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Measurement of Electrolyte Levels in Vegetable Samples using Ion Selective Electrodes (ISE) Technique

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ABSTRACT

Ion Selective Electrodes (ISE) are ideal measurement tools because of their ability to monitor selectively the activity of ions in solution both continuously and nondestructively. This work presents the development of microcontroller based Instrumentation system to measure the sodium-potassium and concentration in vegetables by using an array of ISE. The values measured by using the developed instrument are compared with the other method. A line of comparison is drawn between the two methods, which prove the accuracy of the developed instrument.

Keywords: Atmega 32, Sodium ISE, Potassium ISE, Microcontroller based system, Vegetable samples.

INTRODUCTION

Electrolyte disturbances can affect all systems of the body. For example, Hyperkalemia is the plasma potassium more than 5.5 mEq per liter. It is so common that it happens in 10% of hospitalized patients. More severe hyperkalemia is seen in around 1%. This causes even the death of the patient.

The potassium is a predominantly intracellular ion. If it is not taken inside the cell it can increase potassium level though reduced Renal Potassium excretion is another cause. Sometimes some drugs affecting the Renin-angiotensin-aldosterone axis can cause hyperkalemia. Renal failure patients cannot excrete potassium and hence their intake of potassium must be limited. Low levels of potassium are called hypokalemia. This can cause muscle weakness and even intermittent paralysis of muscles i.e. in between the patient is normal [1]. Even sometimes increased potassium can cause paralysis i.e. Hyperkalemia paralysis but it is rare [2]. Sometimes severe degrees of hyperkalemia can be silent and can cause stopping of the heart in a diastole and lead to death. Serum potassium >7 mmol/l is a medical emergency and is associated with ECG changes. Muscle weakness is often the only symptom unless it is associated with metabolic disease. It is seen in muscles closer to the shoulder and hip region and the bulbar muscles are not affected sometimes this needs dialysis to reduce the potassium levels. In nerve conduction studies there are reduced motor amplitudes. EMG may not be recordable.

There is a lot of methods available to measure the concentration of electrolytes in biological samples, environmental samples, and dietary samples. The real-time analysis method requires an inexpensive, compact and automated system for measuring the concentration of analytic in samples [3]. The potentiometric technique is one of the analytical methods, which uses ISE, the best tool to measure electrolytes like sodium, potassium, chloride, calcium, lithium, and fluoride [4]. In that Sodium and potassium play a vital role to regulate body fluid balance. Potassium is the principal positively charged ion in the fluid inside the cells, while sodium is the principal Cation in the fluid outside of cells. Potassium concentration is about 30 times higher inside than outside cells, while sodium concentrations are more than 10 times lower inside than outside cells. The concentration difference between Sodium and potassium produces cell membrane potential, which is critical for nerve impulse transmission, muscle contractions, and heart functions. Dietary intake of sodium and potassium necessitate avoiding mineral deficiency, which influences some chronic disease. Most foods such as fruits, vegetables, meat, milk, and cheese are good sources of sodium and potassium.

Vegetables are a basic and important food for human beings because it comprises the important nutrients and micronutrients [5]. Vegetable and banana stem juice extract is excellent.

Sources of calcium ions, both qualitatively and quantitatively. In processed banana stem juice powder, essential elements are usually added in order to satisfy nutritional requirements. It has high nutritional value and is also considered as a complete food. Calcium ions (Ca^{2+}) in vegetables and banana stem juice extract are more easily absorbed by the intestine than the calcium ions from the vegetables and cereals. Calcium ions are necessary for the growth and development of young children. Calcium is an essential mineral required for the diverse physiological and biochemical functions in the human body [6].

Routine intake of the required amount of calcium ions is essential to keep healthier the function of a number of physiological processes like intercellular communication, muscular contraction, secretion, and blood coagulation. Calcium ions are required in the homeostatic regulation of inorganic compounds. Institute of Medicine recommended 800 mg of calcium ions per day for children between 4 and 8 years and 1300 mg calcium ion for children of 9-18 years old, 1000 mg per day between 19 and 50 years including pregnancy and lactation period and 1200 mg per day for citizens older than 51 [7].

Recent times many methods used for the determination of sodium potassium and calcium ions, which involve atomic absorption spectrometry, potentiometric with ion-selective electrodes, EDTA titration, and spectrophotometry with a variety of reagents [8-17]. Among these methods, an ion selective electrode is well-suited because of its simplicity and low cost effective.

In this work, a real-time microcontroller based system is implemented using Ion Selective Electrode to monitor the concentration of Sodium and Potassium in vegetables. The analytical performance of the implemented system is compared with other methods.

SYSTEM INTERFACE

The System Interface circuit is shown in Figure 1. The output of integrated assembly of Sodium and Potassium ISE is connected to inverting and the non-inverting input of Instrumentation Amplifier CA3140. Two separate amplifiers are used for two sensors [18]. The output of Amplifier is connected to ADC of microcontroller ATmega32 through PAO and PA1. The Microcontroller ATmega 32 is a low power, high-performance 8-bit microcontroller. It has 32 K bytes of flash and 2 K byte of RAM. Stepper motor is interfaced with PB0, PB1, PB2, and PB3 of the microcontroller for moving the electrode sensor probe up and down for dipping into the sample during measurements. By proper commands, the stepper motor is rotated clockwise and anticlockwise and the rotating motion is converted into a linear motion for moving the electrode probe. A position sensor (IR) is used to identify the home position for the probe. The dc pump motor is interfaced with PB4 of the controller through switching transistor and hex buffer 7407. The motor is activated whenever the electrode moves up or down [19]. While the electrode is moving up the motor pumps the calibrator1 into the electrode and while moving down it pumps the calibrator1 out. Keys are interfaced with PC4, PC5, and PC6. The display is connected to port D of the microcontroller to display the results.

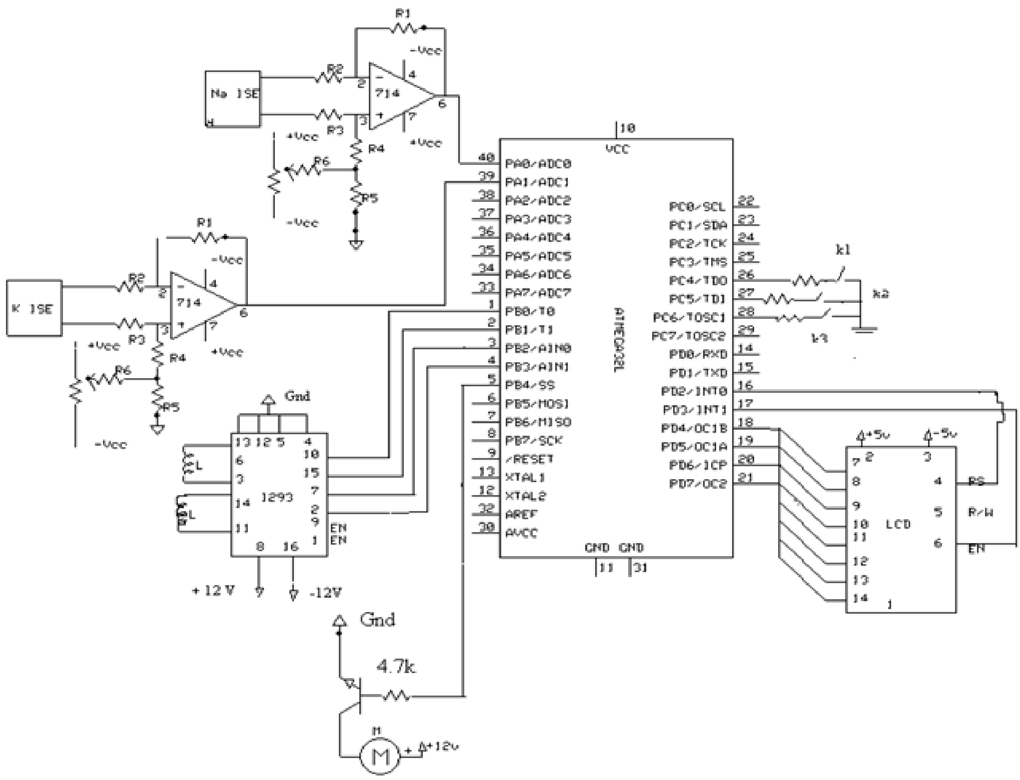

Figure 1: System interfacing with microcontroller ATmega 32.

MATERIALS AND METHODS

The potentiometric membrane composition is taken from the literature [20,21]. For sodium membrane preparation, sodium ionophore VI 6.3% (Sigma), Adipate 56.3% (Fluka), and PVC 37.1% (Aldrich) are used. For Potassium membrane preparation Valinomycin 2.14% (Sigma), Adipate 51.1% (Fluka), PVC 30.9% (Aldrich) and Nitrobenzene 16.3% are used. The prepared membrane solution is coated on PVC tubing in such a way, sodium is on first groove and Potassium is on the second groove. Ag/AgCl is used as a reference electrode. The vegetables are purchased from the market and stored in plastic bags after washing. During the testing time, their extracts were taken and filled in plastic bottles. The instrument needs two-point calibrations. It uses two calibrator solution calibrator 1 and calibrator 2 with low and high concentrations of sodium and potassium is prepared by dissolving appropriate quantities of sodium chloride and potassium Chloride in double distilled water. The electrode is always maintained in calibrator 1 solution. So that it is conditioned and ready for measurement. The pump motor is used to pump in and out the calibrator 1 solution into and out of the electrode. The calibrator 2 is taken in a sample cup during calibration. First, the instrument reads the potential at calibrator 1 and then moves down to read calibrator 2. The slope of this calibration is calculated using the Nernst equation. Then this calibrated value of the slope is used to find out the unknown concentration of the sample. The sample concentration of sodium and potassium is calculated from the relation.

$$\frac{Ex - Es1}{Es1 - Es2}\left(Log\, as1 / as2\right) = Log ax / as1$$

Where Ex, $Es1$, and $Es2$ are the potentials of the sensor at the sample, cal 1 and cal 2 solutions respectively. Similarly $as1$, $as2$, and ax are activities (concentrations in mmol/L).

SOFTWARE

Software is developed in assembly language and C language to read data from keyboard, to start A/D conversion, to check EOC, to read higher and lower byte of data, to take readings for standard1, standard2 and sample, to activate the stepper motor and dc motor, to process data and to display the results in the LCD display (Figure 2).

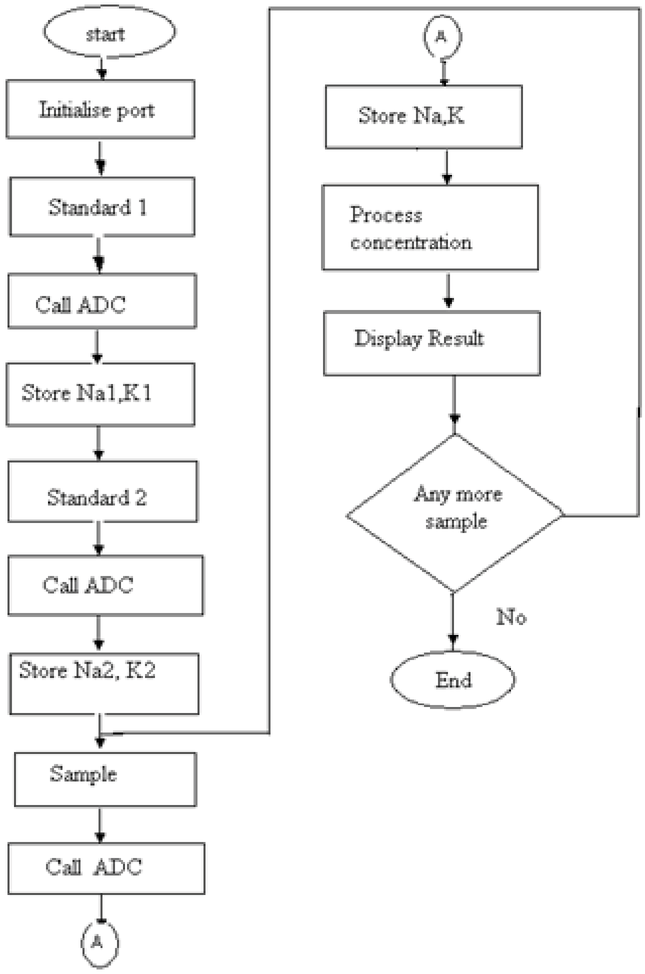

Figure 2: Flowchart.

RESULTS AND DISCUSSION

Tables 1 and 2 gives the concentration of sodium and potassium in vegetables measured by using ISE and Flame Photometry method respectively. The results show that the values obtained by the ISE method are close to the other method. It is observed that the reproducibility of the implemented system is good compared to that of another method. The precision between the two methods is estimated statistically by performing the F-test. The basic assumption or null hypothesis is that there is no significant difference between the variances of the two sets of data at 5% level with d.f of 4. Table 3 gives the mean and S.D of two methods in which there is no obvious difference between the developed instrument and the flame photometry principle. The value of the correlation coefficient between the implemented system and Flame photometry system is quite satisfactory. The maximum and minimum value of sodium and potassium concentration is also well matched between the two methods. Figure 3 shows the comparison of the sodium concentration obtained by using ISE and Flame photometry, which gives the slope of 1.029 and the intercept of -0.62. Figure 4 presents the line drawn for potassium concentration whose slope of 1.158 and intercept of -0.1. In both graphs, the line shows the linearity, which confirms the suitability of the developed instrument with the other method. It is noted from the results the concentration of sodium is high in beetroot and low in potato. The concentration of Potassium is high in Carrot and comparatively low in beetroot. Generally, dietary intake of sodium should not exceed 4000 mg and potassium within 4700 mg/day. People who suffer from hypernatremia, hyponatremia and hyperkalemia, hypokalemia may have the vegetables accordingly.

Table 1: Concentration of sodium in vegetables.

Sample	The concentration of Sodium (mmol/ ltr)	
	By developed system	By Flame Photometry
Carrot	22 ± 1	25 ± 2
Potato	6	7 ± 1
Beans	18 ± 2	15 ± 4
Tomato	7.9 ± 0.2	6 ± 2
Beetroot	127	130 ± 2

Table 2: Concentration of potassium in vegetables.

Sample	The concentration of Sodium (mmol/ ltr)	
	By developed system	By Flame Photometry
Carrot	67 ± 2	70 ± 2
Potato	47 ± 1	44 ± 4
Beans	37 ± 1	36 ± 2
Tomato	57.5	60 ± 3
Beetroot	32	30 ± 3

Table 3: Statistical results obtained between the implemented systems versus flame photometry method.

Variable	Mean		S.D	
	Sodium	Potassium	Sodium	Potassium
Concentration by ISE	180.9	240.5	36.18	48.1
Flame Photometry	183	240	36.6	48
Residual	2.1	0.5	0.42	0.1
R	0.99	0.99	-	-

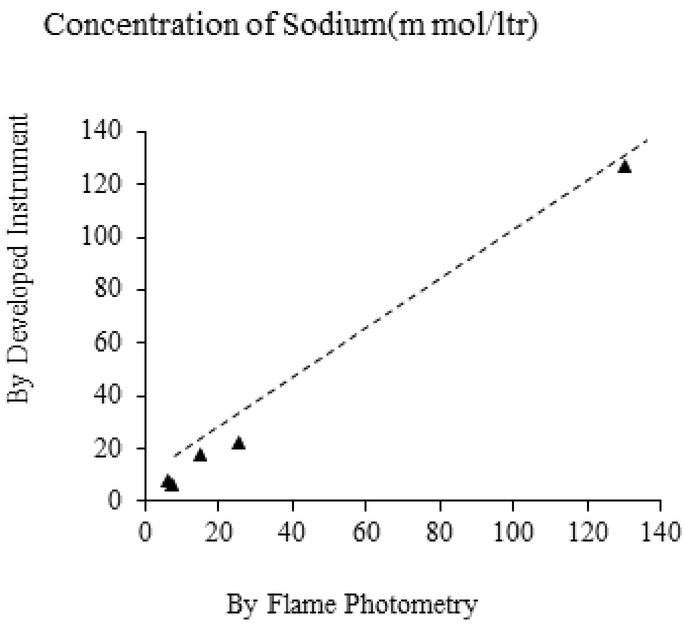

Figure 3: Comparison line between the two methods for sodium concentration.

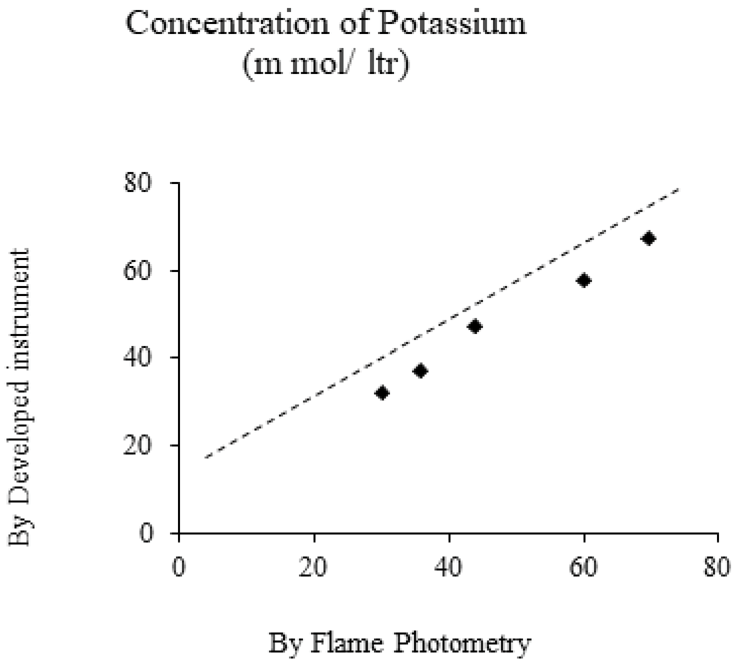

Figure 4: Comparison line between the two methods for potassium concentration.

CONCLUSION

A microcontroller Atmega 32 based instrumentation system to measure the concentration of sodium and Potassium is developed and its analytical performance is compared with the Flame Photometry system. The comparison study showed a good correlation between the two methods. It is easy to use and maintenance free direct potentiometric analyzer. The implemented system can be used to measure the analyte activities of Sodium and Potassium in various food samples. This will be useful to find alternate options of optimal electrolyte content in food stuff like fruits and vegetables.

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