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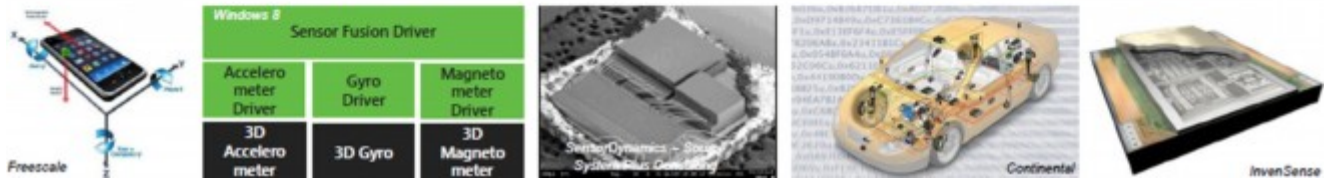
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Measurement and Analysis of Sodium in Vegetables Using ATmega16 Microcontroller Based Spectrophotometer

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Abstract: ATmega16 microcontroller based portable and inexpensive spectrophotometric system is developed to determine sodium ion concentration in vegetable samples. Light Emitting Diode of wave length 530 nm as light source and photodiode as light detector are used for measurement. The developed instrument is used to measure the absorbance, calculate the concentration of sodium in the sample and display the result. This method is based on the reaction between sodium ion with reagent and form a brownish colored complex which gives the maximum absorbance at 530 nm of wavelength. Statistical analyzes are carried out to check the performance of the developed instrument. Linear regression analysis reveals the excellent correlation with correlation coefficient (r) of 0.99864. The developed system shows the good performance characteristics and the results are in good agreement with 98 % of confidence level in comparison with current clinical spectrophotometric method.

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Keywords: Microcontroller, LED, Spectrophotometer, Sodium, Vegetables sample.

1. Introduction

Sodium is the one of the primary electrolytes which involved in the regulation of water content in the body and help to preserve the electrolyte balance inside and outside of cells [1]. It is essential to maintain the correct balance of the body fluids and it is important in nerves and muscles to work. Eating vegetable is one of the tried and true recommendations for a healthy diet. Although all

vegetables likely contribute to benefit, green leafy vegetables such as lettuce, spinach, Swiss chard, and mustard greens; cruciferous vegetables such as broccoli, cauliflower, cabbage, Brussels sprouts, and kale make important contributions [2, 3]. Eating plenty of sodium rich vegetables helps to reduce heart disease and stroke, control blood pressure, avoid a painful intestinal ailment called diverticulitis, and guard against cataract and macular degeneration are the two common causes of vision losses. Health benefits of consuming vegetables have been well documented [4]. Further, greater consumption of high sodium vegetables has been shown to be associated with a reduced risk of cardiovascular disease (heart attack and stroke), kidney disease due to the additional retention of sodium and water necessary for the kidney to excrete excessive amounts of salt [5], and type-2 diabetes. The high sodium intake has been shown negative impact on health sometime. Excessive intake of sodium increases blood pressure and leads to the resulting risk of hypertension [6].

Previous research works have suggested that, the embedded system based instrument for the measurement of nutritionally important mineral sodium concentration is very few. Hence the present work deals with the development of embedded system based spectrophotometer instrument to overcome the difficulties, which prevail in the conventional methods. The designed instrument obeys Beer-Lambert's law. The developed instrument takes the full advantages of microcontroller features and its reprogrammable facility

2. Experimental

2.1. Design of Hardware

The Block diagram of microcontroller based spectrophotometric instrument to measure sodium ions in vegetable samples is shown in the Fig. 1 and the circuit diagram of the instrumentation set up is shown in Fig. 2. Block A contain monochromatic light source LED of 530 nm of wavelength. LEDs offer a number of advantages compared to existing light sources in optoelectronic applications [7]. These include increased lifetime, low cost, reduced power consumption, higher brightness, rugged construction, flexible configuration, enhanced spectral purity, small size, and breadth of spectral range (LEDs in the spectral range ca. 247-1550 nm are commercially available) [8, 9]. The sample holder used to hold the test tube of solutions containing blank, standard and the unknown sample solutions, is kept in Block B. The Block C work as a signal conditioning unit consists of S1336-8BQ photodiode and CA 3140 (PDIP, SOIC) operation amplifier. The optical sensor S1336-8BQ (Si photodiode - HAMAHATSU PHOTONICS) is used to detect the amount of transmitted light from the solutions. These Si photodiode also has sensitivity in the UV to near IR range. Active area of photodiode is 5.8 - 5.8 and photosensitivity of the diode is 0.12 (A/W). It has excellent linearity with respect to incident light, low noise, wide spectral response range and long life [10]. The pins of the photodiode are connected to the inverting and non-inverting input (Pin No 2 and 3) of the CA3140 operational amplifier. The CA3140 is integrated circuit operational amplifiers that combine the advantages of high voltage PMOS transistors with high voltage bipolar transistors on a single monolithic chip. It is a low cost, low inverting current noise op-amp compared with wideband current feedback op amps. High slew rate, very high input Impedance, very low input current and directly replaces industry type 741 in most applications [11]. The Op-Amp convert the current from photo diode to voltage and the output voltage is connected to pin PA0 (ADC0) of the microcontroller (pin number 37).

Block D consists of ATmega16 microcontroller. It is a 44 pin microcontroller (TQFP/QFN/MLF) with In-System Programmable (ISP) facility. It is a low-power CMOS 8-bit microcontroller based on the AVR enhanced RISC architecture. By executing powerful instructions in a single clock cycle, the microcontroller ATmega16 achieves throughputs approaching 1MIPS per MHz allowing the system to optimize power consumption versus processing speed. It has 32 bidirectional I/O lines namely Port A(PA0-PA7), Port B(PB0-PB7), Port C(PC0-PC7) and Port D(PD0-PD7). It has 16 Kb of Flash

Program memory with read-while-write capabilities, 512 Byte of EEPROM, 1KB SRAM data storage, 32 general purpose working registers, a JTAG interface for Boundary-scan, On-chip Debugging support and programming, three flexible Timer/Counters with compare modes and PWM, Internal and External Interrupts, a serial programmable USART, a byte oriented Two-wire Serial Interface, an 8-channel 10-bit analog to digital convertor (ADC) with optional differential input stage with programmable gain [12,13]. It is used to read the voltage for the blank, standard and the sample from the output of operation amplifier.

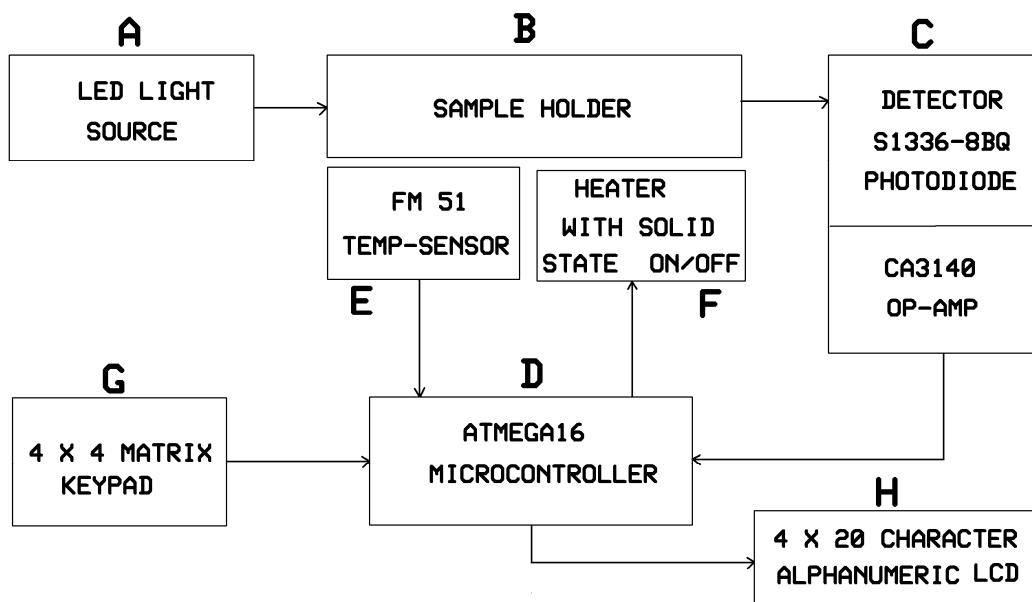


Fig. 1. Block Diagram of ATmega16 Microcontroller based sodium ion analyzer.

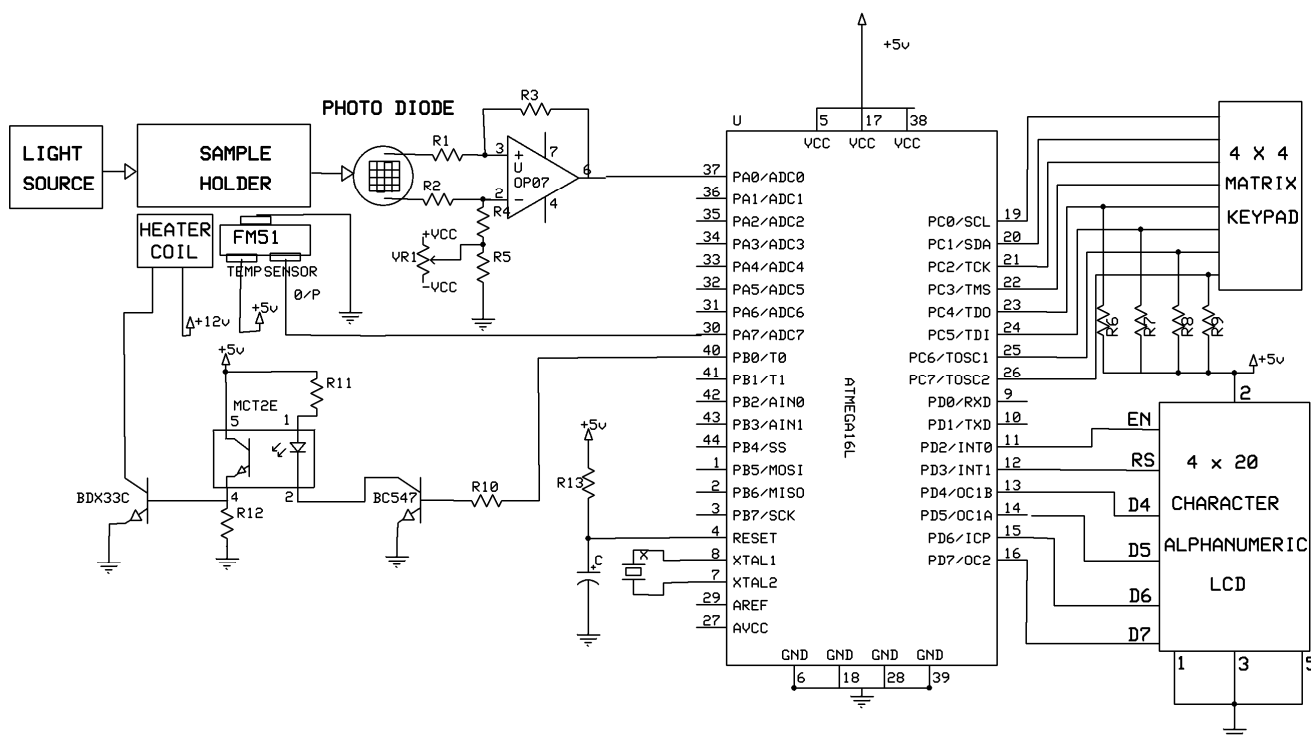


Fig. 2. The circuit diagram of ATmega16 microcontroller based designed instrument setup.

Block E consists of FM51 temperature sensor. Throughout the measurement system, temperature is measured and controlled at 37 °C which is essential for/to incubate the sample for better reagent and sample contact. The FM51 is a high-precision CMOS temperature sensor that provides a cost-effective solution for applications requiring high accuracy temperature monitoring and high drive current. The FM51 output voltage ramp is extremely linear and has a slope of 10 mV/°C [14]. It is typically accurate to ± 1 °C over a temperature range of -40 °C to 125 °C. The signal is generated by the FM51 temperature sensor output (pin number 3 of FM51) is fed to PA7 (ADC7) of the microcontroller (pin number 30). Block F consists of solid-state power controller is constructed with optocoupler MC2TE, BDX33C transistor, and Heater. An optocoupler MCT2E is used to isolate temperature controller section from the microcontroller circuit. Temperature is controlled by on/ off control signal from microcontroller to pin 2 of optocoupler through switching transistor. The temperature is controlled at any temperature by proper command from the PB0 (pin number 40) of ATmega16 microcontroller.

The user enters experimental variables, modes of operation and selection of menu into the system through a 4x4 matrix keypad, which is interfaced with the microcontroller through Port C (PC0-PC7) (Pin number 19 to 26), which is kept in Block G. Block H is a four rows twenty characters alphanumeric LCD (Liquid Crystal Display) to display the experimental results. Port PD4 to PD7 (pin number 13-16) lines are connected to MSB nibble of LCD connector (D4 – D7), PD3 is connected to Register select and PD2 is connected to Enable pin of the LCD. 8 MHz crystal is connected between Pin 7 and 8 of microcontroller. XTAL1 and XTAL2 are input and output of an inverting amplifier which can be configured for use as an On-chip Oscillator. An external reset is generated by a low level (Low active) on the RESET (pin 4). Reset pulses longer than the minimum pulse width will generate a reset, even if the clock is not running. Shorter pulses are not guaranteed to generate a reset. Pull up resistor is connected between +V_{cc} and pin 4 of microcontroller.

2.2. Reagent

All reagents are analytical grade and use without any further purification. Precipitating reagent, standard reagent are the reagent for measuring sodium ion. The color reagent and the precipitating reagent are taken in the pipette of 1 ml and 0.02 ml respectively. These are mixed well and allowed it to stand at room temperature for 5 minutes. The mixed solution is used for the measurement and to calculate the absorbance of blank. All reagents are stable at 2-8 °C temperature till the expiry mentioned on the labels. The reagent kit is purchased from Medsource ozene biomedical, Haryana, India.

2.3. Solutions Preparation

Sodium standard 0.01 ml and precipitating reagent 1ml are taken in the test tube. These are mixed well and allowed it to stand at room temperature for 5 minutes with shaking intermediately and it is centrifuge at 2000 to 3000 RPM for 2 minutes to obtain clear supernatant. The supernatant of 0.02 ml and color reagent of 1 ml is taken in the test tube and then shaken well. This prepared solution is used to determine the absorbance of standard.

Widely consumable and easily available different vegetables samples such as onion, beetroot, beans, cabbage, potato, tomato, radish, carrot and turnip are purchased from local market. Fresh vegetables are cleaned with water and external moisture wiped out with a dry cloth. The edible portion of the individual vegetable is separated, dried in a hot air oven at 50 °C for 1hour. Then, it is boiled in a water bath, extracts it into a test tube and it is filtered using filter paper and it is centrifuge at 2000 to 3000 RPM for 2 minutes to obtain clear sample solution. From the prepared sample solution of

0.02 ml and 1.0 ml of color reagent are taken in the test tube and then shaken well. This prepared solution is used to determine the absorbance of unknown vegetable sample.

2.4. Measurements

The prepared solutions are used to measure the absorbance and concentration of sodium. Blank solution is taken in the test tube and is placed in to sample holder. "V_o" is the measured voltage of the blank solution. Standard solution is taken in the test tube and is placed into sample holder and "V_s" is measured. The sample solution is placed in test tube and "V_t" is measured.

$$\text{Absorbance of standard} = \log_{10}(V_o/V_s) \quad (1)$$

$$\text{Absorbance of sample} = \log_{10}(V_o/V_t) \quad (2)$$

$$\text{Sodium concentration (mmol/l)} = \frac{\log_{10}(V_o/V_t)}{\log_{10}(V_o/V_s)} CS \quad (3)$$

CS is the standard sodium concentration (150 mmol/l)

2.5. Software

When the system is powered up, the microcontroller jumps to the memory location '0000'H, resets all variable values, ports are oriented and the main menu is displayed on the LCD screen. All commands of the microcontroller to control the experimental process are programmed using 'C' language as well as in assembly language. This includes to initialize ports, conversion of analog input into digital, to read blank, standard and sample voltage, to read temperature, to control the temperature of heater at 37⁰C, to accept the data from keypad, setting 4800 baud rate for data transfer/receive with PC, manipulation of data and calculation of concentration, to store and display the result in LCD. Using in-system programmable (ISP) facility, the developed program code is downloaded from PC to microcontroller through PB5 to PB7 (MOS1, MSO and SCK).

3. Results and Discussion

The performance of the microcontroller based instrument in measuring the sodium ions concentration in vegetable samples is investigated. Table 1 shows the sodium concentration of vegetable samples for the designed and conventional instrument ELDEX-3.8. From the Table 1, it is observed that, the sodium concentration of vegetables ranged from 0.13 to 12.38 mmol/l. The onion has low amount of sodium ion concentration of 0.13 mmol/l and corn has high amount of sodium ion 12.38 mmol/l. The graph is drawn between conventional instrument and designed instrument forms a straight line (Fig. 3) which shows the accuracy of the designed instrument. It is observed that the sodium ion concentration is high in corn, Beans, carrot, cauliflower, potato and low in onion, tomato and beetroot. The measured absorption and concentration are compared with the conventional instrument and is found to agree well with correlation coefficient $r = 0.99864$.

Table 1. Concentration of Sodium ion for various vegetables sample using designed and conventional instrument.

Vegetables	Concentration of sodium ion using designed instrument (mmol/l)	Concentration of sodium ion using conventional instrument (mmol/l)
Onion	0.132	0.191
Tomato	0.311	0.393
Beetroot	0.443	0.512
Cabbage	0.826	0.913
Potato	1.047	1.114
Cauliflower	1.315	1.414
Carrot	1.584	1.718
Beans	11.604	11.493
Corn	12.387	13.369

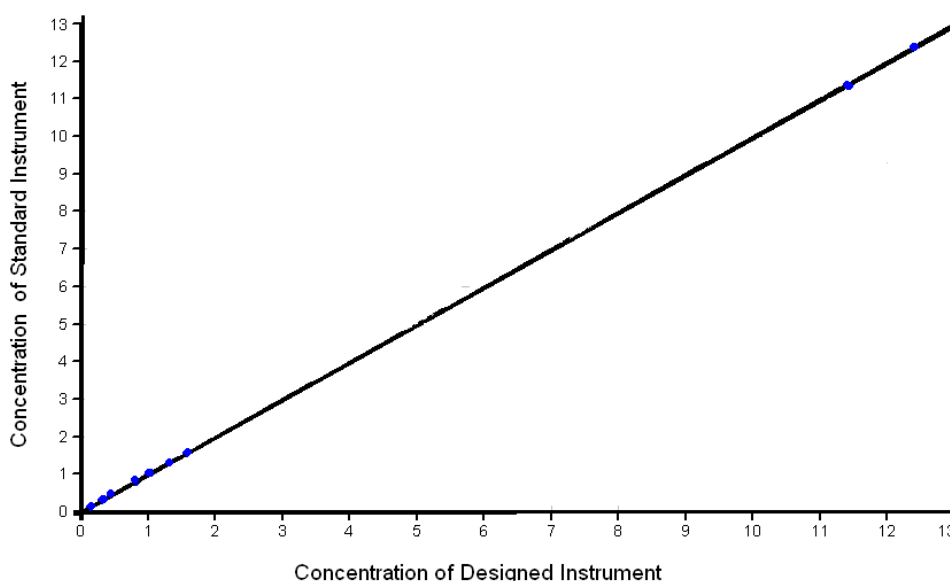


Fig. 3. Regression line for comparison of designed and convention Sodium analyzer.

4. Statistical Analysis

4.1. Linear Regression

The accuracy of the developed system is evaluated by comparing the results with those obtained by using a conventional Instrument. 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. Linear regression analysis is performed and tested for lack of fit to the linear model. The equation for linear regression analysis is follows:

$$Y = a + Bx \tag{4}$$

The values of intercept $a = 0.052796$, $b = 1.033486$ and correlation coefficient $r = 0.99864$ which closes to 1 indicating the perfect correlation. For the developed instrument the standard deviation is 4.959134 and for conventional instrument the standard deviation is 5.132159. The Relative standard deviation (RSD) of designed and conventional instrument is 1.505352 and 1.484379 respectively.

Fig. 3 shows the linear regression drawn (using software ULTIMACALC) between the designed instrument and the conventional instrument, which shows the accuracy of the designed instrument. The developed system produces 99 % of reproducible and also reflected in the results and average differences between these two methods are very small.

4.2. 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, value at 1% level for 9 df is 2.088. The results are compared statistically with those obtained by ELDEX-3.8 by t-test and F-test. From the test, no significant differences are observed. The value of t-test and F-test is 0.473092 and 0.925096 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

ATmega16 microcontroller based optoelectronic instrument is designed and developed to measure the concentration of the sodium ions in vegetables. ATmega16 microcontroller together with another carefully selected low power electronics devices in a design results a system with inexpensive, minimal optical requirement, application specific, over conventional tungsten light spectrophotometer design. The overall relative standard deviation of the measurement is less than 1.5 %, reveals excellent precision properties of the developed instrument. In addition t-test and F-test results show good accuracy and better precision with value of 0.473092 and 0.925096 respectively. The measurement range is found to be linear and accurate with 1 % variation within run. The instrument is advanced one and human involvement is less and the results are more accurate. The designed instrument is measuring concentration of sodium is tested with different samples. Accuracy can be increased by using 12 bit A/D converter can be increase the accuracy of concentration of sodium. By changing the wavelength and software, it can be used to measure the concentration of other minerals for various samples.

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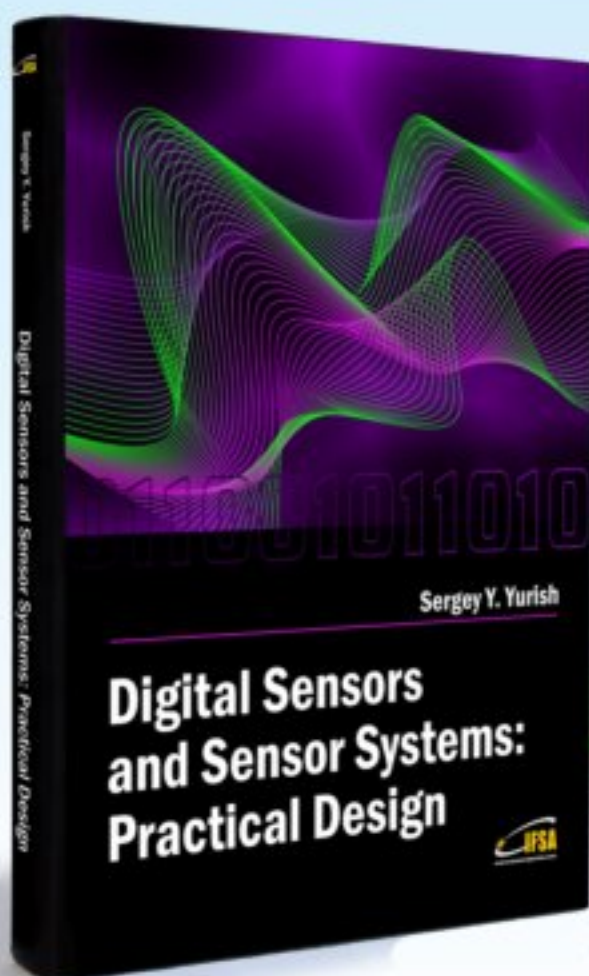
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