Understanding the decay of Proteins: A Study of Time dependent Microwave Response of pM concentration of Insulin in Buffer Solution

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Bio-molecule when isolated from its natural ecological condition is subjected to rapid decay. This decay leads to change in polarization of the molecule and the permittivity. This study presents an experimental analysis of the decay pattern of pM concentration of insulin using whispering gallery mode (WGM) dielectric resonator method. Analysis is carried out by comparing the permittivity, relaxation time and time delay for three days. It is observed that different pM concentrations of insulin solutions start to decay after 24 hours at 5^0C.

Keywords: insulin, buffer solution, whispering gallery mode, dielectric resonator.

I. INTRODUCTION

Study of the response and stability of various bio-molecules in various buffer solution is important [1]-[5], because most of the biological systems such as protein are active in aqua medium, and it is found to closely mimic *in vivo* environment in which protein can be handled. Due to this usually protein solutions are prepared in buffer at high concentration preferably greater than 1 mg/ml for storage, as below this concentration protien degrading becomes comparatively faster [6]. One such important protein is insulin, as it plays a primary role in controlling of human metabolism and is found in picomolar (pM) concentration in human blood. There are many reports on quantification of insulin [7]-[10] but very low concentration of insulin makes such studies very challenging.

Whispering gallery mode (WGM) in dielectric resonator (DR) is well known to have high sensitivity and high Q-factor and have been used for various sensing applications [11]-[12]. In the present study a composite single crystal sapphire DR with a use-and-through type low cost polycarbonate sample holding disk (SHD) is used to study the activity of pM concentration of insulin 25 millimolar (mM) Hepes buffer solution at 17 GHz microwave frequency for WGE_{800} mode. Hepes buffer with 35 pM to 378.78 pM concentration of insulin is used to measure permittivity and relaxation time.

II. EXPERIMENTAL RESULTS AND ANALYSIS

All the experimental observations of the present research work were carried out using Rohde & Schwarz ZVA 50 vector network analyser. For taking experimental results a composite DR is used for which a c-axis oriented single crystal sapphire of dielectric constant 11.5, height 8 mm and diameter 20 mm, was purchased from CrysTech GmbH, Germany and it was composited with a SHD of width 1mm and diameter 20 mm having a ring cavity of width 0.5mm and 0.5 mm depth near the rim of the disk. In experimental measurement sapphire DR was found resonating at 17.59 GHz with Q value 86,122 at room temperature for WGE_{800} mode and composite DR was measured resonating at 17.5485 GHz with a 17,000 Q-factor. Furthermore, calculated loss tangent of sapphire is 3.6×10^{-6} and for SHD is 0.00708.

To study the activity of insulin pM concentrations in solution particular volumes of sample was loaded in 0.5 mm ring of SHD. For this various insulin solutions of desired concentration in the range found in human blood were prepared by diluting 10 mg/mL Insulin Solution (Human recombinant) in 25 mM Hepes buffer solution, both were purchased from Sigma Aldrich. For present study different volumes of 0.2, 0.4, 0.8, 1.2 and 1.6 µL quantities with different concentration of insulin were analysed to study the dielectric relaxation and the time delay behaviour of the pM concentration solution of insulin. These obtained results are discussed in detail as follows:

A. Real and imaginary permittivity of insulin solution

The thought behind the present research work is that with the change in the constituents of solution permittivity and other characteristics of the solution will change and these properties will also depend upon the state of activity of the present biological molecule in solution. Thus, by evaluating these properties it is possible to identify the activity period of any biological material in *in vitro* medium. In the present study real and imaginary parts of

permittivity of different insulin concentrations in hepes buffer solution was calculated by using the cavity perturbation method, according to which real and imaginary part of permittivity can be given by following equations [13]:

$$\frac{f_{SHD} - f_{soln}}{f_{SHD}} = A_1(\varepsilon'_{soln} - 1)\frac{V_{soln}}{V_{DR+}V_{SHD}} \quad (1)$$

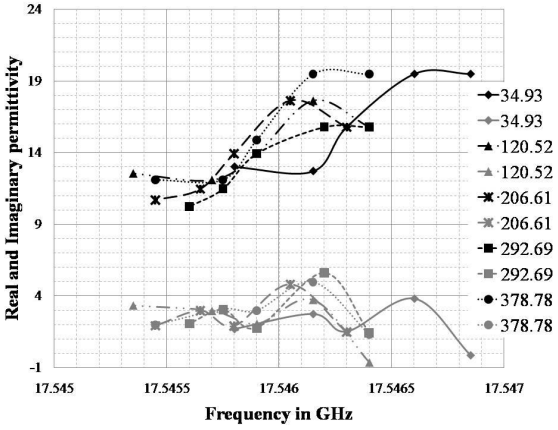
$$\frac{1}{Q_{soln}} - \frac{1}{Q_{SHD}} = A_2\,\varepsilon''_{soln}\,\frac{V_{soln}}{V_{DR} + V_{SHD}} \quad (2)$$

Here ε'_{soln} and ε''_{soln} represents real and imaginary permittivity of solution, *f*, *V* and *Q* represents the frequency volume and quality factor corresponding to its suffix. A_1 and A_2 are constants and calculated values for these are 0.01097 and 0.01903 respectively. But according to Debye dielectric theory real and imaginary part of permittivity of solution can be given by following equations:

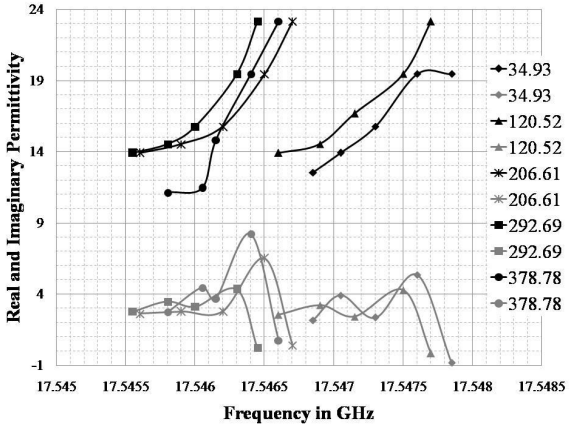
$$\varepsilon'_{soln}(\omega) = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + \omega^2\tau^2} \quad (3)$$

$$\varepsilon''_{soln}(\omega) = (\varepsilon_s - \varepsilon_\infty)\frac{\omega\tau}{1 + \omega^2\tau^2} \quad (4)$$

Here, ε_s and ε_∞ represents the static permittivity and $\varepsilon_\infty = \lim_{\omega\to\infty} \varepsilon'(\omega)$. According to Debye equation (3) and (4) it is clear that permittivity does not depend on sample volume, thus calculated permittivity values using different volume placed on SHD of different pM concentration of Insulin are used to see the frequency dependency of permittivity of this solution (see figure 1 (a) (b) & (c)) and to plot a cole - cole type graph using the corresponding real and imaginary part of permittivity (see figure 2(a) (b) & (c)).



(a)



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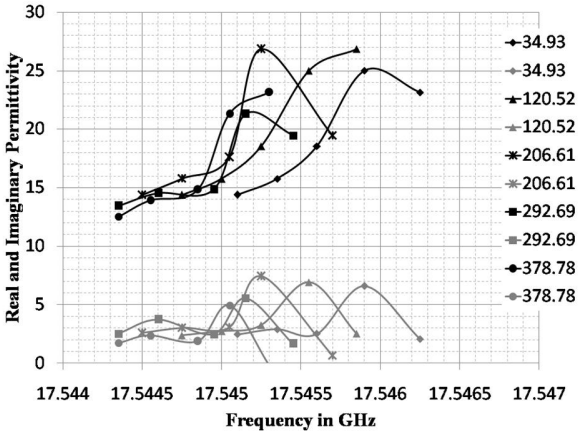
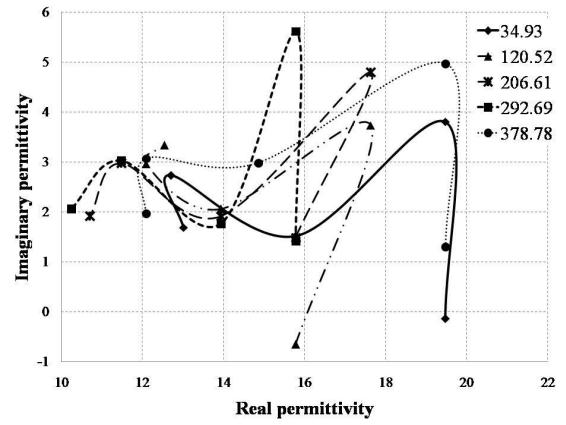


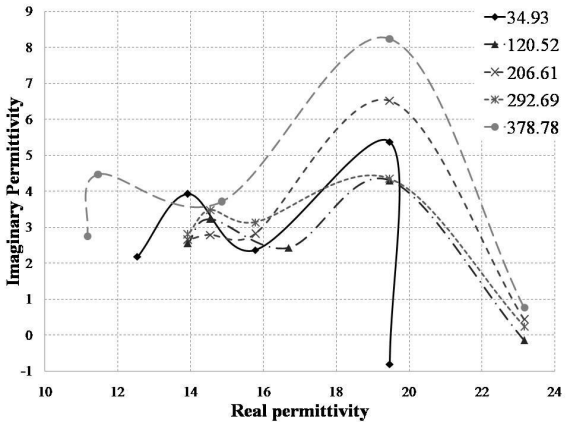
(c)

FIG.1. Real (black) and imaginary (grey) part of permittivity as a function of frequency for (a) fist day data, (b) second day data (c) third day data.

From figure 1 it is observed that when real permittivity is frequency dependent, losses (imaginary permittivity) are showing its maximum value, this indicates that results follow characteristic standard dispersion curve of biological tissues. Furthermore, it also shows that real permittivity of different insulin concentration exhibits similar behaviour for which real permittivity is increasing with increase in frequency similarly imaginary permittivity also shows alike behaviour. Moreover, on comparing the results obtained for three days data shown in figure 1 it is observed that obtained values for real and imaginary permittivity for different concentration solution coincides with each other for first and third day data whereas for second day data it shows a consistent shift in frequency with increase in insulin pM concentration. Using the calculated values of real and imaginary permittivity with increase in resonant frequency a cole – cole graph is plotted in figure 2 for three days data.



(a)



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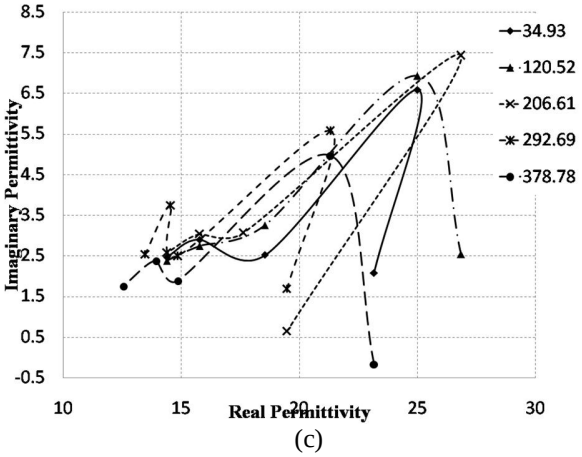


(c)

FIG.2. Cole-Cole type plot between real and imaginary part of permittivity for insulin solution for two days continuous measurement for (a) fist day data, (b) second day data (c) third day data.

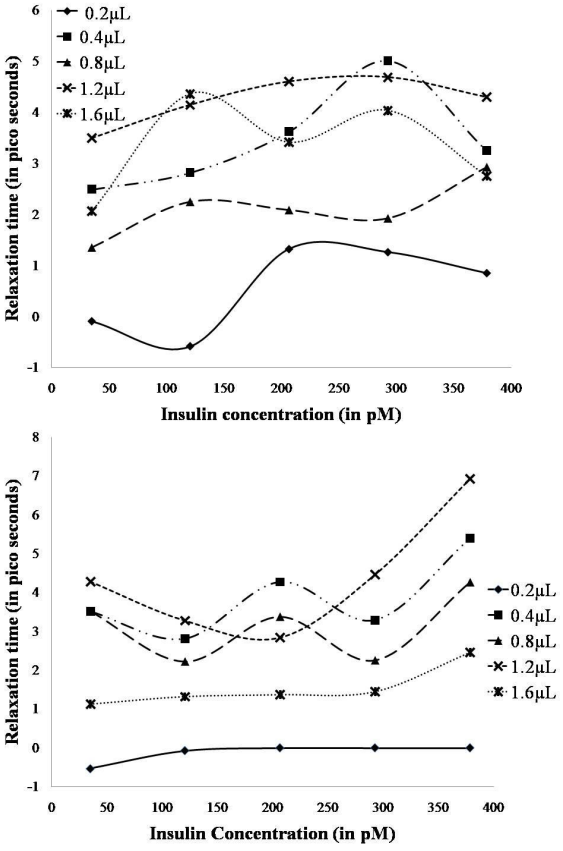
From figure 2 it was observed that the data plotted in figure 2 (b) show a consistent increase in covered area by plot with increase in concentration of insulin in solution. Whereas graph plotted with first and third day data exhibits random values with approximately the same covered area by plots with the increase in insulin concentration.

B. Dielectric Relaxation Study of Insulin Solution

From the measured values of permittivity for different concentration of same solution for three days it is observed that data taken on second day shows consistent behaviour. To further understand these results relaxation time for the same measured data was also calculated using the Debye equations (3) and (4). Therefore, from these equations relaxation time of solution can be written as follows:

$$\tau = \frac{1}{2\pi f} * \frac{\varepsilon''_{soln}}{\varepsilon'_{soln} - \varepsilon_{\infty}} \qquad (5)$$

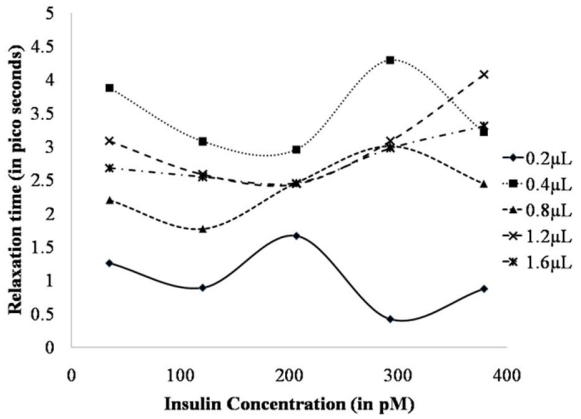
where $\omega = 2\pi f$ represents the resonant frequency as the concentration of Insulin is very low thus ε_{∞} of solution can be taken same as for water. Thus, using $\varepsilon_{\infty} = 5.6$ as found for water [14] at room temperature, relaxation time of solution is calculated by using equation (5).



(b)

Calculated relaxation time is plotted as a function of insulin pM concentration for three days data available shown in figure 3 (a) (b) & (c) and from this it is observed that for second day data with the increase in insulin concentration relaxation time is also increased for all volumes loaded on SHD with the increase in insulin concentration, however for the first day and third day data random results are

obtained for calculated relaxation time with all volumes loaded on SHD.



(c)

Figure 3. Relaxation time as a function of Insulin concentration for (a) fist day data, (b) second day data.

III. DISCUSSION OF RESULTS

in vitro time dependent degradation and stability study of proteins and insulin in medium have been carried out by various researchers to estimate the activity of biomolecule in solution with variation in other constituents of buffer solution and due to other environmental factors [6], [15]-[18]. This experimental study investigates the decay patern of insulin in hepes buffer solution on diluting up to 35 pM concentration. Experimental measurement were spread over three days. On comparing the results for each day it is observed that on first day there is no consistent pattern wheras on the second day consistency was observed in measurement of varying concentrations of insulin. Therefore, it can be concluded that solution takes 24 hours to stabilize. It has been reported that 24 to 37 hours required for homogenisation at 4^{o}C [15] on diluting the protein solution and results obtained here also follows the same. for same insulin solutions. On third day experimental data with same samples of insulin solution again showed random and similar results for all the concentrations of insulin. This indicates that now results are According to Basey et.al [19] it was found that for lower concentration of proteins in solution, ratio of change is static permittivity to change in relaxation time does not depend on change in concentration of protein and only depends on particular protein present in solution. Results presented here was observed that with the increase in insulin concentration there was an increase in permittivity and relaxation time on the second day data. Thus, data observed shows actual results with the increase in insulin concentration.

IV. CONCLUSIONS

Analysis presented in the present research work is to identify the activity of insulin solution with time and for this experimental data for three days are collected and analysed on the basis of calculated value of permittivity, relaxation time and time delay. From these analysed results it was observed that when an insulin solution of pM concentration is prepared in buffer solution it requires 24 hours to be saturated before taking experimentation but after two days it starts decaying as insulin have very short life even if we place solution at 5^{0}C in between time. Furthermore, in the present letter a microwave WGM- DR method is presented which is capable of estimating insulin pM concentration in hepes buffer solution, and can identifying the activity of insulin pM concentration in hepes buffer solution. Moreover, results presented in this letter also shows that by measuring the permittivity, relaxation time, and time delay it is possible to identify that particular biomolecule is in its active state or not.

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