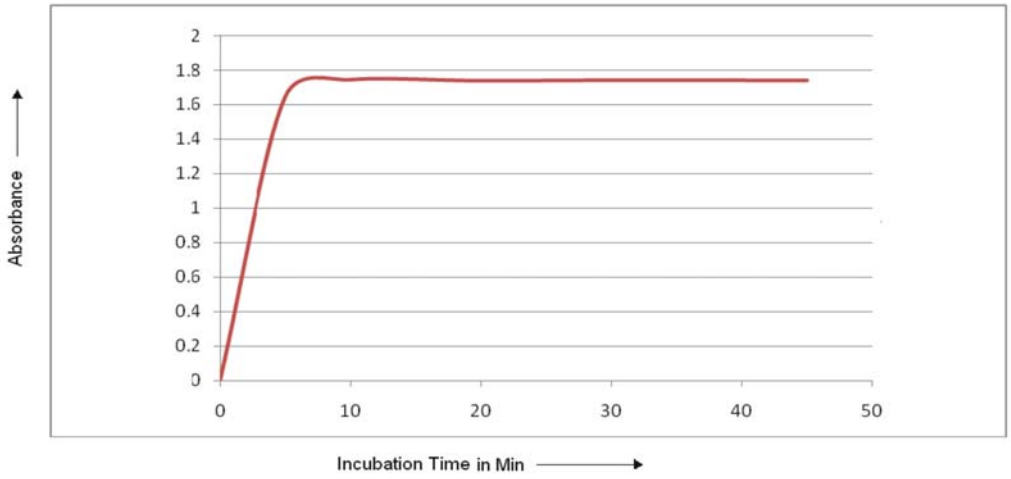

Fig. 4. Optimization of Incubation Period (Time versus Absorbance).

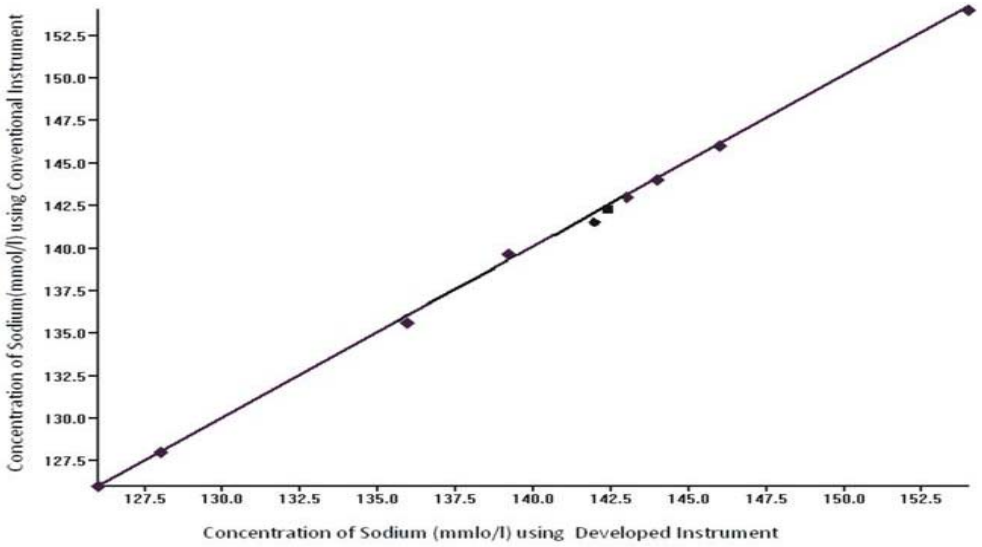

Fig. 5. Regression line for comparison of designed Instrument and ELDEX-3.8 Sodium analyzer.

The values of intercept a = 0.24898, b = *0*.99808 and correlation coefficient r = 0.99889 which closes to 1 indicating the perfect correlation. Fig. 5 shows the linear regression drawn (using software ULTIMACALC) between the designed instrument and the conventional method, which shows the accuracy of the designed instrument. The concentration of the Sodium measured by developed system (n=10) is 140.38±8.2156 with 5.85 % of relative standard deviation and for conventional Instrument is 140.36±8.2089 with 5.84 % of RSD. From the graph, all the points fall in the linear range and near to the straight line with minimal error. This regression line is perfectly positive correction.

4.3. Chi-Square Test for Significant

Chi-square test is used to test the goodness of fit. The test enables to find out whether the difference between the designed instrument concentration and standard analyzer concentration value is significant or not. From the Table 1 it is observed the value at 1% level for 9 df is 2.088. The parametric t-test and F-test is used to check the significant and precision of the instrument. This test compares two distinct statistical populations. The goal of the two-sample t-test and F-test is to determine whether there is any statistically significant difference between the mean of the first population when compared against the mean of the second population, based on the results observed in the two respective samples. The results are compared statistically with those obtained by ELDEX-3.8 by t-test for accuracy and F-test for precision. From the test, no significant differences are observed. The value of t-test and F-test is 0.436619 and 0.998097 respectively. The results measured with the developed system are in good agreement with the value obtained with the ELDEX-3.8. Statistical analyses of measurement with the developed system is best satisfying and produce 98% confidence level keeping all the measurement errors within limits.

5. Conclusion

The Multi Wavelength Illuminator based optoelectronic instrument shows the possibility of the implementation of Sodium ion in blood serum samples. The absorbance detection assembly is sensitive and selective for Sodium ion determination. The LPC2136 microcontroller is a general purpose 32-bit microprocessor, which offers high performance and very low power consumption. The ARM architecture is based on Reduced Instruction Set Computer (RISC) principles and related decode mechanism are much simpler than those of Complex Instruction Set Computer (CISC). The OTLA-0100 is multi wavelengths Nine-LED packaged onto a compact, thermal conductive ceramic substrate and providing wavelengths ranging from UV through Visible range. Reproducibility of the instrument is examined using different time interval for Sodium absorbance value of Sodium standard solution. The RSD values exhibit, excellent precision property of the developed instrument. The linear regression analysis reveals excellent correlation (r) with value of 0.99889. In addition t-test and F-test results show good accuracy and better precision with value of 0.436619 and 0.998097 respectively. The chi-square test shows the good significant of the data coincide with ELDEX-3.8 instrument. This system proves that the suitability of sodium measurement in serum samples and exhibit good spectroscopic properties. In LPC2136 microcontroller the speed of computation of Blood concentration is found to be faster than 8-bit microcontrollers. The microcontroller based instrumentation setup is inexpensive, easy to handle, portable and quit accurate. The same instrument can be used to estimate the concentration of other minerals from various samples by selecting requirement wavelength of LED in multi wavelength illuminator by modification of software.

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