MethodsX 4 (2017) 35–41

Contents lists available at ScienceDirect

MethodsX

journal homepage: www.elsevier.com/locate/mex

Method Article

Understanding the decay of proteins: A method to study time dependent response of pM concentration of insulin at microwave frequencies

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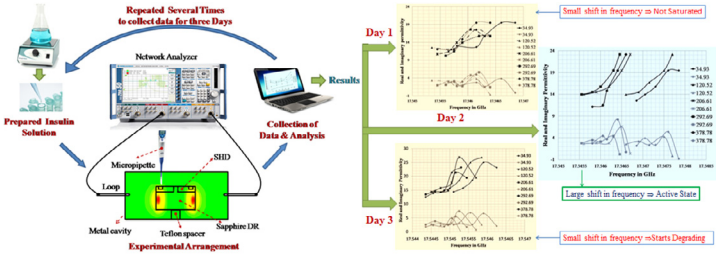
GRAPHICAL ABSTRACT



ABSTRACT

Bio-molecule when isolated from its natural ecological condition is subjected to rapid decay. This decay leads to change in polarization and permittivity of molecule. This study presents an experimental analysis of the decay pattern of pM concentration of insulin using whispering gallery mode (WGM) dielectric resonator (DR) 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 h at 5 °C. Salient features of the present method are:

- This method presents time dependent analysis to determine the activity of protein solution by measurement of permittivity, relaxation time and time delay.
- In the present paper activity of pM concentration of Insulin in buffer solution is tested for three days.

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http://dx.doi.org/10.1016/j.mex.2016.11.004

- This method is a general method and can be a fundamental basis to test the activity of bio-molecules in solution.

ARTICLE INFO
Method name: WGM DR method for time dependent activity analysis of insulin in buffer solution
Keywords: Insulin, Buffer solution, Whispering gallery mode, Dielectric resonator
Article history: Received 3 October 2016; Accepted 30 November 2016

Specifications Table

Subject area	*Materials Science*
More specific subject area	*Microwave Bio-sensing*
Method name	*WGM DR method for time dependent activity analysis of Insulin in buffer solution*
Name and reference of original method	*Microwave WGM DR sensing method (Ref. [11])*
Resource availability	*Not necessary*

Method details

Response of biomolecules in buffer is central in understanding the behaviour of biological systems in their native ecological conditions [1–5], because most of the biological systems such as proteins are active in aqua medium, and they are found to closely mimic their natural behaviour in a buffer medium *in vivo*. Due to this protein solutions are prepared in buffer at high concentration preferably greater than 1 mg/ml for storage. Below this concentration protein degradation becomes comparatively faster [6]. One such important protein is insulin, which plays primary role in controlling 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. To study the biological activity of picomolar concentration of biomolecule in a liquid, the method must be very sensitive to ultra small changes. 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 [11–15]. In the present study a composite single crystal sapphire DR with a disposable 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 35–378.78 pM concentration of insulin is used to measure permittivity and relaxation time.

Experimental results and analysis

All the experimental observations presented here were carried out using Rohde & Schwarz ZVA 50 vector network analyser. Composite dielectric resonator comprised of *c*-axis oriented single crystal sapphire puck with dielectric constant 11.5, height 8 mm and diameter 20 mm and a SHD of thickness 1 mm and diameter 20 mm having a ring cavity of width 0.5 mm 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 maintained at 5°C during experimentation. Temperature was continuously