Chapter 6

Discussion of Results & Conclusion

Consistent increase in type II diabetes cases globally has made quantification of hormone especially insulin a critical requirement in health care sector [1][2]. Complexity of conventional assay methods, long turnaround time and skill required to carry out the analysis have put these methods under doubt for able to meet the future requirement from health care industry [3]. To address the epidemic growth of type II diabetes and for early diagnosis, control and monitoring, an on the fly, simple and robust detection technique is required. Various methods have been reported on detection of insulin by electrochemical and modified electrode sensor for concentration ranging from few nano-moles to 1000 micro-moles [4]-[7]. But methods capable of detecting insulin or any hormone in less than nano-volume concentration in a liquid is challenging though a necessary requirement for possible application in diagnostics. As discussed in chapter 1, according to clinical data available, insulin level can be divided into the three groups viz. for healthy humans fasting Insulin level can lie between range 5-20 µIU/ml, for nonfasting case can lie between 20 to 60 µIU/mL and in abnormal conditions insulin level will be greater than 60 µIU/mL [3][7][8]. Abdollah Salimi et.al have reported a brief experimental
study on quantifiable technique for picomolar (pM) concentration of insulin in phosphate buffer solution [7] but sensitivity of modified electrode sensors generally falls due to decay in response current with time. At high frequencies Shaforost et.al have demonstrated a characterization technique of organic molecule and glucose in a solution ranging from 10 % to 100% concentration [9][10], authors have used waveguide exited whispering gallery mode (WGM). WGM are very high-Q modes which is essential for detection of low concentration of analyte in nanoliter volume of solute using quartz and sapphire. In the present research work a single crystal sapphire WGM dielectric resonator (DR) is used with a low cost polycarbonate use-and-throw type sample holding disk (SHD) for quantifiable of insulin in pM concentration ranging from 35 pM to 378.78 pM in 25 mM Hepes buffer solution as discussed before this range spam the maximum and minimum concentration range of insulin found in human blood.

6.1 Discussion of Results obtained with 1mm and 0.5 mm ring in SHD for composite WGM-DR system

For present research work WGE₈₀₀ mode in composite DR is selected for analysis of Insulin in solution. From chapter 4, it is observed that at this mode maximum field concentrated near the air and dielectric boundary of composite DR and this leads to higher sensitivity in that region. Thus, on the basis of EM field’s patterns, position of ring in SHD is optimized and two types of SHD with ring of 1mm and 0.5 mm width are etched. Simulation and experimental results obtained with both the SHD are given in chapter 5 now in the following subsections I will discuss and compare these results in brief for these two SHD:
6.1.1 Explanations of obtained results on the basis of calculated parameters.

(a) \textit{Q-factor of resonance peak}

From figure 5.3 and 5.7 it is observed that measured change in inverse quality factor increases as concentration of Insulin sample loaded on ring of SHD changes from 35 pM to 378.78 pM in 25 mM Hepes buffer solution. Furthermore, corresponding to variation in concentration of Insulin solution approximately no shift in frequency is observed. These observations can be explained using perturbation theory.

According to perturbation theory, the change in inverse quality factor is proportional to $\tan \delta$ which is found to be proportional to $\omega \tau$, it is expected that change in inverse quality factor should exhibit dependency on $\tau$, for constant shift in frequency. As relation between the viscosity and relaxation time according to Stokes-Einstein-Debye equation [11] is given by

$$\tau = \frac{\pi \eta r l^2}{k_B T}$$ \hspace{1cm} 6.1

In equation 6.1, $l$ is hopping length, $\eta$ is viscosity, $r$ is molecular radius, $k_B$ is Boltzmann constant, and $T$ is temperature. Here $\eta = \eta_w (1+\eta_r)$ and according to Einstein [11] for extremely low concentration solutions relative viscosity can be written as $\eta_r = 2.5k\phi$, where $\phi$ represent the concentration of solute. Therefore from equation 6.1, $\tau$ is directly proportional to $\eta$ which leads to dependency of $\tau$ on concentration on Insulin solution, as $\tau$ increases with concentration and so is the change in inverse Q-factor.
(b) **Real and Imaginary permittivity of sample solutions**

Inset of Figure 5.4 shows real part of permittivity that does not change with Insulin concentration in pM for the three volumes whereas from figure 5.8 it is observed that for 0.2 and 0.4µL real permittivity do not change. Thus, it is clear that very low concentration of solute in solution does not affect the resonance frequency leading to almost constant value of permittivity at given volume, whereas for 0.8, 1.2, 1.6 µL volume it exhibit slight decrease in real permittivity.

Imaginary permittivity of solution linearly depends on change in inverse quality factor (see equation 5.15) this is evident from figure 5.4. This indicates that imaginary permittivity will also increase as a function of insulin concentration in solution or change in inverse quality factor is directly measure of losses in sample solution.

(c) **Time delay**

Time delay due to sample droplets on the ring of both types of SHD is calculated in picoseconds and it is found that calculated time delay usually decreases with the increase in Insulin concentration in solutions and exhibit change in slope of curve for 200 pM to 250 pM Insulin concentration (see figure 5.5 & 5.9). Cause of this variation may be that as number of molecule increases more than 200 pM concentration of Insulin in solution wave takes more time to come out due to increase in collisions between corresponding molecules of Insulin. Whereas, the range of time delay is found to be depend on droplets position in ring of SHD as evident from figure 5.9.
6.1.2  Towards real time Insulin sensor

According to the instrument society of America sensor can be defined as follows [12]:

“A device which provides a useful output in response to a specified measurand”

For real life application of any biosensor must meet certain criteria like linear device response, selectivity, sensitivity and response time. Thus, in the following subsections these criterions are discussed and compared for the technique developed here:

(a)  Linearity

First criteria of good sensor are that calibration curve for the response of measured values from sensing device should be linear in order to determine the concentration of substances and other parameters of biological interest. For present research work it is found that measured losses are sensitive for variation in Insulin pM concentration. From chapter 5 with reference to figure 5.3 and 5.7 response are fairly linear for measured change in inverse quality factor as function of pM concentration of Insulin in solution. Thus, present device follows linear response characteristics of biosensors.

Similar studies are also done on developing Insulin sensing device using modified electrode measuring technique [4]-[7], [13]. Obtained calibration curves for these devices show linear response as a function of Insulin concentration for different working range but these exhibits aging problem as with the time oxidation current on electrode decreases due to which calibration curve drift.
(b) **Sensitivity**

Sensitivity of any biosensor is given by measurable response value of device per analyte concentration. For present research work sensitivity of composite DR system with two types of SHD for different volumes are calculated and shown in table 5.4 and table 5.6. According to these results measured losses with WGM-DR is sensitive to every 10pM change of Insulin concentration in sample solution.

(c) **Detection limit**

Detection limit of any biosensor is also an important characteristic which gives the smallest concentration of analyte that can be detected. For present research work lower detection limit selected for Insulin concentration is 35pM as in normal conditions Insulin level in human blood cannot go below this value. Therefore, for developing a real time Insulin sensor value of detection limit 35pM is sufficient.

(d) **Specificity**

Specificity (or selectivity) is a measure to which response of sensing device can be measured without any interference or with minimal interference. For this in present research work, all the other substances used for preparing sample solution are kept constant. Furthermore, effect of temperature on device is found negligibly variable at room temperature during the experimentation. Thus, all the interfering factors are kept minimal variable.

(e) **Dynamic range**

Upper and lower levels of detection set by dynamic range of sensor. Furthermore, for biosensor it is very important as various biomolecule are found in
particular range or concentration in human. Due to this reason in the present work Insulin concentration ranging from 35 pM to 378.78 pM in 25 mM Hepes buffer solution is used as it is correlated to actual range of Insulin found in human blood.

(f) **Signal stability**

In constant environment of sensor device it is necessary that output signal response should not drift (in absence of sample) because it will affects directly the response of sensor when sample is loaded. In present research work it is observed that in constant conditions output signal values i.e. output resonance of composite resonator (without sample) does not change usually.

(g) **Speed of response and Recovery time**

Speed of response can be defined as time required for a sensor output to change from its previous state to a final settled response. In present research work device is operated manually though it takes hardly 5-10 seconds to load sample and takes less than 5 seconds to give response. Furthermore, recovery time is time required to set sensor for next measurement after interacting with sample solution and in present work only 2-3 seconds required to replace used SHD.

(h) **Reproducibility**

In the present research work, during multiple experimentation approximately 7.84% variation is observed for values of difference in inverse quality factor with same Insulin concentration sample with 1mm ring SHD, whereas using 0.5 ring SHD 10.44% variation is observed. These, results are based on various observations done on different days in our laboratory.
(i) **Immunity to environment**

Change in environment conditions like temperature and humidity affect the response of sapphire dielectric resonator, due to which slight change in Q-factor and resonant frequency observed. Because of this temperature is required to keep constant or minimal variable throughout the experimentation.

(j) **Cost & Size of sensing Device and Amount of sample used**

Above all for a commercially viable biosensor, it should be cheap, small in size, handy and most importantly amount of sample required for testing should be minimal. For device presented in this thesis a single crystalline sapphire is used with very cheap polycarbonate disk and sample volume required for testing is 0.2 µL – 6.4 µL. Testing device used is small and it is portable but used measuring devise (network analyzer) is bulky. However, the whole device can be made handy for a required testing range after doing further optimization and finalization of device design and sample volume used for testing Insulin level in blood.

On the basis of above comparison it can be said that the present WGM-DR method can be a replacement for conventional Insulin assay method.

In table 6.1 several devices or sensor reported for Insulin level testing are compared including present work on the basis of different properties required to be follow for good biosensor. From this comparison table it is clear that all the devices have ability to sense Insulin but present work seems better as for real time application these devices have limitations, as more sample volume required, dynamic range is different and required large overall operating and response time.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Method</th>
<th>Sensitivity</th>
<th>Detection limit</th>
<th>Response time</th>
<th>Sample volume required</th>
<th>Size of sensing device</th>
<th>Working Range</th>
<th>Comment on range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present work</td>
<td>WGM-DR with SHD</td>
<td>Shown in table 5.4 and 5.6</td>
<td>35 pM</td>
<td>5 sec</td>
<td>0.2µL-6.4µL</td>
<td>48mm×48mm×21mm</td>
<td>35 pM-378.78 pM</td>
<td>Lies in Human Insulin range</td>
</tr>
<tr>
<td>[5]</td>
<td>PSA</td>
<td>20ms/0.2µM</td>
<td>20 nM</td>
<td>120 sec</td>
<td>-</td>
<td>33.5mm×101.5mm</td>
<td>100nM-600nM</td>
<td>Not lies</td>
</tr>
<tr>
<td>[6]</td>
<td>MWCNTs modified electrode</td>
<td>50nA/100µM</td>
<td>1µM</td>
<td>-</td>
<td>3µL</td>
<td>1mm sensor head</td>
<td>1µM-1000µM</td>
<td>Not lies</td>
</tr>
<tr>
<td>[7]</td>
<td>Amperometry</td>
<td>100.9pA/pM</td>
<td>22pM</td>
<td>3 sec</td>
<td>-</td>
<td>-</td>
<td>100pM-4µM</td>
<td>Partially lies</td>
</tr>
<tr>
<td>[13]</td>
<td>EIS</td>
<td>1.5R&lt;sub&gt;ct&lt;/sub&gt;/5pM</td>
<td>1.2 pM</td>
<td>30 min</td>
<td>Dip in solution</td>
<td>3 electrode of 1.6mm diameter</td>
<td>5pM-50nM</td>
<td>Includes Insulin level in human</td>
</tr>
</tbody>
</table>

PSA - Potentiometric Stripping Analysis, EIS - Electrochemical Impedance Spectroscopy, R<sub>ct</sub> - Normalized Charge-Transfer Resistance
6.2 Conclusion

Present work is focused on the development of a microwave testing method to sense and quantify the presence of pM concentration of Insulin in hepes buffer solution. WGM-DR is found advantageous as modal fields confined within the small region near the resonator boundary of cylindrical DR at higher modes and makes it more sensitive in that region. Therefore, composite WGM-DR system is designed and optimized through MATLAB programming and simulations on CST Microwave Studio. Based on this approach single crystalline sapphire DR with two types of very low cost disposable polycarbonate SHD are experimentally analyzed with various concentration of Insulin solution. Throughout the experimentation it is considered that presented Insulin testing device should fulfill all the basic need of a practical biosensor.

Developed composite resonators for WGE$_{800}$ mode is resonating in Ku band of microwave frequency. This microwave resonator takes hardly 5 seconds to respond for any perturbation, calculated values of change in inverse quality factor exhibits linear dependence on Insulin pM concentration with both the SHD loaded by different micro liter volume of sample. Measured, results shows that measured losses are sensitive to pM concentration of Insulin in solution with nearly constant shift in frequency for particular volume loaded on SHD. Furthermore, calculated time delay exhibits change in slope of curve as concentration of Insulin increases from 200 pM value. Dynamic range of this resonator for testing Insulin concentration lies in same range as found in human blood with 35pM lower detection limit. Therefore, presented high-Q microwave sensing technique has all the essential characteristics of Insulin (a biomolecule) sensor for possible application in health care sector to address the global
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demand for rapid detection of insulin. Thus, it can be seen as future Insulin sensing device for real time application in place of conventional assay method. Results presented in this thesis can also be a fundamental basis towards the detection and quantification of various hormones and other pathological parameter.
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