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Instrumentation to Measure the Capacitance of Biosensors by Sinusoidal Wave Method

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Abstract: Micro Controller based instrumentation to measure the capacitance of biosensors is developed. It is based on frequency domain technique with sinusoidal wave input. Changes in the capacitance of biosensor because of the analyte specific reaction are calculated by knowing the current flowing through the sample. A dedicated 8-bit microcontroller (AT89C52) and its associated peripherals are employed for the hardware and application specific software is developed in 'C' language. The paper describes the methodology, instrumentation details along with a specific application to glucose sensing. The measurements are conducted with glucose oxidase based capacitance biosensor and the obtained results are compared with the conventional method of sugar measurements using the UV-Visible spectroscopy (Phenol-Sulphuric acid assay method). Measurement accuracy of the instrument is found to be ± 5 %. Experiments are conducted on glucose sensor with different bias voltages. It is found that for bias voltages varying from 0.5 to 0.7 Volt, the measurements are good for this application. *Copyright © 2009 IFSA.*

Keywords: Biosensor, Capacitance, Dextrose, Sinusoidal wave method, Bias voltage

1. Introduction

Electrochemical devices have traditionally received a major share in biosensor development. This is because of the inherent simplicity of obtaining a direct electrical readout and the opportunities for using integrated circuit technology [1].

Enzymes are suitable for use in biosensors that function as continuous monitors of analyte concentration. The basis for the vast majority of enzyme sensors reported to date is the conversion of analytes of interest to a detectable product by an immobilized enzyme. The product will have quantifiable electro-activity (for example, redox-active) or optical properties [2]. Although the optical sensors are the most commonly employed today, the electrochemical ones can be expected to give the lowest detection limits along with the most simple instrumentation. One of the electrochemical transducers with the highest potential is the capacitive one. This type of transducer is suited for large sized analytes, like hormones or DNA fragments, as it measures changes in the dielectric properties or in the thickness of the immobilized sensing layer. An exception is the detection of heavy metal ions, where the heavy metal ion specific proteins immobilized on the surface are believed to change conformation when binding occurs [3]. Enzyme based capacitance glucose sensors also fall under the same category. Glucose measurement has one of the major applications in the diagnosis of diabetics. It has lot of applications in food and dairy industries also. Development of instrumentation which could measure the capacitance of biosensor is very much required, hence is developed. Capacitance biosensor is used to show the applicability of this instrument in glucose measurements.

Glucose oxidase immobilized [4] sensors are used for the study. Conventional method of determination of sugars by phenol-sulphuric acid assay method is used in the first place to alternatively measure the glucose concentration present in the solutions of interest. This method uses a colorimetric method for determination of glucose in solutions [5].

There have been few attempts in the past made on measurement of capacitance of solar cells using impedance spectroscopy technique by applying a small ac signal about the operating point over a wide range of frequencies [6]. But it is an indirect technique, highly complicated and needs expensive instrumentation such as Electro Chemical Interface (ECI) and Frequency Response Analyzer (FRA). Low frequency oscillographic methods for measuring the capacitance have also been reported [7]. The same methods mentioned above are generally used in the measurement of capacitance of biosensors based on ISFETs [8] and other semiconducting substrates using the capacitive sensing in biosensors. In order to avoid the complex instrumentation used in the above measuring systems, instrumentation based on frequency domain technique is developed. In this, the active devices are biased externally using DC voltages at the desired operating level and the sinusoidal wave of desired amplitude and with variable frequencies are applied. The resultant AC current of the device is measured and the capacitance is calculated using the Equation (1):

$$c = \frac{i_+ - i_-}{4\pi V_{in} f_{in}} = \frac{\Delta I}{4\pi V_{in} f_{in}} \tag{1}$$

For an applied sinusoidal waveform with amplitude V_{in} and frequency f_{in} and i_+ & i_- are the current values measured at a single value of applied voltage.

2. Instrumentation

2.1. Instrument Description

The schematic of the instrument is shown in Fig. 1. It is developed based on sinusoidal wave method for the measurement of capacitance in biosensor. The instrument consists of a D/A converter (DAC0808), a waveform generator (MAX038) chip, a power amplifier for biasing, a programmable DC voltage source, a differentiator, an absolute value output circuit, a peak detector, an A/D converter (ICL7135) and an 8-bit Microcontroller (AT89C52) interfaced with (2X16) LCD display with application specific software developed using C-language.

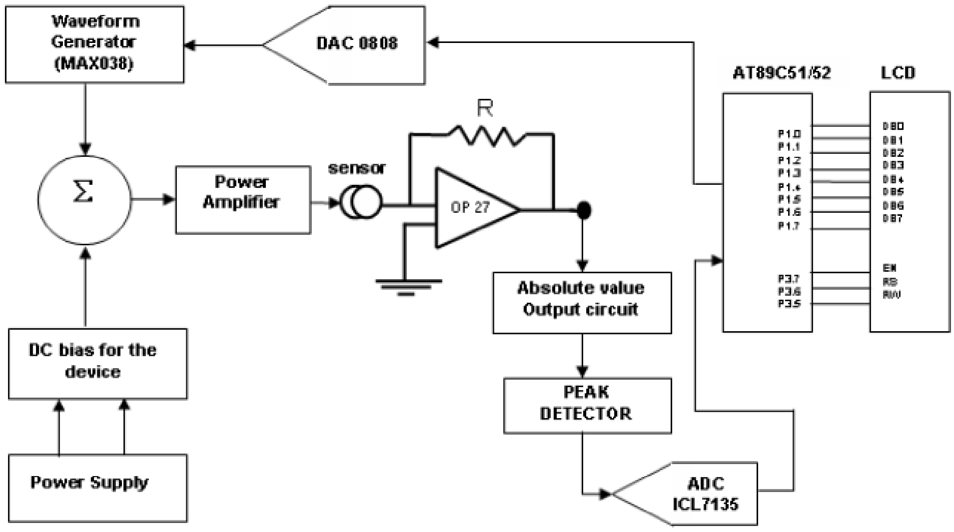

Fig. 1. Schematic of the capacitance measuring instrument.

MAX038 is a precision, high-frequency function generator that produces accurate sine, square, triangle, saw tooth, and pulse waveforms with minimum of external components. Frequency is digitally adjusted by connecting a voltage output of DAC 0808 to MAX038 pin via a series resistor. The converter output ranges from 0 V at zero to 2.0 (255/256) V at full-scale. Differentiator circuit is used for measuring the capacitance of the sample. Periodical sinusoidal voltages of desired amplitude with variable frequencies are applied to the sample. The resultant AC current of the sample is measured and the sensor capacitance is calculated. The current flowing through the sample and the voltage will both be periodic functions of time, so that we can eliminate time as a parameter and solve for the current as a function of the applied voltage. An absolute value output circuit is used in wave shaping. This circuit produces an output signal that swings positively only, regardless of the polarity of the input signal. Peak detector that measures the positive peak values of the input signal and output of the peak detector is fed to the A/D converter input. The output of this A/D converter is then fed to the microcontroller for further processing.

2.2. Testing and Calibration

Sinusoidal method is implemented using the developed software and the measurements are conducted with plastic package capacitors (2 % tolerance) of 100 nF and 220 nF as standards to estimate the errors in capacitance measurements. These measurements are conducted for various frequencies starting from 100 Hz to 5 kHz. The measurements are stable over the frequency of operation. Fig. 2 shows the results for the capacitance measurements conducted on 100 nF and 220 nF. Capacitance measurements in biosensors are conducted at lower frequencies than those for measurements in other applications like the semiconductor devices. The frequency range generally used for the biosensors is less than 10 kHz because the stray capacitances and the double layer capacitances at the electrodes dominate at higher frequencies and overshadow the changes in capacitance measured in the biosensor.

As the capacitances for biosensor are expected to be in the pF to nF ranges, measurements on 33 pf and 1 nF are also conducted and results are shown in Fig. 3.

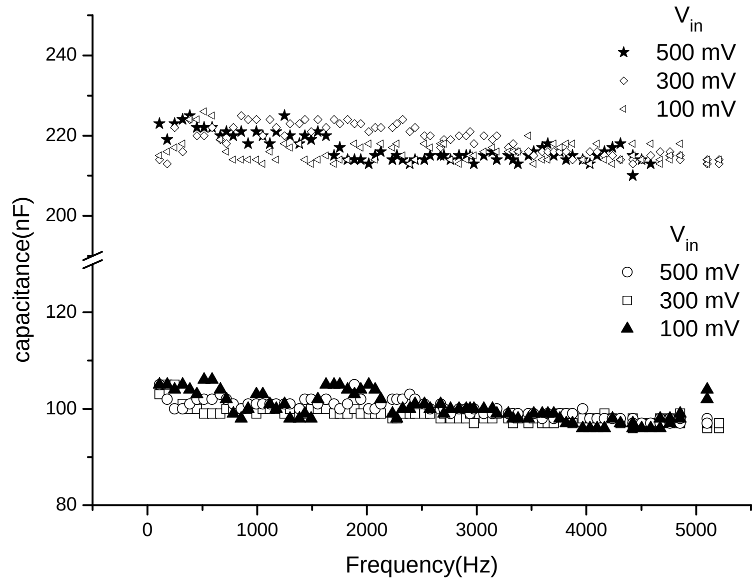

Fig. 2. Capacitance Measurement with sinusoidal wave method in the nF range.

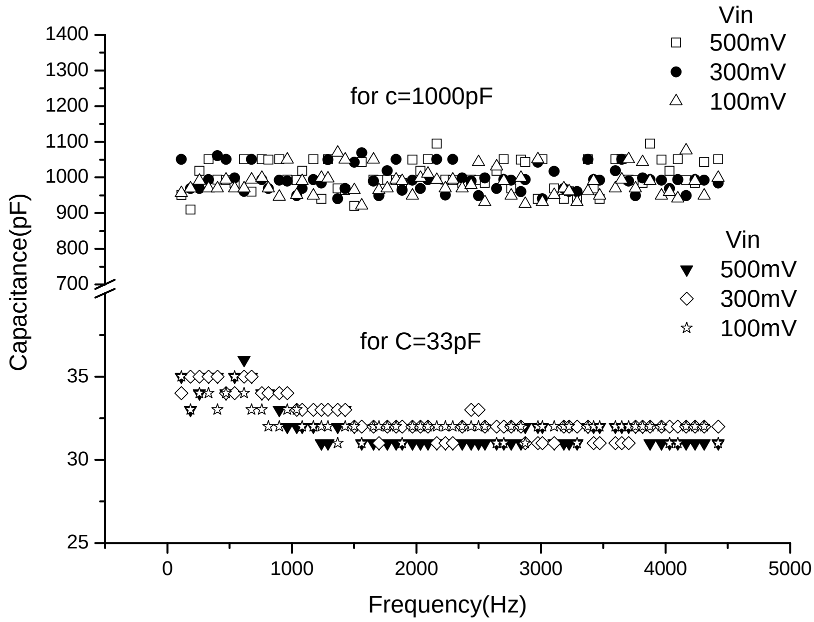

Fig. 3. Capacitance Measurement with sinusoidal wave method in the pF range.

Measurements on the same capacitors are conducted with Quad Tech 7600 Precision LCR meter and the results are compared with those from the sinusoidal wave method. Table 1 shows the comparison of errors in capacitance measurements with both the methods. Accuracy in capacitance measurements is ± 5 %.

Percentage errors in Table 1 show that the sinusoidal wave method performs well in the range of capacitances for biosensor applications. Therefore it is used for the measurements with the capacitance type biosensor. R, C values in the differentiator circuitry of the instrumentation are tuned to select the range of capacitance measurements whereas, the frequency range is decided by the R, C values at the MAX038 waveform generator chip. The instrument is tested with a capacitor in the appropriate range before conducting the glucose measurements.

Table 1. Comparison of Capacitance Measurements.

Capacitance	% Error	
	Developed Instrument	Quad Tech 7600
33 pF	± 5.0	± 4.5
1 nF	± 4.0	± 4.0
100 nF	± 4.0	± 3.0
220 nF	± 3.2	± 1.25

2.3. Glucose Biosensor

Capacitance biosensor (Pulsatom India Pvt Ltd) used here is an enzymatic biosensor with Glucose oxidase immobilized on the nylon matrix housed in between simple parallel plates(fingers) deposited on a polymer base. Glucose oxidase catalyses the reaction of glucose (Dextrose) with oxygen selectively there by enabling a capacitance change which is measured with the developed instrument.

3. Experimentation

Instrumentation is used for the measurements with glucose biosensor in the selected range. The measured capacitance is an indication of the concentration of glucose in the solution. Measurements of capacitance of the biosensor are conducted at various bias voltages and the behavior of the biosensor capacitance is observed. The capacitance versus bias voltage characteristic studies helped in selecting the appropriate DC bias voltage for glucose measurement.

For finding the concentration of unknown glucose samples in the known range, a standard curve for glucose measurement using the capacitance instrumentation is plotted. This is plotted by measuring the capacitances of known concentrations of glucose. Unknown glucose concentrations are measured with the instrument by measuring the capacitance and relating the capacitance value to a concentration in the standard curve.

UV-Visible absorption spectroscopy measurements are used to measure the unknown glucose concentrations alternatively. Phenol-Sulphuric acid assay method is used for this purpose. UV measurements are made after conducting the Phenol-H_2SO_4 method for determining the glucose concentration. For this 0.5 ml of 5 % aqueous phenol, 2.5 ml of concentrated sulfuric acid (98 %) are used along with various concentrations of glucose to obtain the standard curve of absorption versus concentration from the UV-Visible spectroscopic measurements at 490nm. The unknown glucose samples are subjected to Phenol- sulphuric assay method and the resultant orange-yellow colored compound is subjected to UV – Visible spectroscopy and the absorption values are correlated to the concentration values from the standard curve.

4. Results and Discussion

Fig. 4 shows the variation in measured capacitance with applied bias voltages at 200, 300,400 mg/dL of glucose concentrations.

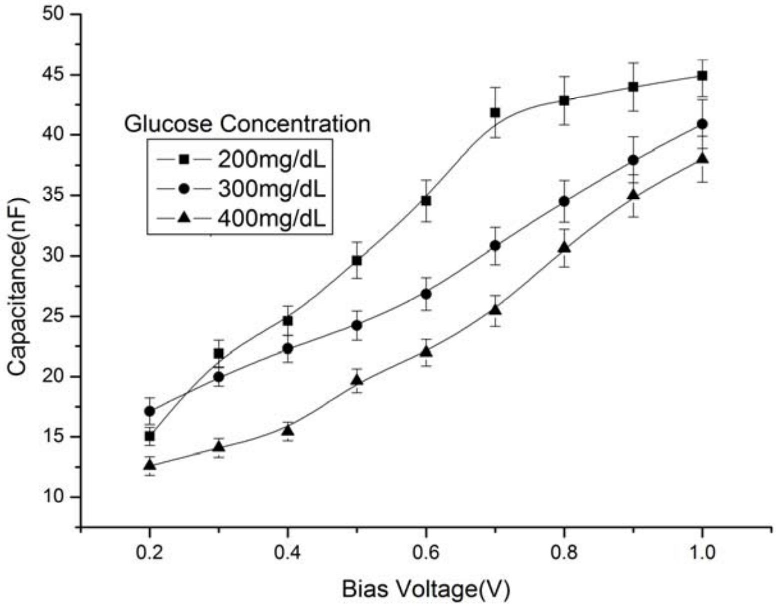

Fig. 4. Variation of capacitance of biosensor with Bias Voltage.

These characteristics are useful in deciding the appropriate bias voltage of operation for biosensor measurements. From the characteristics, it is noted that the bias voltage values in the 0.5 to 0.7 range are appropriate for capacitance measurements with the biosensor. These results suggest that the change in capacitance is due to oxidation of H_2O_2 around 600 mV [9, 10]. Frequencies in the range 10 Hz to 10 kHz are good for measurements with the electrochemical biosensors and the double layer capacitance effects dominate for frequencies above 10 kHz [9, 11]. Therefore, appropriate bias voltage and frequency are selected for measurement of glucose with the capacitance type biosensor.

By taking the known values of standard glucose solutions, with the help of the instrumentation, a standard capacitance Vs concentration curve is plotted and shown in Fig. 5.

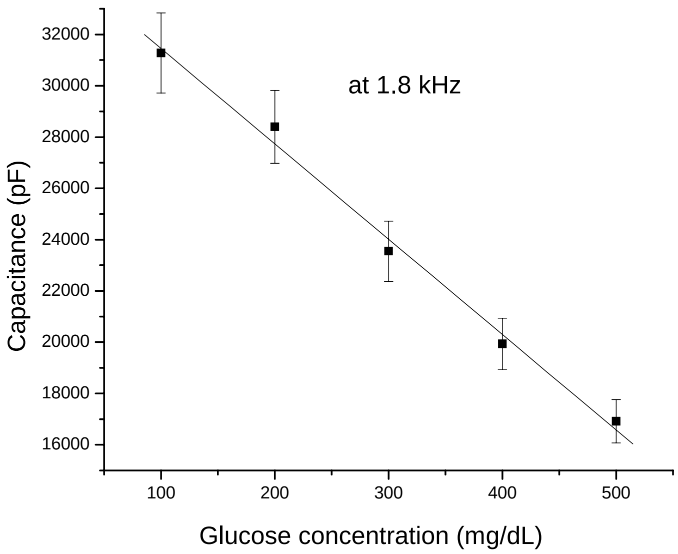

Fig. 5. Capacitance vs. Glucose Concentration.

Appropriate bias voltage and frequency are selected to avoid the double layer capacitance effects at the fingers of the capacitance biosensor. The capacitance variation with glucose concentration follows a linear path with a negative slope. The capacitance calculated is in accordance with the glucose oxidase catalyzed reaction of glucose with oxygen. Current through the sensing circuitry with the application of input sinusoidal signal is used to calculate the capacitance values. Once the standard curve is plotted with a good degree of accuracy, the unknown concentration of glucose is measured by measuring the capacitance and finding the appropriate value of concentration from the standard curve. Glucose measurements are also conducted with Phenol-Sulphuric acid assay method. Finer details about the instrumentation and glucose measurements with the sensor are documented in MSc (Eng.) thesis [12].

Fig. 6 compares the obtained results for measurements of 150, 250, 350, 450 mg/dL glucose concentration with capacitance method and phenol sulphuric acid assay method.

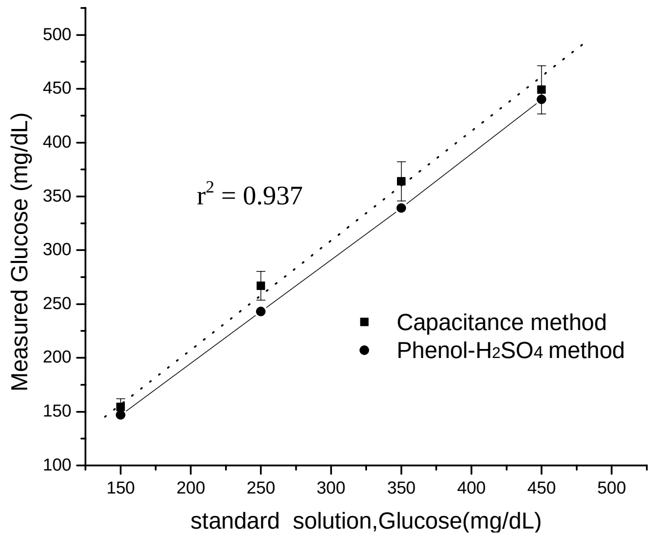

Fig. 6. Comparison between Capacitance and Phenol - Sulphuric acid methods.

The obtained results are very much acceptable for the measurements in the following ranges which include the blood glucose levels.

5. Conclusions

Micro controller based capacitance measuring instrumentation is developed. With this, Glucose measurement is successfully demonstrated with the capacitance based glucose oxidase biosensor in the range of measurements which includes the blood glucose levels. Accuracy in capacitance measurements is found to be ± 5 % and is in agreement with the current state-of –art instrumentation available. Measurement resolutions and the accuracy of the glucose measurement using this method could further be improved with the use of interdigitated sensors as capacitors implementing this scheme. The current instrument can be used for the applications involving cell based biosensors, to study bacterial growth, for capacitance based humidity sensors with appropriate standard curves developed for each of the methods mentioned.

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Guide for Contributors

Aims and Scope

Sensors & Transducers Journal (ISSN 1726-5479) provides an advanced forum for the science and technology of physical, chemical sensors and biosensors. It publishes state-of-the-art reviews, regular research and application specific papers, short notes, letters to Editor and sensors related books reviews as well as academic, practical and commercial information of interest to its readership. Because it is an open access, peer review international journal, papers rapidly published in *Sensors & Transducers Journal* will receive a very high publicity. The journal is published monthly as twelve issues per annual by International Frequency Association (IFSA). In additional, some special sponsored and conference issues published annually. *Sensors & Transducers Journal* is indexed and abstracted very quickly by Chemical Abstracts, IndexCopernicus Journals Master List, Open J-Gate, Google Scholar, etc.

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Contributions are invited on all aspects of research, development and application of the science and technology of sensors, transducers and sensor instrumentations. Topics include, but are not restricted to:

- Physical, chemical and biosensors;
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- Smart sensors and systems;
- Sensor instrumentation;
- Virtual instruments;
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- Nanosensors;
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