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MEASURING CHLORIDE IN SERUM USING SINGLE PROGRAMMABLE SYSTEM ON CHIP (PSoC)

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PSoC devices are dynamically reconfigurable, versatile programming, low-power consumption and multiple interfacing, which motivates the design of portable and inexpensive instruments. The Chloride Analyser is built around a Cypress CY8C27443 PSoC with its analog and digital blocks, which is typically an embedded system, and it is configured for the measurement of Chloride in serum. The principle of Chloride measurement is based on colorimetry with LED as illuminating source and photodiode GASPG1104 as a light sensor. The run to run precision of the implemented system is determined by analysing human control serum Accutrol^{TM} Normal (Sigma) and the Chloride concentration is found to be 104 ± 4 mmol/l (mean $\pm$ SD, $n = 5$), which is close to the certified value. This system has been used successfully for the routine assay of bio-medical samples, with the results in good agreement with values obtained by the commercial clinical analyzer at 95% of confidence level.

1. INTRODUCTION

Chloride is an essential electrolyte for human, which occurs primarily in body fluids. This anion is specially transported into the gastric lumen, in exchange for another negatively charged electrolyte (bicarbonate) in order to maintain electrical neutrality across the stomach membrane. After utilization in hydrochloric acid, some Chloride ion is reabsorbed by the intestine, back into the blood stream where it is required for maintenance of extra cellular fluid volume [1]. A constant exchange of Chloride and Bicarbonate between red blood cells and the plasma helps govern pH balance and transport Carbon dioxide, a waste product of respiration from the body.

Since, Chloride is a highly mobile ion that easily crosses cell membranes and is involved in maintaining proper osmotic pressure, water balance and acid-base balance. Several studies have suggested that the Chloride ion may play a more active and independent role in renal function, neurophysiology and nutrition. Low serum chloride values are found with extensive burns, excessive vomiting, intestinal obstruction, nephritis, metabolic acidosis, and in Addisonian crisis. Elevated serum chloride values seen in dehydration, hyperventilation, congestive heart valve, and prostatic or other types of urinary obstruction [2]. The clinical significance of tests for chloride in the serum are important in the diagnosis and treatment of patients suffering from hypertension, renal failure or impairment, cardiac distress, disorientation, dehydration, nausea and diarrhea.

Plasma or serum Chloride is routinely assayed by a clinical Chloride ion meter that uses a Chloride ion selective electrode [3], or by cyclic voltametry [4]. Because of the difficulty in tuning of the Chloride meter that is usually specialized for human serum or plasma in clinical laboratories, determination of Urinary chloride is based on titration methods using the reaction between Chloride and Silver ions [5]. There is difficulty in judging the correct end point, especially for the Silver Nitrate method. The Coulometric titration solved this problem but requires the special instrumentation [6].

Colorimetry is one of the simplest and best techniques, which have been used for the clinical routine assays. Hence, there is a need to develop a simple, accurate and inexpensive bio-medical analyzer based on Colorimetry principle.

Modern embedded measurement and control systems incorporate a microcontroller as the principal component. As well as the microcontroller, an embedded control system frequently uses external chips such as peripheral device controllers and analog chips for processing input analog signals.

The new generation of re-configurable PSoC controllers, which integrates all the above components, will become the dominant system architecture for the majority of micro-based designs, by employing advanced lithography and FLASH-based programming technology. A single PSoC device can integrate as many as 100 peripheral functions with a microcontroller [7]; saving customers design time, board space, power consumption. Using the development tools, library elements can be configured to provide analog functions (from Analog blocks), such as Programmable Gain amplifiers, filters, ADCs with exceptionally low noise, input leakage and

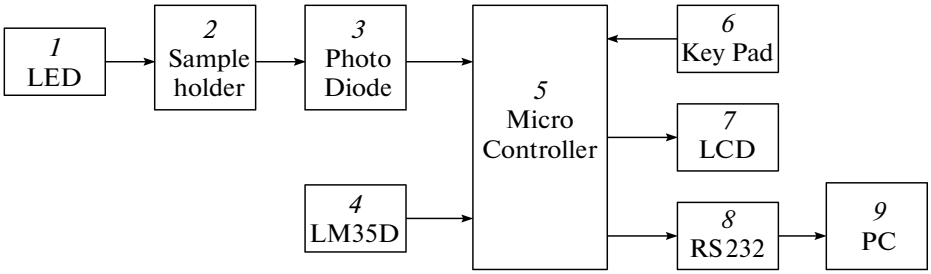

Fig. 1. Block diagram of the Implemented Bio-Medical Analyser.

voltage offset, DACs, comparators, etc. Digital functions such as timers, counters, PWMs, SPI and UARTs can be configured from the digital blocks of Digital systems.

Due to the flexibility of configuration (peripheral features), low power and other advantages of PSoC, it is selected for the design of bio-medical analyser to determine the Chloride concentration in serum. This instrument has been applied to measure the absorbance and hence the concentration of Chloride in serum samples, with the results comparable with a commercial clinical analyser.

2. INSTRUMENTATION

2.1. Design of the Implemented System

The Design Scheme of the Implemented system is shown in Fig. 1. Block *1* represents the Green LED, which acts as an illumination source with a dominant wavelength of 480 nm. Block *2* indicates the sample holder to hold the blank, standard, and sample solution tube with a diameter of 1 cm. Block *3* is a photodiode GASPG1124 used to detect the amount of transmitted intensity of the sample. The temperature sensor LM35D kept in Block *4* is a precision semiconductor temperature sensor giving an output of 10 mV per degree centigrade. It is capable of measuring temperature between 2°C and 100°C. The output signals are fed to the CY8C27443 microcontroller for signal amplification. After that the analog signals are converted into digital signals to compute absorbance and hence concentration of Chloride.

Block *5* represents the Microcontroller PSoC CY8C27443 [8]. It's analog system is composed of configurable blocks as Programmable Gain Amplifiers (PGA) up to 4, with gain up to 48*x* and Instrumentation amplifiers up to 2, with selectable gain up to 93*x*, Analog to Digital Converters up to 4, with 6 to 14 bit resolution, filters with 2, 4, 6 and 8 pole band pass, low pass and notch, Digital to Analog Converters with 6 to 9 bit resolution and Comparators up to 4, with 16 selectable thresholds. It has configurable Digital blocks of Timers (8 to 32 bit), Counters (8 to 32 bit), PWMs (8 to 32 bit), SPI slave and master up to 2, and 8 bit UART with selectable parity up to 2. It also contains 16 KB of flash memory, 256 bytes of SRAM, an 8 × 8 multiplier with 32-bit accumulator, power and sleep monitoring circuits, and hardware I^2C communications.

A keypad denoted by Block *6* is used to give input data to the microcontroller. Block *7* is LCD, a two row alphanumeric display, which is used to display Patient's ID number, measured data and the results. The data are transmitted from the microcontroller to the computer represented by Block *9* via RS232 denoted by Block *8*.

2.2. Description of the Microcontroller based system

The Microcontroller PSoC CY8C27443 and interfacing circuit of the developed instrument system, based on colorimetry principle is shown in Fig. 2. Here a colorimeter measures the intensity of light shining through a coloured solution compared to the intensity of light passing into the solution. A detector measures the transmittance T (% of light passing through) of the solution. This is mathematically converted to absorbance ($A = -\lg T$) and the absorbance is directly proportional to the concentration (Beer-Lambert law). The photo detection assembly is well insulated from outer light and the output signals are detected by photo to diode of GASPG1124.

The Analog blocks in CY8C27443 are configured as Programmable Gain Amplifier and ADC for the processing of analog signals (Fig. 3). The output signals from the photodiode sensor are fed to analog input P0(7) as shown in Fig. 2. The two stages of Programmable Gain Amplifier (PGA) are used for amplifying the light sensor's signals. The configured 12 bit dual ADC is used for converting analog signals to digital quantities.

The temperature sensor LM35D is connected to pin P0(3), the other input for the 12 bit dual ADC of microcontroller (Fig. 2). The Temperature Controller circuit is implemented with PWM (Pulse Width Modulation), Opto isolator (IC MOC3021), and TRIAC (BT136), to control and maintain the Temperature of sample at 37°C for incubation. The IC MOC3021 is used to isolate low voltage section from high voltage section.

The data lines of LCD are interfaced with Port 2 (Fig. 2) of Microcontroller to display the patient ID number, user information, and results. A digital block

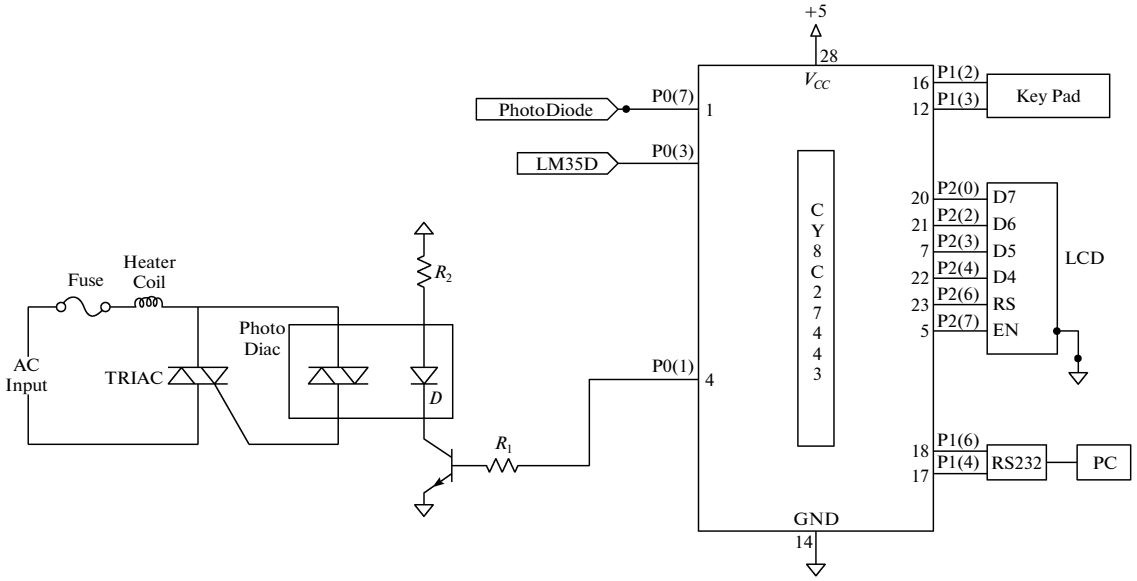

Fig. 2. Microcontroller and interfacing circuit.

in the Microcontroller is configured as UART for RS232 interface to transmit/receive data from the PC.

2.3. Software

In the present work, Software is developed in C, to configure analog and digital Blocks as peripherals, to initialize LCD, to start ADC, to read 12 bit data signals, to measure and maintain temperature, to measure voltages for blank, standard, and sample, to compute absorbance and concentration, to display the result in LCD, to get the data from the keyboard and to send data to PC for further processing. The implementation of the above tasks is given in the flowchart (Fig. 4).

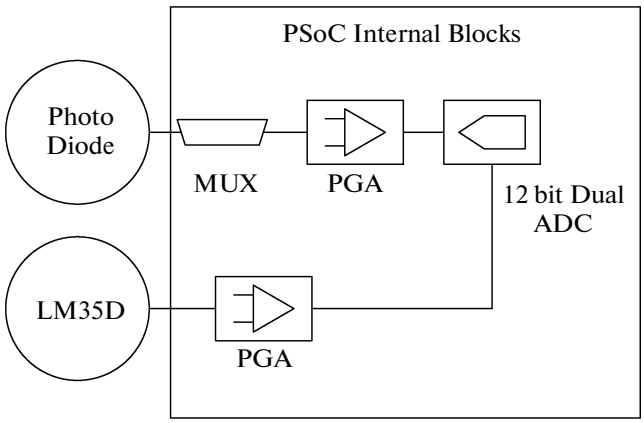

Fig. 3. Internal blocks of PSoC.

3. MATERIALS AND METHODS

Serum samples are collected from the patients and are separated from the blood clot soon after drawing. Grossly hemolyzed serum should not be used, as it will create falsely decreased values. The collected Serum samples are stored at room temperature. The colorimetry principle is used for the measurement of absorbance and concentration of Chloride electrolyte.

3.1. Principle

Chloride ions form a soluble, non-ionized compound, with mercuric ions and will displace thiocyanate ions from non-ionized mercuric thiocyanate. The released thiocyanate ions react with ferric ions to form a color complex that absorbs light at 480 nm. According to Beer-Lambert's law, the intensity of the color produced is directly proportional to the chloride concentration [9].

$$2Cl^- + Hg(SCN)_2 \longrightarrow HgCl_2 + 2SCN^-$$
$$3SCN^- + Fe^{+++} \longrightarrow 4Fe(SCN)^{+++}.$$

3.2. Reagents

Two reagents R1 and R2 are used in this measurement system. The reagent R1 consists of Mercuric thiocyanate (2 mmol/l), Ferric nitrate (20 mmol/l) and nitric acid (29 mmol/l). The reagent R2 is Chloride standard solution (NaCl 100 mmol/l). Blank solution is prepared by mixing 1 ml of reagent R1 with 10 μl of distilled water. To prepare standard, 1 ml of reagent R1 is added with 10 μl of standard (R2). For sample preparation, 1 ml of reagent R1 is added with 10 μl of Serum sample. The above solutions are thoroughly mixed and

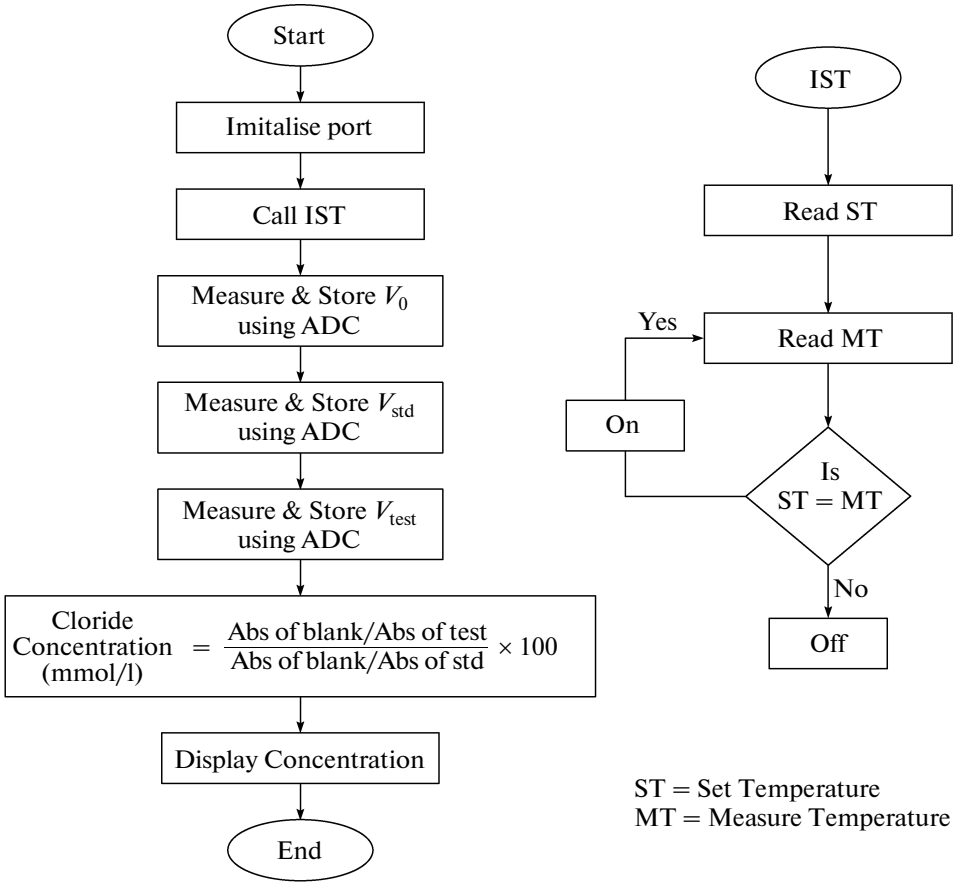

Fig. 4. Flowchart.

left for incubation for 5 min at 37°C, before the absorbance is measured at 480 nm.

3.3. Measurement

The designed Instrument is used to measure the blank, Standard and sample voltages and to compute absorbance and concentration. The test tube labeled blank is placed in a sample holder and the measured voltage is V_0. By holding the standard solution test tube in a sample holder, the voltage V_{std} is read by the microcontroller. The sample solution is placed in a sample holder and voltage measured as V_{test}. The concentration of Serum Chloride is computed using the formula:

$$\text{Concentration of Chloride ion} = \log(V_0/V_{test})/\log(V_o/V_{std}) \times 100,$$

where $\log(V_0/V_{test})$ – Absorbance of sample, $\log(V_0/V_{std})$ – Absorbance of standard, $\log(V_0/V_{std}) \times 100$ – Concentration of standard Chloride.

The concentrations of Serum Chloride measurements are made for 20 patient's sample using the developed instrument. The same samples are tested using the commercial clinical analyser. The absorbance of sample solution is measured and repeated for five times to check the reproducibility. Chloride is stable in serum for one day at room temperature, up to one week at refrigerator temperature and for three months frozen when stored as tightly capped.

4. RESULTS AND DISCUSSION

The absorption curve of the ferric thiocyanate complex formed is shown in Fig. 5. Since the curve has a rather broad peak, the color can be read over a wide spectral range. A wavelength of 480 nm is used in the developed analyser. However, any wavelength chosen between 450 nm and 480 nm gave satisfactory results. This is an advantage for laboratories using photometers with fixed filter systems.

The concentration of nitric acid (HNO_3) in the color reagent has a marked effect on color development. When HNO_3 is added to give a concentration above 0.25N, proteins in the specimen are precipitated. A nitric acid concentration of approximately 0.03N gave optimum conditions for the determination of physiologic serum chloride levels.

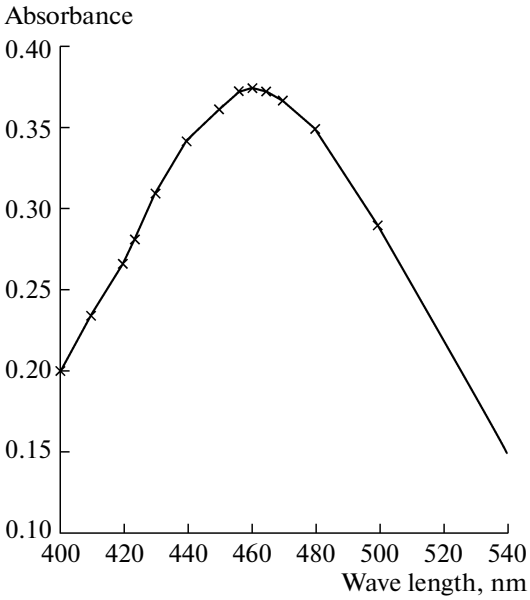

Fig. 5. Absorption Curve.

4.1. LINEARITY AND SENSITIVITY

To check the linearity of the designed instrument, different types of serum sample ranging between 90–150 mmol/l have been used. The absorbance increases with the Chloride concentration, and shows the linearity of the instrument up to 150 mmol/l. For the wavelength of 480 nm, the absorbance change of 0.001 typically corresponds to Chloride concentration of 0.25 mmol/l, which gave the sensitivity of the analyzer.

4.2. Precision

Run-to-run precision is obtained by assaying commercial human control serum Accutrol™ Normal (Sigma) gave the results of Mean 104.4, S.D. 4.0, C.V (%) 3.3 and elevated results for a period of thirty (30) days produced the results of Mean 91.7, S.D. 3.8, C.V (%) 4.1. Within Run precision is obtained by assaying control Normal serum twenty (20) times having Mean 86.9 (104.4), S.D. 1.3 (4.0), C.V (%) 1.0 (3.3).

Statistical Analysis for the data arrived using Developed Instrument and the Clinical Analyzer

Variables	Developed Instrument	Clinical Analyser
Standard Deviation	5.07	5.40
Standard Error of Standard Deviation	0.82	0.85
Mean value	100.84	100.5
Standard Error of Mean	1.16	1.20
Median value	101	100.5
Standard Error of Median	1.45	1.51
Mean Deviation	4.37	4.5
Coefficient of variation	5.02	5.38
Standard Error of Coefficient of variation	0.81	0.85

4.3. Recovery

To measure the recovery of Chloride, pooled serum is diluted approximately two-fold with distilled water. To 0.5 ml of diluted Serum, various amounts of 0.1 N Sodium Chloride (NaCl) solutions are added to bring the total Chloride concentration within normal range. Using the developed Instrument the recovery values of added Chloride ranged from 99.4% to 97.5% with an average recovery of 98.44%, this indicates the suitability of the designed instrument for biomedical tests.

4.4. Interferences

No significant interferences are observed with the exception of Bromide and Fluoride and they can cause falsely elevated chloride values. Bromide at 10 and 20 mmol/l produced positive bias of 20 and 42 mmol/l, respectively, for chloride measurements. However, the Assays can be directly performed on raw biological samples i.e., in the presence of lipid, protein and minerals such as magnesium, iron and zinc without any pretreatment. Lipemic and/or icteric sera do not interfere in the reaction.

4.5. Linear regression analysis

To examine the accuracy of the developed Instrument, Linear regression is performed and tested for the results obtained using designed Bio-Medical analyser and the other clinical analyser. All the samples fall into the linear range, and there is sufficient precision in the data to continue with the linearity study. There is no outlier in the data sets. The strength of the linear association between two variables is quantified by the correlation coefficient. The Regression line equation arrived is,

$$y = 0.95x + 4.6,$$

the value of slope $m = 0.95$ (close to ideality) and the intercept $c = 4.6$ indicated that the developed instrumentation system is well suited to determine the Chloride in serum. The correlation coefficient $R = 0.95$ ($n = 20$), shows that the present method is well correlated with the clinical analyzer.

4.6. Statistical Analysis

The performance of the PSoC based Bio-Medical Analyser using colorimetry principle is investigated by comparing its results with the results obtained by other clinical analyser. There is no obvious difference between the results obtained by two Instruments. The Table represents the statistical reports for the results arrived using the developed Instrument and the Com-

mercial clinical Analyzer. It is noted that the values of Standard Deviation, Standard Error of Standard Deviation, Mean Value, Standard Error of Mean, Median value, Standard Error of Mean, Mean Deviation, Coefficient of variation and Standard Error of Coefficient of variation for the designed analyzer is close to the clinical analyzer.

5. CONCLUSION

A rapid, precise, and inexpensive Programmable System on Chip based biomedical analyser has been fabricated to measure the Chloride concentration in serum. The PSoC design increases flexibility of configuration of peripherals, lower part count, and provides in system performance improvement, design security, and field upgrades. The developed Instrument is sensitive and suitable for determining Chloride concentration in serum and it is a user friendly one, as no special training is required to use it. Usual spectrophotometers have optical lenses and filter, which makes the system clumsy, and difficult to use, as incandescent lamps are used as light sources, it generates lot of heat and consumes more power. All these problems are rectified in this PSoC based system and it can be used as an alternative to the commercial clinical Analyser. The instrument can be used to measure the Chloride concentration of other biological samples like serum, blood, plasma and Cerebro Spinal Fluid by changing reagents. Since, the method of measuring Chloride in Serum is based on colorimetry principle, any of the branded reagents, which are available in local pharmaceuticals, can be used. Comparing the present results on analytical performance with that of other clinical analyzer, the developed Instrument gives compatible analytical results in all approaches.

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