Electronic Readout System for Interfacing Impedance Biosensor Responses

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ABSTRACT

Rapid pathogen detection is an urgent necessity in order to ascertain contamination and diseases caused by pathogens such as Salmonella typhimurium or Escherichia coli. Rapid detection of pathogen is essential in several critical fields such as national security, food safety, and human and animal healthcare, to name a few. Thus, a rapid and controlled mechanism to counter the threat of mass contamination is needed.

A low power, low cost, and simple electronic readout system has been simulated and implemented for rapid bacteria detection for impedance biosensor responses. Such an interface will enable the label free and rapid impedance biosensors convenient for field use. Experimental reading shows that the variation in sensitivity is not affected significantly by a 10% fluctuation in frequency but the signal amplitude has to be maintained within 30 mV. The readout system generates automatically sinusoidal waveforms of discrete values in the wide range 100–100 kHz by interfacing a high-Q band pass filter with a rectangular waveform of maximum frequency 15 kHz obtained from ATMEGA128 microcontroller, by configuring the PWM and Timer modules. The duty cycle of the rectangular waveform generating from microcontroller is tuned so that the control of voltage is maintained within 30 mV amplitude. Also, the power consumption of the sine wave generator is maintained within 30mW.

Keywords

Electronic interface, impedance biosensor responses, minimum cost, low power

1. INTRODUCTION

One of the greatest challenges in medical field is the rapid and sensitive detection of pathogenic bacteria in physiological fluid like blood, bioterrorism, urine and others to prevent risk of infection, and enteric diseases [1]. Culture technique which is the most common method of detection is where the test samples are first centrifuged for initial purification followed by the microbiological growth process which takes around 2 days.

Enzyme Linked Immunosorbent Assay (ELISA), relatively rapid method of detection requires secondary labels and can be either colorimetric or fluorometric.To obtain high sensitivity with the rapid detection method called ELISA, the entire optical instrumentation system possess a high signal. This makes the method high cost and also requires skilled manpower for operation.

Some label free optical techniques based on changes in refractive index upon biomolecule [4]–[6] binding which are based on the use of grating couplers, optical waveguides and Surface Plasmon Resonance (SPR) .SPR has several drawbacks like to support the surface Plasmon wave, the limited to the highly lossy visible spectral regime (400–800 nm), and the most of false positives due to input power fluctuations and/or connector losses associated with the intensity based measurements.

Commercialization of impedance biosensors is the other option. Biosensors are label free, rapid and sensitive. To improve the sensitivity and specificity of the impedance biosensors, thereare many attempts. A recent literature on an optimized macro porous silicon based biosensor reports a detection of 102 CFU/ml E.coli O157 within 15 minutes which is 10 times more sensitive than the refractive index based method. This paper thus focuses on the study of a portable electronic readout for impedance biosensors.

At present most of the impedance measurements are being conducted with a desktop LCR meter where computer interface is required for automatic impedance spectroscopy measurements and no bacteria concentration display present. Thus it is required to develop a portable electronic interface with an adaptive readout for such impedance biosensors. Similar requirements for development of portable readout have been addressed for flexural plate wave type [7] and lateral flow strip type biosensors.

The major requirement in the electronic interface of the impedance biosensor is the generating sinusoidal wave forms in a automatic manner in the wide range of 100 Hz–100 kHz within amplitude of 30 mV with low power and optimum cost budget. For such applications, ANALOG DEVICE5933 and ANALOG DEVICE5934 by Analog Devices are the commonly used off the shelf components which consume at least 85 mW powers at 5 V. Frequency generations can be done by using pulse width modulation techniques using microcontroller, which is the most common method. But generating frequencies greater than 20 kHz would require high frequency microcontrollers which are expensive and consume high power . There exists some approaches with current based sensing schemes which lead to a high voltage for a high impedance sensor. A recent report interfaces carefully designed 3rd order low pass filters with rectangular outputs of an ordinary PIC16F688 microcontroller to generate 20 kHz and 50 kHz waveforms but the input to the sensor is more triangular in nature and hence discrete Fourier Transform has been carried out for extraction of the components corresponding to the fundamental and the harmonics. It may provide some erroneous results of impedance biosensor as sensitivity for computation of sensitivity is dependent on both frequency and amplitude of sinusoidal waveform.

In this paper, we ARE simulating a simple electronic interface which automatically generates sinusoidal waveforms of frequencies in discrete ranges (between 100Hz to 100KHz)by interfacing a high-Q band pass filter with a rectangular output of maximum frequency 15 kHz obtained from an ordinary 16 MHz low power ATMEGA128 microcontroller. Each waveform is always maintained within 30 mV with negligible dc offset to reduce ionic fluctuations at the interface, which is obtained by tuning the duty cycle of the rectangular signal at the microcontroller output. If Wien Bridge oscillators would be used, this would be difficult. The readout system has been simulated for the impedance biosensor responses.

2. ELECTRONIC INTERFACE DESIGN

From the description of the impedance biosensor, the operational requirements for a portable, simple and low power electronic interface device are as follows:

・Provisions should be provided to the user to enter the different signal ranges corresponding to target pathogen concentration. These ranges are sensor specific and hence flexibility is required to key in the values.

・ An option is provided to the user to select control and test reading. When the control option is selected, the amplitude of the signal should be first checked. If within 30 mV, then impedance magnitude values have to be automatically recorded for the different frequencies. Similarly after the processed infected sample is provided, the test reading is to be recorded for different frequencies. If the amplitude is out of range, then it is to be adjusted.

・Both the test and control readings have to be recorded after a certain time gap to allow the electrical reading to stabilize. Around 50 readings should be taken and average value is to be stored.

・From the average control and test readings corresponding to the different frequencies, first it is to be observed whether the impedance values decrease with frequency. If so, then the signal value has to be computed at 100 Hz and compared with the data entered and the concentration reading will be displayed.

The elements which are necessaryin the electronic readout are

- microcontroller interfaced with switches and LCD,
- signal generator,
- sensor drive circuit and
- RMS value generator of the sinusoidal output of sensor.

The basic block diagram is shown in Fig.1.

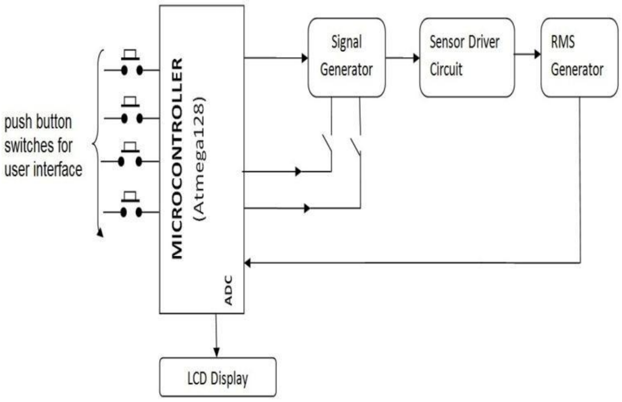


Fig.1 Block diagram of the electronic interface system.

2.1 Analog signal processing

In this paper, the operational amplifiers OP07used which are low noise, low offset and low power. The bandwidth, noise and offset voltage are 600 kHz, 600 nV and around 75 μV respectively which are sufficient for our scheme. These amplifiers are operated with either +5 V or ±5 V supply. Using three 1.5 V AAA rechargeable batteries connected in series,the supply of -4.5v is generated which help to ensure a single supply since the LCD used requires a minimum of 4.2 V. The passive components with low temperature coefficient precision metal film resistors and polyester capacitors are used. They ensure stability of the waveforms.

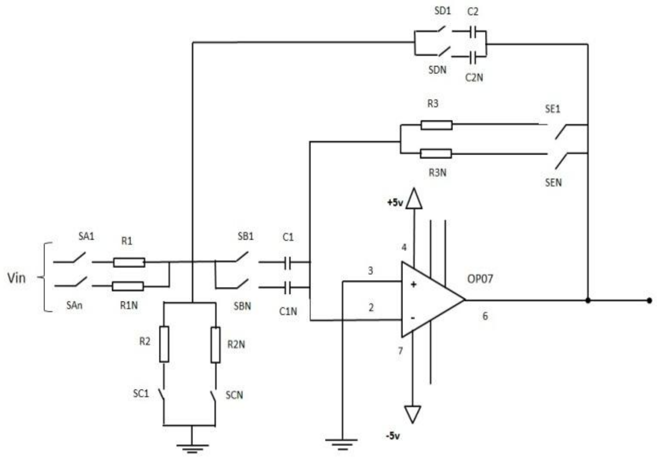


Fig.2 Schematic of the signal generator

The relationships between different components and the central frequency to be generated along with amplitude of the output sine wave are given by following equations

$$R_1= \frac{Q}{2\pi f_c C\, A_f} \tag{1}$$

$$R_2 = \frac{Q}{2\pi f_c C\,(2Q^2-A_f)} \tag{2}$$

$$R_3 = \frac{Q}{\pi f_c C} \tag{3}$$

$$A_f= \frac{R_3}{2R_1} \tag{4}$$

$$V_{out}= V_{in}[\frac{\tau}{T}+\sum_{n=1}^{\alpha}\frac{1}{n\pi}\sin(2n\pi\frac{\tau}{T})] \tag{5}$$

Where $f_c$is the central frequency, Q is the quality factor of the filter, $A_f$is the gain of the filter, τ *is* the on time and T is the time period of the rectangular waveform.

The signal generation is based on the principle that a rectangular waveform when passed through a high Q narrowband pass filter tuned to the nth harmonic produces a sinusoidal waveform. Frequencies of 100 Hz, 2 kHz and 15 kHz have been generated from the corresponding rectangular waveforms of fundamental frequencies. Frequencies of around 5 kHz, 50 kHz and 80 kHz have been generated from the 15 kHz rectangular waveform by designing the filter tuned to the 3rd and 5th harmonics with a Q value of around 30. The corresponding values of resistors and capacitors for the filter are selected by microcontroller controlled programmable switches as shown in Fig .2

In Fig2 values of C1 and C2 are identical. For six frequencies, four sets of R1 and R3, three sets of R2 and six sets of C1 and C2 have been selected. The gates of the switches SA1, SC1 and SE1 are shorted; those of switches SA2, SC2 and SE2 are shorted and so on and connected to the pins of one output port of the microcontroller. Similarly the gates of switches SB1 and SD1 are shorted, SB2 and SD2 are shorted and so on which are connected to the pins of another output port of the microcontroller. For all the six frequencies, two amplifier chips OP07 have been used to reduce the loading effect of the components. One of them has been connected to generate frequencies 100 Hz, 2 kHz and 5 kHz and the remaining frequencies are generated by the other chip. The total power consumption in this signal generator is within 30 mW for a 4.5 V power supply which is almost a one-third reduction compared to the ANALOG DEVICE5933 and ANALOG DEVICE5934 chips.

For measurement of the sensor impedance, first the amplitude adjustment is carried out by an inverting amplifier with six sets of resistors connected in feedback. The resistors are connected to six switches (SLR1-6) for adjusting the amplitude corresponding to six frequencies. Similar scheme has been reported for wide dynamic range measurement of gas sensor .The gates of switches SLR (1–6) are connected with that of SB and SD in Fig3. This stage is required since the amplitude of the sine waves generated at the output of the filter is different. Two switches HS and LS have been included to select the output of only one amplifier of the filter circuit. The gates of these switches are controlled by a separate output port of the microcontroller. Then the dc offset of the waveform generated is eliminated using a low pass filter configuration with *RL* and *CL*. Switches *Sc* and *Sm* have been incorporated in the sensor measurement circuit for calibration and measurement and are connected to another port of the microcontroller. When switch *Sc* is closed, the amplitude of the output voltage is checked and if it is within 30 mV, then switch *Sm* is closed for measurement. If the amplitude is above 30 mV owing to drift in any component of the circuit, then it is adjusted by tailoring the duty cycle depending on the frequency of the waveform according to equation in (1) .The sensor impedance measurement circuit is in the negative feedback configuration. The feedback resistor (*Rs*2*)* has been selected so that the output voltage is within 200 mV for a wide range of sensor impedance (*Zsens)* from 500 _ to 10 k_. This is required to interface directly with the succeeding circuit. The output voltage is related to the sensor impedance by equation (6):

$$V_{04} = \frac{V_{03}}{1+j\omega}\left(\frac{j\omega R_L C_L R_{s2}}{Z_{sens}} + 1\right) \qquad (6)$$

Where V_{03} and V_{04} are the input and output of the sensor circuit respectively.

The output of the sensor circuit is a sinusoidal signal and hence before directly interfacing with the built-in ADC of the microcontroller, the output is first interfaced with a true RMS generating chip AD736 which operates on ±5 V supply with a loaded power consumption of 0.18 mW. The bandwidth of the chip is around 200 kHz and hence is suitable for our application. The schematic is shown in Fig3.

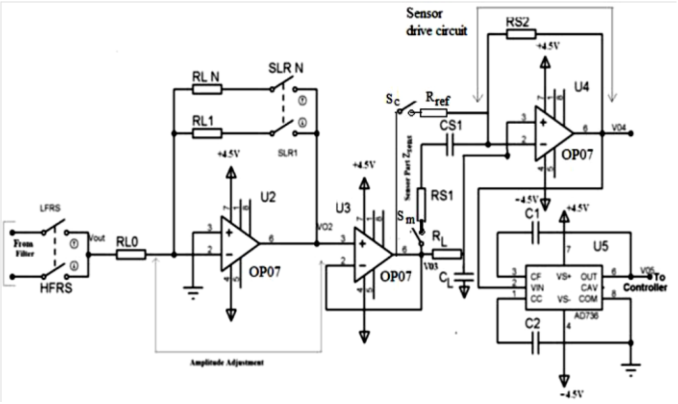


Fig.3 Schematic of sensor impedance adjustment circuit

2.2 Microcontroller interface and algorithm

The microcontroller used in this scheme is ATMEGA128.which is a 8-bit low-power ATMEGA AVR RISC-based microcontroller. It combines 8 KB of programmable flash memory, 1 KB of SRAM, 512 K EEPROM, and an 8 channel 10-bit A/D converter. This device supports throughput of 16 MIPS at 16 MHz and operates between 2.7–5.5 volts.

2.2.1 Algorithm for the readout system

- LCD displays “REFERENCE RANGE” at the beginning. The user is asked to enter the different signal ranges for the pathogen concentration corresponding to the sensor being used. This is done by pressing three push button switches- two for selecting the value and one for setting them. An ‘ESC’ switch has been kept to skip this portion if the correct calibrated data has already been loaded.

- The LCD displays “CONTROL/TEST READING” after this. The user selects the appropriate choice by pressing a push button switch.

- After the selection of the mode, the microcontroller starts generating the rectangular waveforms sequentially with a time gap required to complete the task for a particular frequency.

- The corresponding switches in the signal generator circuit are activated to generate the required sine wave, for a rectangular waveform of frequency 100 Hz. The output of the sine wave passes through the sensor impedance measurement circuit where switch *Sc* is first activated by the microcontroller and ultimately the root mean square (rms) value enters the microcontroller through its in-built ADC. If the value is within a range, then *Sm* is activated and the ADC takes in 50 such samples at a fixed interval and microcontroller computes the average which is stored in a reserved place in the general purpose registers.

- The microcontroller then jumps to the next frequency generation timer program and again fourth step is executed.

- In this way the rms values for the six frequencies are stored at different register locations. If the values stored continuously increases, then the microcontroller waits for the rms values for “TEST READING” if CONTROL was selected in step 2. If the “TEST READING” was selected in step 2, then steps 3 to 4 are executed and the fractional change in rms voltage is computed at 100 Hz.

The fractional change in voltage is matched with the reference ranges entered in step 1 and the corresponding pathogen concentration range is displayed in the LCD.

- The LCD displays “REPLACE SENSOR” and the user has to start from first step, if the rms values are not observed to increase continuously at least up to 15 kHz.

The microcontroller is programmed with an intelligent algorithm in BASIC language where three rectangular waveforms of frequencies 100 Hz, 2 kHz and 15 KHz are generated. PWM module is configured to generate the other set of waveforms.. In ATMEGA128 controller I used a 8 bit timer (timer2). However the program length is not affecting the frequencies of the waveforms. In the timer2, four different prescale values 1, 8, and 128 are used for generating 15 kHz, 2 KHz and 100 Hz frequencies respectively. To get variable duty cycle we have kept the provision of tuning the timer count.

3. SIMULATION RESULTS

The electronic interface has been implemented to generate automatically sinusoidal waveforms of six discrete values in the wide range of 100 Hz–100 kHz with low power and moderate cost budget by interfacing a high-Q band pass filter with a rectangular waveform of maximum frequency 15 kHz obtained from an ordinary 16 MHz ATMEGA128 microcontroller. This scheme enables the control of voltage within 30 mV amplitude by tuning the duty cycle of the rectangular waveform.

3.1 Interface verification and characterization

The entire circuit the performance of the interface has been tested in Proteus 7 Professional. Figure 4.1 shows the simulated signal generator circuitry which is designed using the set of equations in chapter 3, as mentioned.

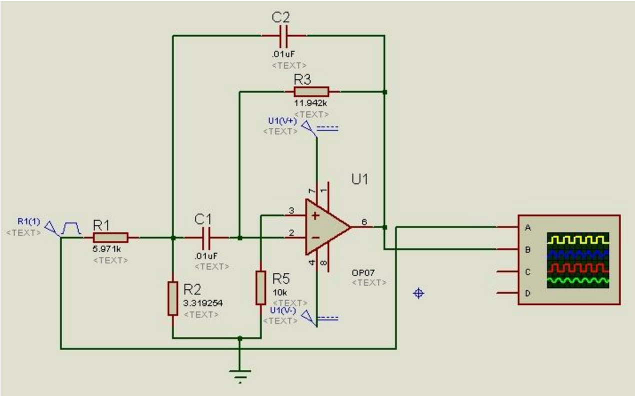


Fig.4 simulated signal generator for 80Kz

Fig.4 shows the sinusoidal waveforms for 80 kHz. It is observed from the simulated waveforms that the spectral quality of the signal is quite satisfactory but the amplitude of the 80 kHz signal varies within 30 mV which is acceptable.

The output of the sensor circuit is a sinusoidal signal and hence before directly interfacing with the built-in ADC of the microcontroller, the output is first interfaced with a true RMS generating chip ANALOG DEVICE736 which operates on ±5V supply with a loaded power consumption of 0.18 mW.

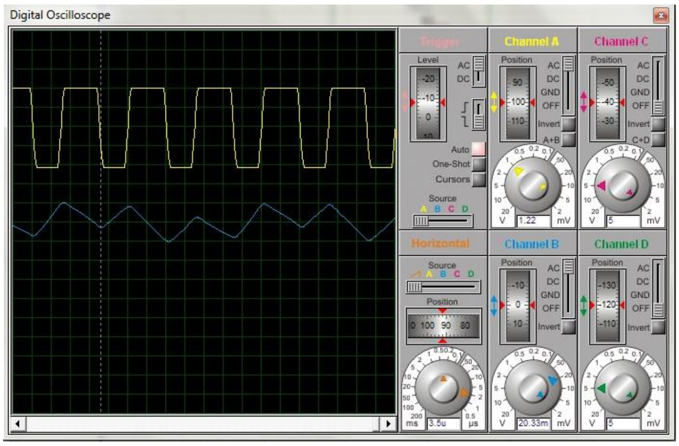


Fig.5 sinusoidal waveforms for 80 kHz

The outputs of the microcontroller ports enabling the different switches for frequency generation of 80 kHz are shown in Fig 6. It is observed that port PA5 is activated to switch on the corresponding switches in the signal generator.

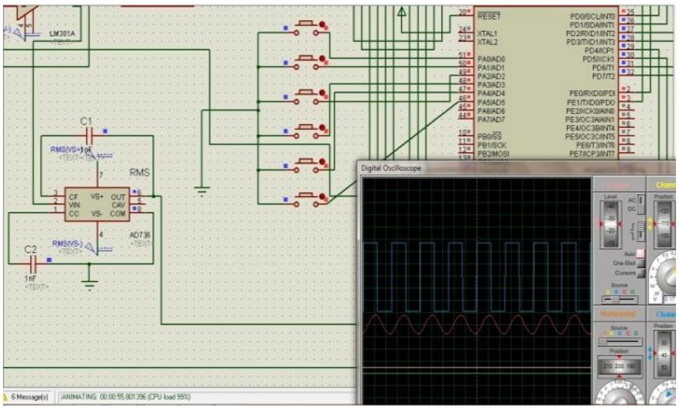


Fig. 6 Output ports of microcontroller for 80 kHz frequency.

The simulated signal generator circuit for the set of frequencies is given in Fig.7

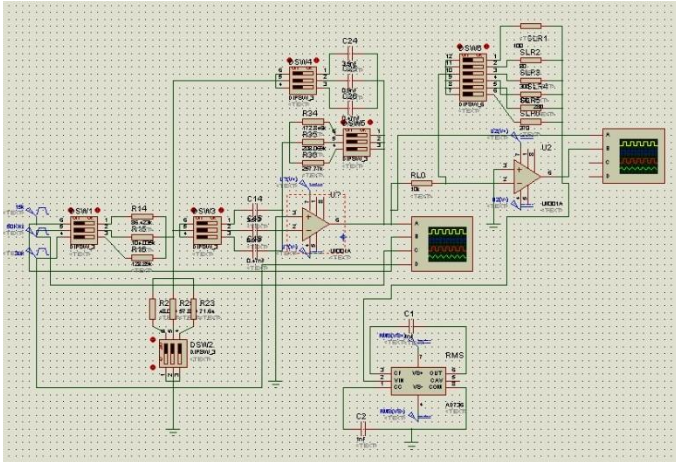


Fig. 7 simulated signal generator for set of frequencies 15K_50K_80K

After simulating for the six frequencies, the outputs of the both the signal generators are tabulated as shown in Table.1.

Table 1 signal generator outputs

Frequency (kHz)	Q-Factor	R1 (KΩ)	R2 (Ω)	R3 (KΩ)	C (μF)	Output Amplitude (mV)
0.102	30	95.541	53.108	191.08	500	1.0
2.07	30	95.541	53.108	191.08	25	0.84
5.162	30	106.15	59.01	212.31	9	1.06
15.793	30	86.423	48.039	172.84	3.5	2.2
51.02	30	104.03	57.829	208.06	0.9	1.0
78.86	30	128.89	71.64	257.78	0.47	0.8

The readout system has been simulated with sensor impedance circuit along with the ANALOG DEVICE736 and microcontroller interface as shown in Fig.8.

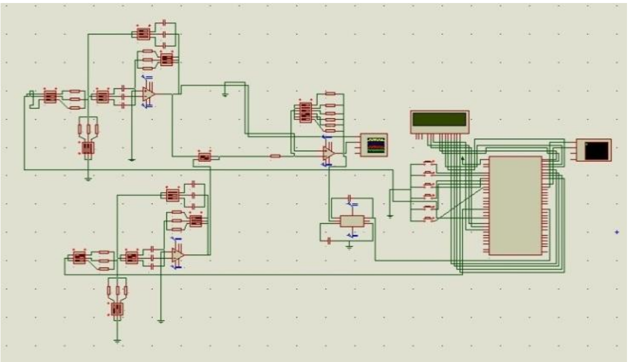


Fig.8 Schematic representation of the simulated impedance meter

During the simulation of the system, the initial conditions has been displayed and one of the frequency has been selected from the different frequency components with the microcontroller interfaces, as shown in Fig.9

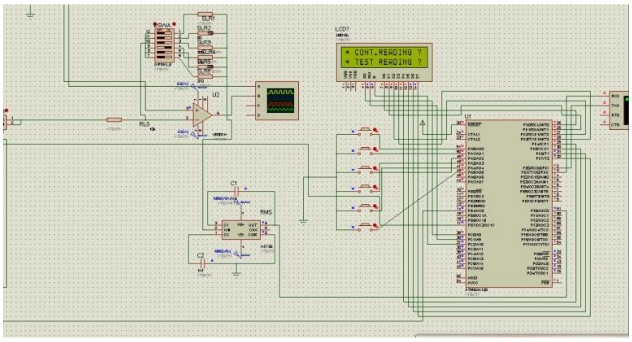
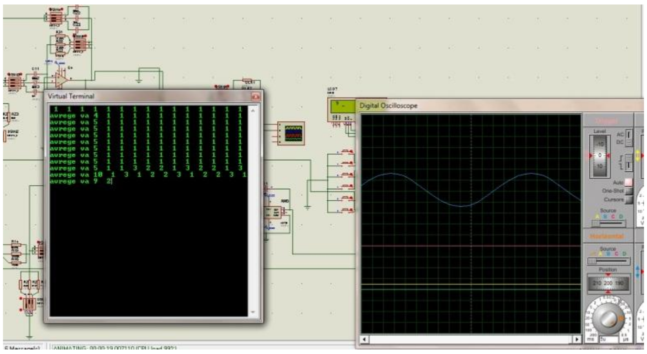


Fig.9 initial stages of simulation

The schematic Figure for simulation for the read out system for the impedance sensor responses is shown in Fig. 10



```
 1  1  1  1  1  1  1  1  1  1  1  1  1  1  1
avrege va 4  1  1  1  1  1  1  1  1  1  1  1
avrege va 5  1  1  1  1  1  1  1  1  1  1  1
avrege va 5  1  1  1  1  1  1  1  1  1  1  1
avrege va 5  1  1  1  1  1  1  1  1  1  1  1
avrege va 5  1  1  1  1  1  1  1  1  1  1  1
avrege va 5  1  1  1  1  1  1  1  1  1  1  1
avrege va 5  1  1  1  1  1  1  1  1  1  1  1
avrege va 5  1  1  1  1  1  1  1  1  1  1  1
avrege va 5  1  1  3  2  2  1  3  2  2  1  3
avrege va 10  1  3  1  2  2  3  1  2  2  3  1
avrege va 9  2
```

Fig. 10 displaying outputs of the read out system

From Fig.10, the waveforms from the sensor impedance adjustment circuitry as well as those corresponding average rms values has been obtained and the simulated rms values for the different components at two different frequencies are shown in Table.2.

During simulation,it is observed that the signal increases with concentration but decreases with frequency. Calibration facilities and User selectable zero offset features are incorporated. The impedance value is reliable and almost steady only if the amplitude is within 30 mV.

Table 2 simulated read out system outputs

Frequency (kHz)	Output Amplitude of Signal generator (mV)	Amplitude adjustment (when RL0=10K)		Output Amplitude of sensor impedance adjustment circuit(mV)	Average rms (mV)
		A1	R_f(Ω)		
0.102	1.0	0.03	300	29.584	10
2.07	0.84	0.03	300	28.96	10
5.16	1.06	0.025	250	27.0	9
15.79	2.2	0.013	130	28.6	8
51.02	1.0	0.028	280	28.0	7
78.86	0.8	0.037	370	29.6	7

4. CONCLUSION

In this paper, a low power low cost and simple electronic interface has been simulated for the impedance biosensor responses. This system will make the label free and rapid impedance biosensors convenient for field use.

The electronic interface generates automatically sinusoidal waveforms of six discrete values in the wide range of 100 Hz–100 kHz with low power and moderate cost budget by interfacing a high-Q band pass filter with a rectangular waveform of maximum frequency 15 kHz obtained from an ordinary 16 MHz ATMEGA128 microcontroller. This scheme enables the control of voltage within 30 mV amplitude by tuning the duty cycle of the rectangular waveform. User selectable zero offset features and calibration facilities have also been incorporated. The power consumption of the sine wave generator is maintained within 30 mW. The simulated rms values for the different frequency components are obtained and the results are satisfactory.

Thus, the electronic readout system interfaced with biosensor responses for the measure of accurate pathogen concentration has been simulated.

5. REFERENCES

[1] L. Kant, “Combating emerging infectious diseases in India: Orchestrating a symphony,” J. Biosci., vol. 33, no. 4, pp. 425–427, 2008.

[2] Y. Liu and Y. Duan, Encyclopaedia of Sensors, vol. 1. Stevenson Ranch, CA, USA: American Scientific Publishers, 2006, pp. 371–400.

[3] Full Microbiology Lab Automation [Online]. Available: http://www.biomerieux.com (2008).

[4] R. Horváth, H. C. Pedersen, N. Skivesen, D. Selmeczi, and N. B. Larsen, “Optical waveguide sensor for on-line monitoring of bacteria,” Opt. Lett.vol. 28, no. 14, pp. 1233–1235, 2003

[5] C. Herath, C. Wang, M. Kaya, and D. Chevalier, “Fiber loop ringdown DNA and bacteria sensors,” J. Biomed. Opt., vol. 16, no.5, pp.050501-1–050501-3, 2011.

[6] J. Homola, Surface Plasmon Resonance based Sensors. New York, NYUSA: Springer-Verlag, 2006.

[7] L. Bissi, P. Placidi, and A. Scorzoni, “Offset voltage evaluation of analog blocks in a conFigurable mixed architecture for smart capacitive sensor applications,” Sens. Actuators A, Phys., vol. 140, no. 2, pp. 162–167,2007.

[8] C. Roy Chaudhuri, R. Dev Das, S. Dey, and S. Das, “Functionalized silicon microchannel immunosensor with portable electronic readout for bacteria detection in blood,” in Proc. IEEE SENSORS Conf., Oct. 2011, pp. 323–326.

[9] Vivek Nandakumar, Daniel Bishop, Eric Alonas, Jeffrey LaBelle, Lokesh Joshi, and Terry L. Alford, “A Low-Cost Electrochemical Biosensor for Rapid Bacterial Detection” in IEEE SENSORS JOURNAL, VOL. 11, NO. 1, JANUARY 2011

[10] AVR Atmega 128, Data sheet

[11] S. P. Mohanty and E.Kougianos, “Biosensors: A tutorial review,” IEEE Potentials, vol. 25, no. 2, pp. 35–40, 2006.

[12] Nirmalya Samanta, Olyvia Kundu, and Chirasree Roy Chaudhuri, Member, IEEE “A Simple Low Power Electronic Readout for Rapid Bacteria Detection With Impedance Biosensor” IEEE SENSORS JOURNAL, VOL. 13, NO. 12, DECEMBER 2013

[13] L. Bissi, P. Placidi, and A. Scorzoni, “Offset voltage evaluation of analog blocks in a conFigurable mixed architecture for smart capacitive sensor applications,” Sens. Actuators A, Phys., vol.140, no.2, pp. 162–167, 2007.