Closure Report

File Number : EMR/2016/007445

Project Title : Study for label free optical detection of snake venom protein using Surface Plasmon Resonance technique

Principal Investigator : Dr. Biplob Mondal
Tezpur University
Distt. sonitpur p.b.no.72 napaam, tezpur, Tezpur, Assam-784011

Total Sanctioned Amount : 47,52,100 (INR)

Total Released Amount : 44,80,000 (INR)

Start Date of the Project: 10 Jul, 2018

Date of completion: 09 May, 2022 (46 months)

Approved Objectives :

- Design and development of portable SPR measurement setup for simultaneous detection of multiple protein
- Performance enhancement of the SPR chip like sensitivity, FOM with the introduction of conducting metal oxide based layer
- Proof of concept SPR measurement system with various IgG
- Study for qualitative and quantitative detection of complex protein samples

Deviation made from original objectives (If Any) :

NA

Ph.D. Produced/ Likely to be : 1

Technical Personnel Trained : 14

Total Expenditure : 44,70,798 (INR)

Concise Research Accomplishment :

The salient points of the finding in this research work are as under Label-free detection of Human Immunoglobulin-G (H-IgG) on a multiple protein-patterned SPR biochip using a portable SPR measurement device is done. H-IgG antigen in phosphate buffered saline fluid exhibited a detection limit of 15µg/ml and a linear response through a wide concentration range (15µg/ml to 225µg/ml) having a high coefficient of determination (R2=0.99661). Design of multi layer mathematical model of SPR sensor for improvement in its performance characteristics such as sensitivity using 2D material and metal oxide layer is done. Proposed 5 layer mathematical model design of SPR sensor consists of BK-7 prism, Zinc Oxide (ZnO), Gold(Au), BlueP-MoS2 and sensing medium providing higher sensitivity (220/RIU) in comparison to the 4-layer and 3-layer mathematical model having sensitivity values of 200/RIU and 180/RIU respectively. Low concentration detection of snake venom protein (Naja naja) in the range of 0.08-1µg/ml on polyvalent anti venom (1 µg/ml) is achieved. Study investigating improvement in hydrophilicity of PDMS substrates using dielectric barrier discharge oxygen plasma treatment at 40W (15kV, 2.66mA) for different duration of time ranging from 60-360s is done. Contact angle of the untreated PDMS chip was found to be 115.3±0.1(indicative of its hydrophobic nature) while that of the 240s plasma treated sample was found to be 50.2±0.3 (indicative of improved hydrophilic behavior).

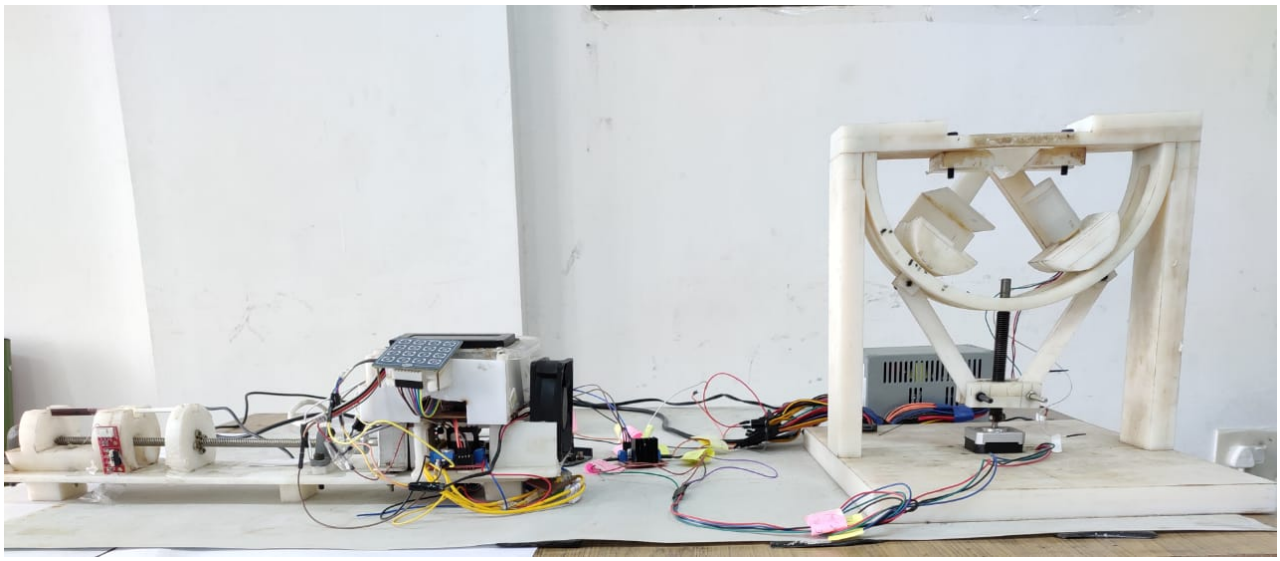
SNo.	CO-PI Details
1	**Robin Doley** doley@tezu.ernet.in Professor(Department of Molecular Biology and Biotechnology) **Tezpur University** Napaam, Tezpur, Assam, ASSAM, SONITPUR

Closure Details

Experimental/ Theoretical Investigation carried out

Fabrication of Experimental set up: The design and fabrication of a portable and robust Surface Plasmon Resonance (SPR) measurement prototype was established based on the Kretschmann's angular interrogation scheme. Fig. 1 shows the experimental set up of the portable SPR measurement device with syringe based fluid infusion system. Robust design and miniaturized structure was emphasized during the design process in order to eliminate possible handling error and error due to vibration, misalignment of optical components etc. The overall mechanical structure, mounts for optical components, all Allen bolts etc were fabricated in a CNC lathe machine (MTAB, Model: MAXTURN) using nylon material. The system also eliminates the use of expensive goniometric stages, linear array of photodiodes conventionally used in single-prism configuration. Primary focus was on achieving high precision with a wide angular scanning range. A horizontal beam (250mm length) was hinged to two vertical arms (250mm length) on either side standing atop a base of nylon sheet. An Allen Bolt (12mmdiameter, 120mmlength) was attached to the shaft of a microcontroller governed stepper-motor(3.2 Kg- cm torque). The body of the motor was embedded in the nylon sheet lying beneath to remove any effects of vibration occurring in the set-up. Two separate cylindrical stages were constructed to mount a laser and a image sensor. The cylindrical stages were hinged to two arms using Allen Bolts (5mmdiameter, 50mmlength) which were, in turn, screwed to an 'H-shaped' block embedded in the Allen Bolt. A triangular prism measuring 30mm x 30mm x 42.4mm (Edmund Optics) with its apex facing downwards was mounted on a platform over the horizontal beam. The position of the prism could be adjusted readily in order to achieve a wide scanning range of 30-80. A laser (632.8 nm, 5mW, 36mm length) affixed to a polarizer (20mm diameter) and a CMOS image sensor (512 pixel) were thereafter mounted on the two separate cylindrical stages. A few drops of index matching liquid (Refractive index 1.51, Norland Optical Adhesive) was then poured on the flat surface of the prism and the BK 7 glass substrate was placed on it with the metal film facing upwards in order to minimize the optical transmission loss. A syringe based fluid infusion system was used for passing a stream of samples over the surface of the sensor. Microcontroller driven stepper motor is used to automate the displacement of a syringe plunger. The rotational motion of the motor was converted into linear motion of the syringe piston with the help of a sliding support after coupling a linear slider to the shaft of the motor. The exertion of force at one end of the syringe piston enabled the infusion of fluids through a flexible tube. The system had a provision of mounting syringes of different volumes to flow liquids at various flow rates ranging from 60µL/min to 3000µL/min. Following the passage of samples over the sensor, the shaft of the programmable stepper-motor was used to turn the Allen Bolt in the clockwise direction causing the 'H-shaped' block to move upwards, which in turn results in synchronized angular increment of the laser and the camera at 0.005per step. The sensor was illuminated by p-polarised light after passing through the glass prism under the conditions of total internal reflection. The reflected light from the sensor chip was collected using the CMOS image sensor and recorded continuously. The clock and timing signals to the linear CMOS detector chip was provided using Arduino Uno which subsequently received the signal voltages from the detector, performed analog-to-digital conversion and serially transmitted it to the computer. The collected data were used for the analysis of resonance condition. All the measurements involving the calibration of the device were performed at room temperature. Methods: The portable surface plasmon resonance (SPR) measurement prototype was used for the label-free detection of Human Immunoglobulin-G (H-IgG) on a multiple protein- patterned SPR biochip. For the fabrication of the multiple protein patterned SPR biochip, BK7 glass substrates (50mm x 26mm x 1.25mm, n=1.51) coated with a thin layer of gold (Au) (~50nm) using DC magnetron sputtering was used. Prior to the deposition of gold ~5 nm titanium was deposited by RF magnetron sputtering to have better surface adhesion. Amine coupling chemistry was followed to bio-functionalize the gold coated substrates using 11-mercuptoundecanoic acid (11MUA), 1-ethyl-3 (3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS). The fabrication of polydimethylsiloxane (PDMS) based microfluidic channel was done next. The PDMS based microchannels were fabricated using an inexpensive technique that uses a master mold with copper wires affixed to a metal plate to replicate the microchannels measuring 20mm in length and 1.5mm in diameter. A mixture of silicone elastomer and curing agent was vacuum desiccated for two minutes and poured directly over the master mold to replicate the channel. The sample was heated in an oven at 100C for one hour followed by cooling at room temperature for a period of 45 minutes. The PDMS was then peeled off from the master mold after curing leading to the formation of three cylindrical shaped microchannels. This was followed by the covalent immobilization of polyclonal antibodies i.e, anti-Human immunoglobulin (aHIg-G) derived from mouse, rabbit, goat etc. over the gold coated SPR biochip through the PDMS microchannels to study the interaction capacity of the SPR sensor with Human Immunoglobulin-G (HIg-G). Thereafter, a multi layer mathematical model based on the Kretschmann angular interrogation technique was designed targeted at the improvement of some of the performance characteristics of the SPR biochip such as sensitivity, detection accuracy etc. The simulations were carried out in a MATLAB based programme for the analysis of the SPR Reflectance curve. The use of metal oxides etc. as different layers of materials in the model were explored in this regard. The research work also involved the optimization of the thickness of different layers in the multi layer mathematical model for the enhancement of the SPR signal. On the basis of the designed model, a suitable structure of SPR biosensor with improved SPR characteristics was proposed. Thereafter, the SPR biochip immobilized with polyvalent antivenom was tested for the detection of complex proteins such as snake venoms. Data collected: The SPR measurement prototype was calibrated using various standard solutions of known refractive index. The reflection intensity is measured using ethanol solutions with different concentrations viz., 2%, 4%, 6%, 8%, 10% and 12% to calibrate the SPR measurement device. Water is considered as the reference which produced a SPR dip at 46.1 for silver film. The SPR dips for different concentrations of ethanol namely, 2%, 4%, 6%, 8%, 10% and 12% occurred at 46.7, 46.9, 47.1, 47.4, 47.9 and 48.1 respectively. It is observed that the increasing shifts in the resonance angle occur with the increment in the index of

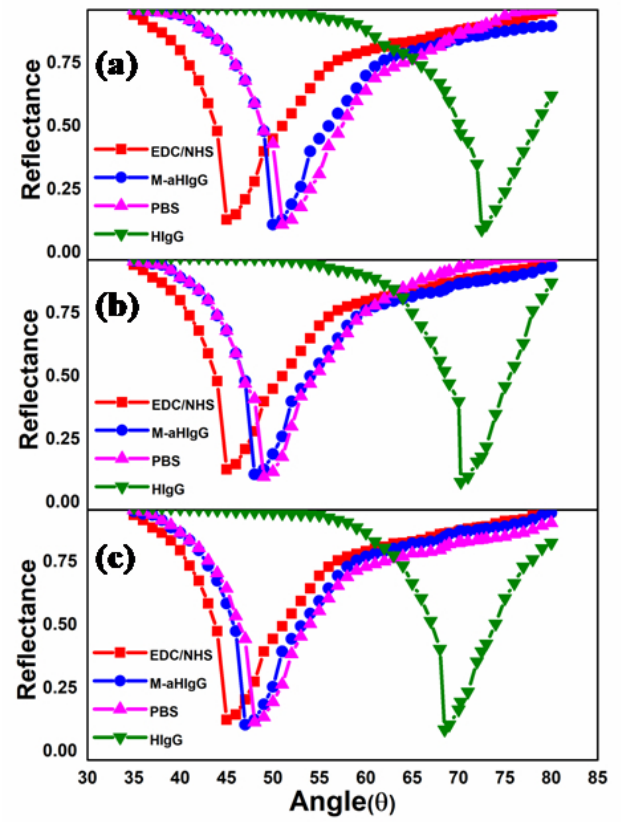
refraction of the ethanol due to the rise in its concentration. Thereafter Surface Plasmon Resonance (SPR) measurement prototype was implemented for the detection of antigen on a multiple protein patterned SPR biochip. The target antigen, Human Immunoglobulin-G (H-IgG) of different concentrations viz; 15- 450µg/ml prepared in PBS was thereafter injected through the microchannels immobilized with polyclonal antibodies viz, Mouse anti Human Immunoglobulin-G (M-aHIgG), Goat anti Human Immunoglobulin-G (G-aHIgG) and Rabbit anti Human Immunoglobulin-G (R-aHIgG) of different concentrations (500µg/ml, 750µg/ml and 1000µg/ml). Figure 2 shows the shift in the SPR angle due to the interaction of 450µg/ml H-IgG antigen with the SPR biochip immobilized with 500µg/ml anti H-IgG derived from Mouse (M-aHIgG), Goat (G-aHIgG) and Rabbit (R-aHIgG). The successive deposition and bonding among various stacked layers, viz., 11-MUA, EDC, NHS and the antibodies on the sensor surface results subsequent shifts in their resonance angle. The resonance dip at 68.5±0.25, 71.5±0.5 and 73.75±0.25 corresponds to interaction of H-IgG with antibodies derived from mouse, goat and rabbit respectively. The existing three layer mathematical model for the numerical analysis of SPR based on the Kretschmann angular interrogation technique comprising of prism, metal and sensing medium exhibit either low sensitivity or poor detection accuracy. Hence, in this research work design of multi layer mathematical models using MATLAB based simulation programme was carried out to propose suitable structure of SPR sensors with reasonably good performance characteristics. The role of different materials like oxides, two-dimensional (2D) materials etc. as different layers in the mathematical model were explored in this regard to target the sensitivity improvement of the SPR chip in addition to other performance parameters such as Full Width at Half Maxima (FWHM), quality factor etc. The simulations were carried out for three, four and five layer mathematical models with respect to different types of prisms of high refractive index such as BK7, SF10 and SF11. Some of the obtained simulation data is represented in Figure 3. Figure 3a, 3b and 3c shows the sensitivity and FWHM data for the three, four and five layer mathematical models for BK7, SF10 and SF11 prisms respectively. Finally to meet the last objective of the project, the SPR biochip was used for the detection of complex proteins such as snake venoms. Polyvalent antivenom of concentration (1µg/ml) was immobilized on the SPR biochip and exposed to different concentrations of crude venom in the range 0.01-1µg/ml to study the interaction capacity of the SPR sensor. Figure 4 shows the variation of the SPR resonance angle with the concentration of venom.



Detailed Analysis of result

Development of portable and easy to use systems for rapid diagnose of various biological fluids remains as an open area of biomedical research. Integrated biosensors capable of detecting the presence of multiple proteins/toxins in the biological fluids would ease medical diagnosis process for proper treatment, lab testing and quality control in food and allied industries etc. In this regard, the research work presents the development of optical biosensor and the associated measurement system to provide a proof of concept for highly sensitive detection of multiple proteins. The design of the portable SPR measurement prototype with a wide angular scanning range of 30-80and an angle scanning accuracy of 0.005eliminates the conventional requirement of expensive goniometric stages or photo-detector arrays used in SPR measurement prototypes involving bulky and complex instrumentations. The design architecture provides proper synchronization between the light source and detector by using only a single stepper- motor. The robust and miniaturized design with a simple stand-alone operation allows it to be operated by semi-skilled person in laboratories with limited facilities. The label-free detection of Human Immunoglobulin-G (H-IgG) on a multiple protein- patterned SPR biochip was achieved using a portable SPR measurement device. The target antigen, Human Immunoglobulin-G (H-IgG) of different concentrations viz; 15- 450µg/ml prepared in PBS were tested against polyclonal antibodies raised in Mouse, Goat and Rabbit immobilized in a SPR biochips and their interaction capacities were analysed. The SPR biochips demonstrated reliable detection of H-IgG antigen in phosphate buffered saline fluid with a detection limit of 15µg/ml and linear response through a wide concentration range (15µg/ml to 225µg/ml). The resonance shifts due to the interaction of 450µg/ml H-IgG with M-aHIgG immobilized with 500µg/ml, 750µg/ml and 1000µg/ml concentration respectively was compared and analyzed (Fig 5). The highest SPR shift (22.5±0.25) was obtained for SPR biochip immobilized with 1000µg/ml M-aHIgG in comparison to the 750µg/ml (21±0.5) and 500µg/ml M-aHIgG (20.5±0.75). This is because M-aHIgG concentration of 1000µg/ml allows larger biomolecular interaction compared to 750µg/ml and 500 µg/ml concentration of M-aHIgG due to the presence of relatively large immobilized M-aHIgG producing larger red shift of the SPR characteristics. The experimental results also indicated that the antigen, H-IgG was selective to all the three varieties of antibodies raised in mouse, goat and rabbit, however, the affinity and the degree of the interaction capacity varied with the type of antibody. This resulted in varying degree of shifts in resonance angle on antigen-antibody interaction. The binding kinetic of SPR Biochip was also studied using H-IgG protein of concentration 225

µg/ml. The obtained sensorgrams indicates the establishment of an initial a baseline value by injecting PBS over the sensor chip for 5min. As H-IgG is introduced, the association of the protein causes the resonance angle to rise subsequently reaching a steady state value. The highest steady state value of the resonance angle (77±0.25) was obtained for the association of H-IgG protein with R-aHIgG, followed by G-aHIgG (72.75±0.25) and M-aHIgG (70.25±0.5), which is due to relatively larger biomolecular interaction between antigen and antibody. After an interval of 15 minutes from the introduction of antigen protein, PBS was injected to replace the protein. The dissociation of protein from the sensor surface results the resonance angle to return close to the baseline value. The results indicates that the dissociation of H-IgG protein initiated earliest for M-aHIgG (~21mins) followed by G-aHIgG (~23 mins) and R-aHIgG (~25 mins). Finally to meet the last objective of the project, the SPR biochip was used for the detection of complex proteins such as snake venoms. Polyvalent antivenom of concentration (1µg/ml) was immobilized on the SPR biochip and exposed to different concentrations of crude venom in the range 0.01-1µg/ml to study the interaction capacity of the SPR sensor. The SPR biochip was immobilized with polyvalent antivenom and tested for the detection of complex proteins such as snake venoms. Crude venom in the concentration range of 0.01-1µg/ml was passed through the sensor chip to evaluate the interaction capacity of the SPR biochip. The results indicate a red shift of the SPR resonance angle with increment of the venom concentration. The binding of the venom protein with the polyvalent antivenom resulted in local refractive index change near sensor surface producing corresponding shifts in resonance angles. Initially, the response shows linear increase of the SPR angle with the increase in venom concentration due to an abundant number of active binding sites present on the sensor surface. Later as the binding kinetic progresses, gradual decrease in the available active binding sites occurs, leading ultimately to a saturated sensor response. The results obtained from the MATLAB based simulations indicated that the five layer mathematical models comprising of prism, oxide, gold, 2d materials and sensing medium etc. reported better values of sensitivities in comparison to the three and four layer mathematical models. The design of suitable structure of SPR sensor in the five layer mathematical model provides higher sensitivity (220/RIU) in comparison to the 4-layer and 3-layer having sensitivity values of 200/RIU and 180/RIU respectively. This was due to the use of 2d materials of suitable thickness and use of oxide as adhesive layers enhancing the surface plasmon effect. Among the different types of prisms used, BK7 prism reported higher values of sensitivity in comparison to the SF10 and SF11 prisms for the three, four and five layer mathematical models. The importance of the research work lies in its usefulness for the analysis of fluids containing multiple varieties of proteins as for example, snake venom which is a mixture of numbers of protein acting as a signature of the snake family or in the quality control of food products etc. The use of multichannel microfluidics in this regard for the patterning and detection of multiple proteins is of significant importance particularly for cases, where analysis is to be conducted with extremely small test samples. The use of microfluidics also provides precise fluidic control, offering benefits like enhanced sensing efficiency.



Conclusions

The label-free detection of Human Immunoglobulin-G (H-IgG) on a multiple protein- patterned SPR biochip was achieved using a portable SPR measurement device. SPR biochips demonstrated reliable detection of H-IgG antigen in phosphate buffered saline fluid with a detection limit of 15µg/ml and linear response through a wide concentration range (15µg/ml to 225µg/ml). Thereafter, multi layer mathematical models of SPR sensor were designed for improvement in the performance characteristics of the SPR sensors such as sensitivity. The design of a 5 layer mathematical model of SPR sensor was proposed consisting of BK-7 prism, conducting metal oxide, Gold(Au), 2D materials and sensing medium which provided higher sensitivity(240 0 /RIU) in comparison to the 4-layer and 3-layer mathematical model having sensitivity values of 230 0 /RIU and 180 0 /RIU respectively. This was followed by the low concentration detection of snake venom protein on polyvalent anti venom (1 µg/ml) in the range of 0.08-1µg/ml. In addition to the approved objectives, the hydrophilicity improvement of the PDMS substrates was also carried out using dielectric barrier discharge oxygen plasma treatment at 40W (15kV, 2.66mA) for different durations of time ranging from 60-360s. The contact angle of the untreated PDMS chip was found to be 115.3±0.1 (indicative of its hydrophobic nature) while that of the 240s plasma treated microfluidic chip was obtained to be 50.2±0.3 (indicative of improved hydrophilic behavior).

Scope of future work

Regarding the future scope of work, the research work can be extended for the detection of molecules in addition to proteins such as nucleic acids like DNA, RNA etc. for the understanding and analysis of genetic information within the cell. In the long run, the system can be used as a diagnostic tool for the detection of multiple toxins/venoms in biological fluids to ease the medical diagnosis process for proper treatment of patients of snake-bite. The research work also finds application in the quality control of food products for the analysis of multiple varieties of proteins in food samples.

List of Publications (only from SCI indexed journals) :

Title of the Paper	List of Authors	Journal Details	Month & Year	Volume	Status	DOI No	Impact Factor
Not Available							

List of Papers Published in Conference Proceedings, Popular Journals :

Title of the Paper	List of Authors	Journal Details	Month & Year	Volume	Status	DOI No	Impact Factor
Syringe based automated fluid infusion system for surface plasmon resonance microfluidic application	Surojit Nath, Kristina Doley, Ritayan Kashyap and Biplob Mondal	8th International Conference on Pattern Recognition and Machine Intelligence (PReMI 2019) (International)	Dec-2019	(571)	Published	https://doi.org/10.1007/978-3-030-34872-4_63	
Portable surface plasmon resonance (SPR) measurement device for sensing applications	Ritayan Kashyap, Ananya Bhattacharjee, Khargeswar Rangpi, Noman Hanif Barbhuiya, Biplob Mondal	INDICON 2020 (International)	Dec-2020	(NA)	Published	10.1109/INDICON49873.2020.9342276	

List of Patents filed/ to be filed :

Patent Title	Authors	Patent Type	Country/Agency Name	Patent Status	Application/Grant No.
Not Available					

Equipment Details :

Equipment Name	Cost (INR)	Procured	Make & Model	Utilization %	Amount Spent (INR)	Date of Procurement
DAQ card	31,968	Yes	National Instruments USB6002	100	49,114	02 Mar, 2021
Ph meter	28,350	Yes	ACZET APHS-3W	30	18,900	27 Apr, 2019
Digital weight balance	3,27,600	Yes	ACZET CY205C	50	2,12,100	27 Apr, 2019
Sonicator	2,67,062	Yes	OSCAR SONAPROS PR-250MP	50	2,08,299	07 May, 2019
Spin Coater	7,44,640	Yes	SUSS MICROTECH	40	6,99,500	10 Apr, 2019
Film thickness measuring instrument	10,51,900	Yes	Filmetrics F20	50	11,47,961	04 Apr, 2019

Plans for utilizing the equipment facilities in future:

The facilities created under this project will be used to undertake extended research work. Currently, research is underway for the enhancement of detection capacity like sensitivity, detection accuracy etc. of complex proteins such as snake venom. SPR biochip immobilized with polyvalent antivenom would be exposed to different types of crude venoms of various dozes to study the quantitative detection of complex proteins.

In future, the research work can be extended for the detection of biomolecules such as nucleic acids like DNA, RNA etc. for the understanding and analysis of genetic information within the cell. The measurement prototype can be used as a diagnostic tool for the detection of multiple toxins/venoms in different biological fluids to ease the medical diagnosis process for proper treatment of patients of snakebite.

A PhD student is being engaged to accomplish some of these target research. Also, training will be continued to be imparted to undergraduate and master's students. The research work will also find application in the quality control of

food products for the analysis of multiple varieties of molecules such as protein, in food samples.
The facilities is also being utilised for various research activities in the lab by other PhD students who are working in the synthesis of 2D materials like transition metal dichalcogenides (TMDC). The project has resulted in a basic research infrastructure and many PhD, M.Tech, B.Tech students will be benefited by this.

SCIENCE & ENGINEERING RESEARCH BOARD (SERB)
(Statutory Body Established Through an Act of Parliament : SERB Act 2008)

Science and Engineering Research Board
3rd & 4th Floor, Block II
Technology Bhavan, New Mehrauli Road
New Delhi - 110016

File Number: EMR/2016/007445

Dated: 13-Jan-2022

Subject: Project titled "Study for label free optical detection of snake venom protein using Surface Plasmon Resonance technique".

Dear Dr. Biplob Mondal,

This is reference to your request for extension of the project duration; the undersigned is directed to convey approval for the extension of the above mentioned project duration for 04 months till 09-05-2022 without any additional cost.

The aforementioned extension of the project duration is subjected to the same terms and conditions as mentioned in the earlier sanction letter of even number sanctioning the project

Yours sincerely,
(Dr. Anima Johari)
Scientist D
Ph:
Email: anima.johari@serb.gov.in

Dr. Biplob Mondal
Electronics And Communication Engineering
Tezpur University , Distt. sonitpur p.b.no.72 napaam, tezpur, Tezpur, Assam-784011

REQUEST FOR ANNUAL INSTALMENT WITH UP-TO-DATE STATEMENT OF EXPENDITURE

Annexure-II

1. SERB Sanction Order No and date : EMR/2016/007445/EEC Date: 18-06-18
2. Name of the PI :DR BIPLOB MONDAL
3. Total Project Cost :Rs 47,52,100/-
4. Revised Project Cost (if applicable) :
5. Date of Commencement :10-07-2018
6. Statement of Expenditure :

(Month wise expenditure incurred from 01-04-22 to 09-05-22)

Month & year	Expenditure incurred/ committed
April 1- April 30 2022	Nil
May 1- May 09 2022	Rs 23190.00 (consumable)

1. Grant received in each year:

a. 1stYear :Recurring Rs 789500.00
Non- recurring Rs 2340500.00
Total Rs 3130000.00

b. 2ndYear : Recurring Rs 750000.00

c. 3rd Year :Nil

d. 4th Year : Recurring Rs 600000.00

e. Interest, if any :Rs 32767.00 (FY:2018-19) + Rs. 11134.00 (FY:2019-2020)+Rs2248(F.Y 2020-21)+Rs 1961(F.Y 2021-22)

f. Total (a + b + c + d+e): Rs 4528110.00

(150)

Statement of Expenditure
(01.04.2022-09.05.2022)

S r N o (I)	Sanctioned Heads (II)	Total Funds Allocated (indicate sanctioned or revised) (in Rs) (III)	Expenditure Incurred 1st Year (DOS to 31st March next year) (in Rs) (IV)	2nd Year (1st April to 31st March next year) (in Rs) (V)	3rd Year (1st April to 31st March next year) (in Rs) (VI)	4th Year (1st April to 31-03-2022 (in Rs) (VII)	5th year (1st April 2022 to 09-05-2022)	Total Expenditure till date (in Rs) (VIII = IV + V +VI+ VII)	Balance as on (date) (in Rs) (IX= III - VIII)	Requirement of Funds upto 31st March next year(in Rs)	Remarks (if any)
1.	Manpower costs		175667.00	146774.00	336800.00	176129.00	Nil	835370.00			
2.	Consumables			198790.00	308023.00	142745.00	23190.00	672748.00			
3.	Travel		18054.00	31136.00	Nil	Nil	Nil	49190.00			
4.	Contingencies		49824.00	29614.00	18692.00	48756.00	Nil	146886.00			
5.	Others, if any										
6.	Equipment		1147961.28	1138799.00	Nil	49114.00	Nil	2335874.28			
7.	Overhead expenses		143750.00	90000.00		196980.00	Nil	430730.00			
8.	Total		1535256.28	1635113.00	663515.00	613724.00	23190.00	4470798.28			

Name and Signature of Principal Investigator:

Date: 3.6.22
Dr. B. Mandal

Signature of Competent financial authority: 24/6/22
(with seal) Finance Officer
Tezpur University
Date:

*** DOS – Date of Start of project**

Note:

1. Expenditure under the sanctioned heads, at any point of time, should not exceed funds allocated under that head, without prior approval of SERBi.e.FiguresinColumn(VIII)shouldnotexceedcorrespondingfiguresinColumn(III)
2. Utilization Certificate (Annexure III) for each financial year ending 31st March has to be enclosed along with request forcarry-forward permission to the next financial year.

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E

RECURRING

GFR 12 – A

[(See Rule 238 (1))]

UTILIZATION CERTIFICATE (UC) FOR THE YEAR 2022-23

in respect of *RECURRING*

as on 09-05-2022 to be submitted to SERB

Is the UC ..... *(Provisional/Audited)*

(To be given separately for each financial year ending on 31stMarch)

1. Name of the grant receiving Organization :TEZPUR UNIVERSITY
2. Name of Principal Investigator(PI): DR BIPLOB MONDAL
3. SERB Sanction order no. & date: EMR/2016/007445/EEC 18-06-18
4. Title of the Project: Study for label free optical detection of snake venom protein using surface plasmon resonance technique
5. Name of the SERB Scheme: **CRG** (CRG/NPDF/ECR etc.)
6. Whether recurring or non-recurring grants : Recurring
7. Grants position at the beginning of the Financial year (Grants released by SERB)

   (i) Cash In Hand/Bank /Carry forward from previous financial year : Rs 42374.14 (recurring I, II and III)

   (ii) Others, If any :

   (iii) Total : Rs 42374.14

8. Details of grants received, expenditure incurred and closing balances:(Actuals)

Unspent Balance of Grants received previous years [figure as at Sl. No. 7(iii)]	Interest Earned thereon	Interest deposited back to the SERB	Grants received during the year			Total Available funds (1+2-3+4)	Expenditure incurred	Closing Balances (5-6)
1	2	3	4			5	6	7
			Sanction No. (i)	Date (ii)	Amount (iii)			
Rs 42374.14	Nil		Nil	Nil	Nil	Rs 42374.14	Rs 23190.00	Rs 19184.14

Component wise utilization of grants:

Grants-in-aid- General	Total
Consumable	Rs 23190.00

Details of grants position at the end of the year

(i) Cash in Hand/Bank : Rs 19184.14

(ii) Refunds to SERB, If any :

(iii) Balance (Carry forward to next financial year) : Rs 19184.14

Signature of PI 8.6.22	**Signature with Seal** :..... 24/6/22 **Name:** ..... **Chief Finance Officer (Head of Finance)** Finance Officer Tezpur University	**Signature withSeal**..... 24/6/22 **Name:**..... **Head ofOrganisation** Registrar Tezpur University

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GFR 12 – A
[(See Rule 238 (1))]
UTILIZATION CERTIFICATE (UC) FOR THE YEAR 2022-23
in respect of *RECURRING*
as on 09-05-2022 to be submitted to SERB
Is the UC ..... *(Provisional/Audited)*
(To be given separately for each financial year ending on 31stMarch)

Certified that I have satisfied that the conditions on which grants were sanctioned have been duly fulfilled/are being fulfilled and that I have exercised following checks to see that the money has been actually utilized for the purpose for which it was sanctioned:

(i) The main accounts and other subsidiary accounts and registers (including assets registers) are maintained as prescribed in the relevant Act/Rules/Standing instructions (mention the Act/Rules) and have been duly audited by designated auditors. The figures depicted above tally with the audited figures mentioned in financial statements/accounts.

(ii) There exist internal controls for safeguarding public funds/assets, watching outcomes and achievements of physical targets against the financial inputs, ensuring quality in asset creation etc. & the periodic evaluation of internal controls is exercised to ensure their effectiveness.

(iii) To the best of our knowledge and belief, no transactions have been entered that are in violation of relevant Act/Rules/standing instructions and scheme guidelines.

(iv) The responsibilities among the key functionaries for execution of the scheme have been assigned in clear terms and are not general in nature.

(v) The benefits were extended to the intended beneficiaries and only such areas/districts were covered where the scheme was intended to operate.

(vi) The expenditure on various components of the scheme was in the proportions authorized as per the scheme guidelines and terms and conditions of the grants-in-aid.

(vii) It has been ensured that the physical and financial performance under **CRG(CRG/NPDF/ECR.....etc.)** (Name of the scheme has been according to the requirements, as prescribed in the guidelines issued by Govt. of India and the performance/targets achieved statement for the year to which the utilization of the fund resulted in outcomes given at Annexure – I duly enclosed.

(viii) The utilization of the fund resulted in outcomes given at Annexure – II duly enclosed (to be formulated by the Ministry/Department concerned as per their requirements/specifications.)

(ix) Details of various schemes executed by the agency through grants-in-aid received from the same Ministry or from other Ministries is enclosed at Annexure –II (to be formulated by the Ministry/Department concerned as per their requirements/specifications).

Date:

Place:

Signature of PI : 2.6.22	**Signature with Seal :** 2/6/22 **Name:** ..... **Chief Finance Officer** **(Head of Finance)**	2/6/22 **Signature withSeal**..... **Name:**..... **Head ofOrganisation**

Finance Officer
Tezpur University

Registrar
Tezpur University

(Strike out inapplicable terms)

NON-RECURRING

GFR 12 – A

[(See Rule 238 (1))]

UTILIZATION CERTIFICATE (UC) FOR THE YEAR 2022-23

in respect of *NON-RECURRING*

as on 09-05-2022 to be submitted to SERB

Is the UC ..... *(Provisional/Audited)*

(To be given separately for each financial year ending on 31stMarch)

1. Name of the grant receiving Organization : TEZPUR UNIVERSITY
2. Name of Principal Investigator(PI): DR BIPLOB MONDAL
3. SERB Sanction order no. & date: EMR/2016/007445/EEC 18-06-18
4. Title of the Project: Study for label free optical detection of snake venom protein using surface plasmon resonance technique
5. Name of the SERB Scheme: **CRG**(CRG/NPDF/ECR etc.)
6. Whether recurring or non-recurring grants: Non–Recurring
7. Grants position at the beginning of the Financial year (Grants released by SERB)

   (i) Cash In Hand/Bank /Carry forward from previous financial year :Rs 38127.57

   (ii) Others, If any:

   (iii) Total : Rs 38127.57

8. Details of grants received, expenditure incurred and closing balances:(Actuals)

Unspent Balance of Grants received previous years [figure as at Sl. No. 7(iii)]	Interest Earned thereon	Interest deposited back to the SERB	Grants received during the year			Total Available funds (1+2-3+4)	Expenditure incurred	Closing Balances (5-6)
1	2	3	4			5	6	7
			Sanction No. (i)	Date (ii)	Amount (iii)			
Rs 38127.57	Nil		Nil	Nil	Nil	Rs 38127.57	Nil	Rs 38127.57

Component wise utilization of grants:

Grant-in-aid-creation for capital assets	Total
Equipment	Nil

Details of grants position at the end of the year

(i) Cash in Hand/Bank : Rs 38127.57

(ii) Refunds to SERB, If any :

(iii) Balance (Carry forward to next financial year) : Rs 38127.57

Signature of PI : 3.6.22	**Signature with Seal:** 3/6/22 **Name:** ..... **Chief Finance Officer (Head of Finance)**	**Signature withSeal**..... **Name:** ..... **Head ofOrganisation**

Finance Officer
Tezpur University

Registrar
Tezpur University

GFR 12 – A
[(See Rule 238 (1))]
UTILIZATION CERTIFICATE (UC) FOR THE YEAR 2022-23
in respect of *NON-RECURRING*
as on 09-05-22 to be submitted to SERB
Is the UC ..... *(Provisional/Audited)*
(To be given separately for each financial year ending on 31stMarch)

Certified that I have satisfied that the conditions on which grants were sanctioned have been duly fulfilled/are being fulfilled and that I have exercised following checks to see that the money has been actually utilized for the purpose for which it was sanctioned:

(i) The main accounts and other subsidiary accounts and registers (including assets registers) are maintained as prescribed in the relevant Act/Rules/Standing instructions (mention the Act/Rules) and have been duly audited by designated auditors. The figures depicted above tally with the audited figures mentioned in financial statements/accounts.

(ii) There exist internal controls for safeguarding public funds/assets, watching outcomes and achievements of physical targets against the financial inputs, ensuring quality in asset creation etc. & the periodic evaluation of internal controls is exercised to ensure theireffectiveness.

(iii) To the best of our knowledge and belief, no transactions have been entered that are in violation of relevant Act/Rules/standing instructions and schemeguidelines.

(iv) The responsibilities among the key functionaries for execution of the scheme have been assigned in clear terms and are not general innature.

(v) The benefits were extended to the intended beneficiaries and only such areas/districts were covered where the scheme was intended tooperate.

(vi) The expenditure on various components of the scheme was in the proportions authorized as per the scheme guidelines and terms and conditions of thegrants-in-aid.

(vii) It has been ensured that the physical and financial performance under **CRG(CRG/NPDF/ECR.....etc.)** (Name of the scheme has been according to the requirements, as prescribed in the guidelines issued by Govt. of India and the performance/targets achieved statement for the year to which the utilization of the fund resulted in outcomes given at Annexure – I duly enclosed.

(viii) The utilization of the fund resulted in outcomes given at Annexure – II duly enclosed (to be formulated by the Ministry/Department concerned as per their requirements/specifications.)

(ix) Details of various schemes executed by the agency through grants-in-aid received from the same Ministry or from other Ministries is enclosed at Annexure –II (to be formulated by the Ministry/Department concerned as per their requirements/specifications).

Date:

Place:

Signature of PI :.....	**Signature with Seal :**..... **Name:** ..... **Chief Finance Officer** **(Head of Finance)**	**Signature withSeal**..... **Name:** ..... **Head ofOrganisation**

(Strike out inapplicable terms)

Finance Officer
Tezpur University

Registrar
Tezpur University