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Research Article

COLORIMETRIC DETERMINATION OF CHLORIDE ION IN ORAL REHYDRATION SALTS USING MICROCONTROLLER P89C51RD2

P. NEELAMEGAM[a], R. VASUMATHI[b]

[a]Department of Electronic and Instrumentation Engineering, SASTRA University, Tanjore, Tamilnadu, India, [b] PG and Research Department of Physics, AVVM Sri Pushpam College, Poondi, Tanjore, Tamilnadu, India -613503

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ABSTRACT

A simple, precise and inexpensive Microcontroller P89C51RD2 based Instrument set up has been designed, developed and validated to find the Concentration of Chloride ion concentration in Oral Rehydration Salts (ORS). The present method is based on the quantitative reduction of thiocyanate ions by Chloride ions by means of Colorimetry principle. Hardware and software are developed for implementing the absorbance measurement and to calculate the Chloride concentration. There is statistically no significant difference between the results obtained with the developed Instrument and the Chloride Ion Selective Electrode (ISE). It is found that the Concentration of Chloride is well within that of World Health Organisation (WHO) certified values. The Correlation coefficient r= 0.99 (n=6) is obtained between the developed instrument and the Ion Selective Electrode method. The sensitivity and the precision are high enough to determine the concentration of Chloride ion without any significant interference. The developed Instrument could be routinely used for the determination of Chloride ion in bulk drugs.

Keywords: Chloride, Dehydration, ORS, drugs, Microcontroller P89C51RD2.

INTRODUCTION

Dehydration is one of the most common problems of infancy and early childhood. They are affected by dehydration during prolonged vomiting, diarrhea and in cases of some diuretic medications. Because vital body fluids and minerals are lost during the above illness. These fluids and minerals contain electrolytes. They must be replaced quickly in order to prevent dehydration. Dehydration affects the body's electrolyte balance. Electrolytes give your body the electrical support necessary[1] for your heart, muscles and nervous system to work properly. When electrolytes are out of balance many organs cannot function properly, and lead to life threatening condition. Chloride is the main extracellular anion. With Sodium it accounts for most of the osmotic pressure of plasma and contributes to maintenance of electroneutrality. Chloride ions are ingested with food and absorbed in the intestinal track. It absorbs minerals and vitamin B12, enables normal muscle contraction[2], relaxation and nerve impulse transmission. Decreased levels of Chloride (Hypochloremia) occur with any disorder. It occurs with prolonged vomiting or gastric suction, chronic diarrhea, emphysema or other chronic lung disease. In order to Rehydrate, Doctors recommend ORS therapy immediately.

The modern era of oral replacement of fluid and electrolytes in pediatric diarrheas had its beginnings in reports from Baltimore using Sodium, Potassium, Chloride and lactate to replace losses in infantile diarrheas in the 1950's with subsequent addition of sugar to spare protein[3]. The science of ORS is advanced when Phillips and colleagues determined the composition of fluid lost in diarrhea[4, 5]. Oral Rehydration Therapy is proposed as a viable alternative for cholera in areas of the world with short supplies of intra-venous fluids and needles forcing clinicians to deliver oral solutions to those with cholera. This reduced mortality rates to only 3% compared to 30% of those treated in other camps with intravenous fluids. Based on this evidence, WHO and UNICEF recommended a single standard ORS formula for all ages. There are lot of brands of ORS therapy is available in local pharmacy. The accurate analysis of minerals in drugs is very important in medications.

Over the years, numerous analytical methods for Chloride in a variety of samples have been developed, such as Ion Chromatography[6, 7], Spectroscopy[8], Ion Selective Electrode (ISE) method[9], Turbidimetric method[10], and so on. Ion Chromatography is an accurate laboratory method, but cannot produce real time data needed for rapid decisions in the field. Spectroscopy is one of the tedious methods. ISEs are accurate when recently calibrated, but are sensitive to drift, fouling and are not ideal for field monitoring. Turbidimetric is popular and regarded relatively reliable for quantification of Chloride. But it suffered from long experimental time, lower sensitivity and complexity. Hence, there is a need for an analytical tool to quantitate the Chloride ion concentration in an inexpensive way.

Moreover, low cost but powerful Microcontrollers are used in many types of portable and hand held Instruments. These electronic circuits can be programmed with the measurement algorithms and the calibration function needed for full instrument operation with the numeric result appearing on a screen at the end of the measurement process. Their internal memory, versatility of programming, possibility of multiple interfaces and low power function make it possible to design the system with high accuracy and high speed response.

In the present method, a Microcontroller P89C51RD2 based Instrument set up has been designed and developed with colorimetric principle to measure the absorbance and hence to determine the Concentration of Chloride in ORS samples, with the results comparable to the other analytical technique (Ion Selective Electrode method).

INSTRUMENTAL

Design scheme of the implemented system

The functional block diagram of Microcontroller based Instrument set up is displayed with a clear depiction of its different blocks is shown in figure 1.

LEDs have been widely studied and applied to this kind of Instruments as monochromatic light source and solid state photo detectors are used for absorbance measurements. The Block A in Figure 1 consists of Green LED with a wavelength of 480nm to optically illuminate the sample solution to measure the Chloride concentration. Block B represents sample holder, which is used to hold the Blank, Standard and Sample solutions. A photo diode (GASPG 1124) is used to detect the amount of light falling on the sample, which acts as a photo detector represented, by a Block C. This ideal detector has the characteristics of long term stability, Short response and high sensitivity to allow the detection of low level radiant energy[11]. Block D consists of OP-AMP, which acts as an instrumentation amplifier that amplifies the weak signal. The output of instrumentation amplifier is connected to the 12 bit A/D converter, which is in Block E. The Microcontroller P89C51RD2, which is kept in Block F, reads data from ADC for processing. Block G represents the keypad to give data for processing and to compute the Chloride concentration. Block H represents LCD, which is used to display the output of Microcontroller (Chloride concentration in ORS samples)[12].

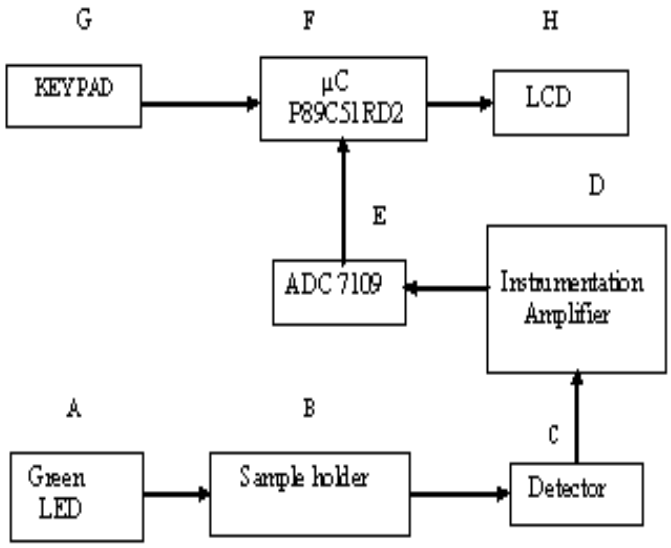

Fig. 1: Design of the implemented system

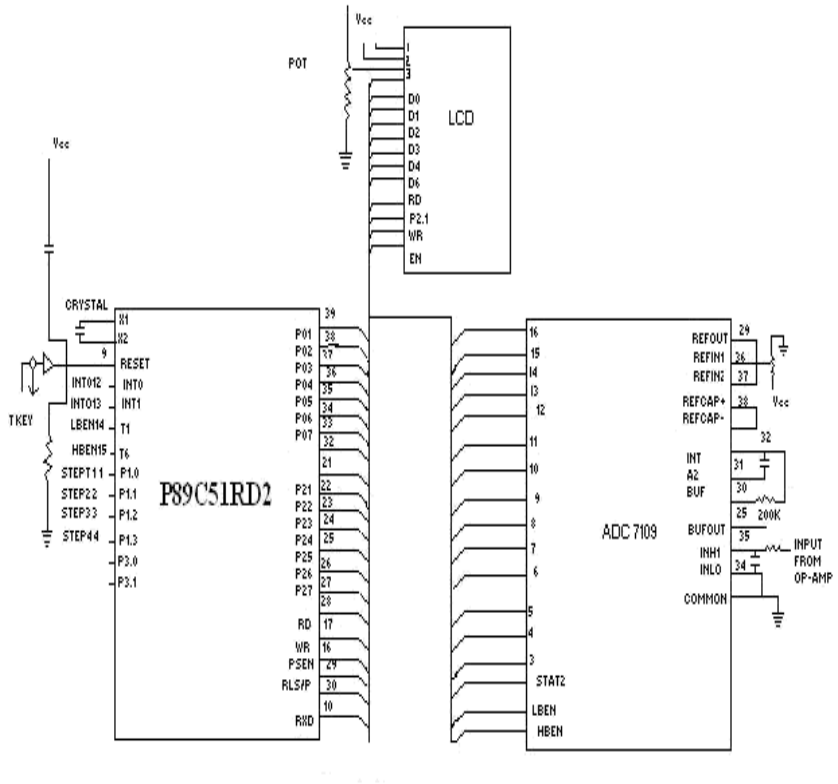

Fig. 2: Microcontroller and interfacing circuit

Description of the microcontroller based system

The Microcontroller and interfacing circuit is shown in Figure 2, which measures the concentration of Chloride ion based on colorimetry principle.

According to this principle, the absorbance of light (which passes through the colored solution) is proportional to the concentration of the ion. The Instrument system is incorporated with the Green LED source. A photodiode GASPG1124 is used as a sensor to detect the amount of light and convert into current. The output current of

photo detector is converted into voltage by an operational amplifier. The output of OP-AMP is connected to the pin 35 of ADC 7109 through 1 MΩ resistance to convert analog to digital value. The lower and higher bytes of ADC 7109 are interfaced with port 0 of Microcontroller. The pin 29 of ADC 7109 generates the internal reference voltage. The Microcontroller P89C51RD2 contains a non-volatile 64KB flash program memory and 1 K bytes of RAM. It has four 8 bit I/O ports, three 16-bit timer/counters, a multi-source four-priority-level, nested interrupt structure, an enhanced UART and on-chip oscillator and timing circuits[13]. A reset switch is provided at pin 9, so that the program can be executed from 0000 after the power is switched on. A tap from preset is given to pin 36 of ADC 7109, the reference input pin. By adjusting this preset it is possible to get a full scale, which means that inputs between –4 and +4 can be converted. A two row Alphanumeric LCD is interfaced to port 0 to display the measured concentration of Chloride.

Software

Fully dedicated Software for the data acquisition and computing the Chloride concentration in different ORS samples is developed in C and assembly language and linked to the application program. The structure of the software is elaborated as flowchart in Figure 3. Digital conversion), to check EOC (End of Conversion) to read lower byte enabling LBEN signal, to read higher byte enabling HBEN signal, to measure the readings for blank, standard, and sample, to compute absorbance and Chloride concentration, to display the result in the LCD and to get data from the keyboard.

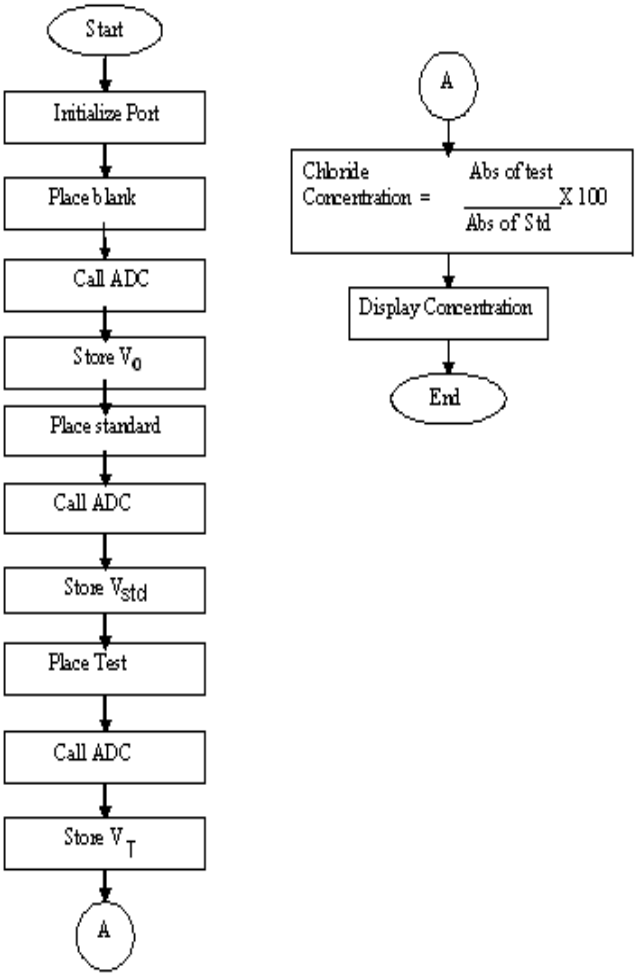

Fig. 3: Flow chart

Software for the implemented system is written to initialize LCD, to start ADC (Analog to It is observed that the minimum time for data acquisition and computation is 0.6 s, taking into account the implemented algorithm.

MATERIALS AND METHODS

Oral Rehydration Salts of six different brands are collected from local pharmaceuticals in Tanjore, Tamil Nadu, South India. The samples of ORS are dissolved in 1L of deionized water without any precipitation. Since the samples are colorless, there is no need to add any chemical reagent to remove the color. The colorimetry principle is used for the measurement of absorbance and concentration of Chloride electrolyte

Principle

The Chloride reagent is based on the method of Zall, Fisher and Garner[14]. When Chloride is mixed with a solution of undissociated mercuric thiocyanate, the Chloride preferentially combines with the mercury to form mercuric Chloride. The thiocyanate that is released combines with ferric ions present in the reagent to form ferric thiocyanate, which can be measured spectrophotometrically. The procedure is very sensitive and needs to be reduced for routine

clinical applications by the addition of mercuric nitrate. The mercuric nitrate binds a fixed amount of Chloride ion and therefore makes them unavailable for reaction with mercuric thiocyanate. Only the Chloride present in excess of that bound by the mercury from mercuric nitrate is reacted with mercuric thiocyanate and produced the red ferric thiocyanate.

$$2Cl^- + Hg(SCN)_2 \longrightarrow HgCl_2 + 2SCN^-$$

$$3SCN^- + Fe^{+++} \longrightarrow 4Fe(SCN)^{+++}$$

Reagents

In this measurement system, two reagents R1 and R2 are used. The reagent R1 consists of Mercuric thiocyanate (2mmol/l), Ferric Nitrate (20mmol/l) and Nitric acid (29mmol/l). The reagent R2 is Chloride standard solution (NaCl of 100 mmol/l). All the solutions are prepared in a well cleaned dried test tube of same diameter. Blank solution is prepared by mixing 1 ml of reagent R1 with 10 µl of distilled water. For the preparation of standard solution, 1 ml of reagent R1 is added with 10 µl of standard (R2). The sample is prepared by adding 1 ml of reagent R1 with 10 µl of prepared ORS sample.

Measurement

The designed Instrument is used to measure the blank, Standard and sample voltages and to compute absorbance and concentration of Chloride. The test tube labeled blank is placed in a sample holder and the measured voltage is V_o. 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_t. The concentration of Chloride is computed using the formula,

Concentration of Chloride ion = $\log (V_o/V_t) / \log (V_o/V_{std}) \times 100$

Where, $\log (V_o/V_t)$ = Absorbance of sample, $\log (V_o/V_{std})$ =Absorbance of standard

100 = Concentration of standard Chloride.

The concentration of Chloride for six different brands of ORS samples is made using the developed instrument. The same samples are tested using the Chloride Ion Selective Electrode (ISE). The absorbance of sample solution is measured and repeated for five times to check the reproducibility.

RESULTS AND DISCUSSION

This study reveals the development of Microcontroller based Instrument set up to measure the concentration by colorimetry method in ORS samples. The Table 1 shows the readings for blank and standard of Chloride reagent.

Table 1: Voltage of blank and standard

S.No	Blank V_o (mV)	Standard (mV)
1	0.22	0.32

The measured readings of absorbance and concentration of Chloride in different ORS samples are given in Table 2.

The Table 3 gives the concentration of Chloride measured using the developed instrument and ISE method in different ORS samples.

Table 2: Absorbance and concentration of chloride in oral rehydration powder

S.No	Types of sample	Sample (V_T)	Absorption	Chloride concentration m mol/l
1	ORS 1	0.175	0.099	61.87
2	ORS 2	0.174	0.101	63.12
3	ORS 3	0.174	0.101	63.12
4	ORS 4	0.173	0.104	64
5	ORS 5	0.173	0.104	64
6	ORS 5	0.175	0.099	61.8

Table 3: Comparison of results obtained using the developed instrument and chloride ISE

Sample id	Chloride concentration (m mol/l)	
	Developed instrument	Chloride ISE
ORS 1	61.87	62
ORS 2	63.12	63
ORS 3	63.12	63
ORS 4	64	64
ORS 5	64	64
ORS 6	61.87	63

The concentration of Chloride is varied from (61.87-64 mmol/l). The Certified Concentration of Chloride in ORS is 65mmol/l[15]. It is found that the range is well within the safe limits and also it is observed that there is no significant difference between the concentration values of different samples, which depicts that they are prepared by following the ORS formula given by W.H.O. The people who have suffered by dehydration having symptoms like dry mouth, loss of body weight greater than 10%, extreme thirst, sunken eyes, no tears when crying, decreased urination, fussiness, weakness, skin that stayed compressed and pinched should have the habit of in taking ORS to avoid some chronic condition. The dosage of ORS depends on age and severity of dehydration. Generally, for infants and children 1-2 litres over a period of 24 hours. For Adults it differs from 2-4 litres over a period of 24 hours.

Linearity and sensitivity

As the absorbance increases the Chloride concentration also increases, which shows the good fit of colorimetry principle. To check the linearity of the developed instrument set up various samples having different Chloride concentration have been measured at the wavelength of 480nm. The Absorbance change of 0.1 typically corresponds to Chloride concentration of 42 m mol/l of the sample solution, which gave the sensitivity of the developed Instrument.

Recovery

To test the feasibility of the procedure and instrument, the recovery of the developed Instrument is studied by the standard addition method. Because the samples may produce some interference during the color development. To 0.5ml of ORS sample, various amounts of 0.1N Sodium Chloride (NaCl) solutions are added to bring the total Chloride concentration within normal range. The Table 4 shows the recovery values of added Chloride ranged from 98.13% to 99.15% with an average recovery of 98.42%, which indicates the suitability of the designed instrument for bulk drugs and assay tests.

Table 4: Recovery of added chloride from ORS chloride (mmol/l)

S.No	In ORS sample	Added	Total content	Total determined	Difference	Recover %
1.	62	40	102	100.5	1.5	98.52
2.	62	45	107	105	2.0	98.13
3.	63	50	113	111	2.0	98.23
4.	63	55	118	117	1.0	99.15
5.	63	45	108	107	1.0	98.07

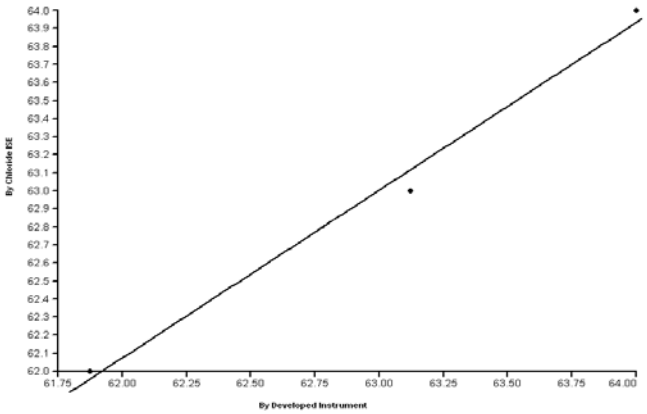

Fig. 4: Linear regression between the developed instrument and the chloride ISE

Linear regression analysis

Linear regression is plotted for the results obtained using designed Instrument and the ISE method which is shown in Figure 4.

Linear regression analysis attempts to model the relationship between two variables by fitting a linear equation that closely fits a collection of data points. 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 = 0.92X+4.43, the value of slope and the intercept (closes to ideality) indicated that the developed instrumentation system is well suited to determine the Chloride in ORS samples. The Correlation Coefficient R=0.99 (n=6), shows that the designed Instrument is well correlated with the Chloride ISE method.

Statistical analysis

The Statistical reports of Chloride electrolyte in different brands of ORS samples is given in Table 5.

Table 5: Statistical analysis for the data arrived using developed instrument and the ise

Variables	Developed instrument	Chloride ISE
Mean	62.99	63.16
Std err of mean	0.356	0.280
Median	63.12	63
Std err of median	0.445	0.350
S.D	0.873	0.687
Std err of S.D	0.252	0.187
Mean deviation	0.751	0.555
Coefficient of variation	1.38	1.08
S.E of Coeff of Variation	0.400	0.314

It is noted from the table that the mean value 63.30 and median value 63.43 are also within the safe limits. The data reported in this study refers that the concentration levels of electrolytes are fairly within the recommended levels, which confirms the pharmaceutical integrity. There is no considerable difference between the results obtained (Mean value, Standard Error of Mean value, Median value, Standard Error of Median value, Standard Deviation, Standard Error of Standard Deviation, Mean Deviation, Coefficient of Variation, Standard Error of Coefficient of Variation) using the designed Instrument and the ISE method which corroborates the validity of the implemented Instrument set up. The accuracy of the Microcontroller based Instrument is confirmed by the less residual between the two methods.

CONCLUSION

The instrument fabricated has been used to measure the concentration of Chloride electrolyte, which uses the colorimetry principle. The implemented system requires simple and low cost electronics component. Normal 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 developed Instrument and it does not require any programming expertise. The measurement system is tested with different samples to check the reproducibility. The application of this Instrument for the determination of Chloride in ORS is very important, due to the high levels of production in the world where these therapies contain considerable amounts of this ion. The same instrument can be used to measure the other analytes like Sodium and Potassium in bulk drugs by changing the light sources.

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