President :
Shri Amrishbhai R. Patel
M.L.A.

Principal :
Dr. S. B. Bari
M.Pharm. Ph.D., D.I.M.F.J.C.

TO WHOM SO EVER IT MAY CONCERN

This is to certify that, Mr. Sopan Namdev Nangare is working as a Ph.D. research scholar at H. R Patel Institute of Pharmaceutical Education and Research, Shirpur. I am Dr. Pravin Onakr Patil (Ph.D. Research Supervisor) certifies that the research work under reference has been done by the applicant.

In this proposed work, Mr. Sopan Nangare (Nominee) is particularly focused on developing an exceptionally sensitive, highly selective, cost-effective, non-invasive, label free surface plasmon resonance (SPR) biosensor-based detection chip for the early-stage prognosis of Alzheimer's disease (AD). To date, the modification of the golf (Au) layer with an amine group, graphene oxide (GO) nanosheet, and AgNPs-CS-PSS-CS (CS: Chitosan, PSS: Polystyrene sulfonate) for '$A\beta_{1\text{-}42}$' peptide detection utilizing GO@AgNPs-Anti-$A\beta$ SPR biosensor still has not been implemented. Therefore, the first approach demonstrates the surface decorated AgNPs-CS-PSS-CS assembly-based affinity surface plasmon resonance (SPR) biosensor for highly sensitive recognition of AD biomarker $A\beta_{1\text{-}42}$ using GO nanosheet as an advanced non-plasmonic nanomaterial. Interestingly, the utilization of AgNPs-CS-PSS-CS assembly furnishes the high selectivity and sensitivity assured by sensorgram. The utilization of 'CS' as an external layer of layer-by-layer (LbL) assembly furnishes the amino groups that offer the plenteous sites for the immobilization of anti-$A\beta$ antibodies with specific directions. The application of GO nanosheet forms the bonding with the NH_2 group on an 'Au' coated sensor chip via carbodiimide chemistry. Mainly, it offers a high surface area for the immobilization of Anti-$A\beta$ antibodies on the surface of the GO nanosheet. Moreover, owing to their optical properties, they help to heighten the overall presentation of GO@AgNPs-Anti-$A\beta$ SPR biosensors in terms of sensitivity and selectivity. It is worth divulging that the combined approach of silver nanoparticles (AgNPs), GO, and LbL assembly resulted in the lowest detection limit of about 1.21 fg/mL and a good linear range from 2 fg/mL to 400 ng/mL. Moreover, it provides the speedy identification of $A\beta_{1\text{-}42}$ peptides without any influence on detection ability, and also it requires a small volume of samples. The anti-interference study and real-time analysis in AD-induced animals

confirmed the applicability of the sensor as well as selectivity towards the $A\beta_{1-42}$ in CSF, blood, and saliva. Moreover, the good stability and reproducibility of GO@AgNPs-Anti-$A\beta$ SPR biosensors make them an exceptional candidate for practical biosensing applications. Herein, the application of 'CS' as coating materials for plasmon materials coating in SPR biosensors will open a new preference for the recognition of disease biomarkers. In winding up, this high-throughput SPR-centered GO@AgNPs-Anti-$A\beta$ biosensor presents a reliable foundation for the early revealing of the '$A\beta_{1-42}$' AD biomarker. In addition, it can be beneficial for the diagnosis of clinical dementia as well as Parkinson's disease dementia. In the upcoming, the actual utilization of the described SPR-assisted biosensor for the identification of $A\beta_{1-42}$ in clinical samples is anticipated.

A poly(allylamine hydrochloride (PAH) and PSS -based AuNPs-PAH-PSS-PAH LbL (AuNPs: Gold nanoparticles) assembly with GO nanocomposite adorned SPR-based astonishingly sensitive and specific affinity biosensor was designed for Tau-441 antigen detection. Briefly, plasmonic AuNPs were obtained adopting an environmentally pleasant green method and then treated to the construction of a polyelectrolyte-based AuNPs-PAH-PSS-PAH LbL arrangement. In this circumstance, opting for a greener strategy for AuNPs synthesis might enable the elimination of harmful interactions with biomolecules, consequently improving the holistic appearance of an SPR biosensor. Surprisingly, AuNPs-PAH-PSS-PAH assembly including cationic 'PAH' presents an excess of immobilization positions for anti-Tau monoclonal antibodies. Because of the abundance of amino binding sites, a precise direction in the immobilization of affinity receptors serves to improve the overall efficiency of the SPR biosensor. The adoption of GO as a 2D carbon spine has assorted advantages, notably large surface area, biocompatibility, and the greatest number of binding sites for the deposition of constructed AuNPs-PAH-PSS-PAH facilitated AuNPs-PAH-PSS-PAH@Anti-Tau conjugates. The hydrogen-bonding, π-π stacking interactions, electrostatic interaction, and specifically hydrophilic affinity of GO strengthened biomolecule sorption possibilities. The projected GO@LbL-AuNPs-Anti-Tau SPR biosensor's sensitivity assessment delivers a broad concentration range from 5 fg/mL to 150 ng/mL and an exceedingly low detection limit of upto 13.25 fg/mL for the Tau-441 antigen. As well, the anti-interference performance and practical applicability of GO@LbL-AuNPs-Anti-Tau SPR biosensor were demonstrated by the selectivity and real-time analysis. Furthermore, the coupling of plasmonic AuNPs-PAH-PSS-PAH@Anti-Tau bioconjugates and GO nanocomposites culminated in complementary improvements for sensitivity and selectivity

of GO@LbL-AuNPs-Anti-Tau SPR biosensor than formerly published SPR biosensors. Finally, the constructed AuNPs-PAH-PSS-PAH@Anti-Tau bioconjugates LbL assembly facilitated GO nanocomposite has numerous advantages such as quick sensing, streamlined method, sustainable strategy, high sensibility, high specificity, reproducibility, and so on. As a result, in the coming days, the hypothesized GO@LbL-AuNPs-Anti-Tau SPR biosensor would bring up a revolutionary viewpoint for *in-vitro* diagnosis of AD as well as other severe health ailments.

The 'CS' and sodium alginate (SA)-coated platinum nanoparticles (Pt-NPs)-based nanobioconjugate was preferred to fabricate Anti-BACE-1-LbL@Pt-NPs-GO-SPR biosensor for highly sensitive and selective detection of Beta-secretase 1 (BACE-1). In concisely, the green-synthesized, stable, and nanosized Pt-NPs were successfully employed as plasmonic nanomaterials, whereas non-plasmonic GO nanosheets were produced via the modified hummers approach. The synthesis of nanomaterials was confirmed by several spectral analyses. For the first time, LbL assembly was constructed for SPR biosensors employing 'CS' and 'SA' as cationic and anionic coating polymers on the surface of green-prepared Pt-NPs, respectively. Here, the affinity biorecognition substrate Anti-BACE-1 was immobilized on the surface of CS-SA-CS@Pt-NPs, wherein the amine functionality of 'CS' provides an immobilization site and increased biocompatibility with Pt-NPs for Anti-BACE-1. Carbodiimide chemistry was used to decorate GO nanosheets on the surface of an amine-functionalized 'Au' coated sensor chip. The non-specific binding sites of GO were masked using BSA as a blocking agent. As an output, the increased performance of the SPR biosensor has been demonstrated wherein it revealed the lowest detection limit upto 5.63 fg/mL. As well, the Anti-BACE-1-LbL@Pt-NPs-GO-SPR biosensor has demonstrated very sensitive monitoring of BACE-1 antigen in the concentration range of 150 ng/mL to 5 fg/mL. The high selectivity of the proposed Anti-BACE-1-LbL@Pt-NPs-GO-SPR biosensor was confirmed in the presence of different interfering agents. Importantly, these use of CS and SA-based LbL family provides plenty of advantages that contributed to improving the SPR biosensor performance towards the BACE-1 sensing. In addition, it verified the excellent stability and reproducibility of the developed BACE-1 detection. The analysis of spiked samples utilizing the Anti-BACE-1-LbL@Pt-NPs-GO-SPR biosensor demonstrated a successful use for BACE-1 recognition in complicated clinical samples such as blood and saliva. Overall, the designed Anti-BACE-1-LbL@Pt-NPs-GO-SPR biosensor exhibited improved performance because of the

synergistic effects of CS-SA-CS@Pt-NPs LbL assembly, non-plasmonic GO nanosheets, plasmonic Pt-NPs, and affinity bioreceptor anti-BACE-1. Therefore, in the future, the projected LbL-based novel, high sensitivity, stability, reproducibility, highly selective, label free, and fast SPR biosensor concept will enable a competitive replacement for measuring $A\beta_{1-42}$, Tau 441, and BACE-1 in clinical samples

The anticipated outcomes of Mr. Sopan Nangare's research are highly promising. It is my optimistic expectation that he has successfully engineered an innovative SPR biosensor chip that holds the potential to revolutionize the early detection of Alzheimer's disease. This advancement could represent a safe and viable alternative to current methods. In essence, the SPR biosensor conceptualized by Mr. Sopan Nangare is poised to introduce a paradigm shift in Alzheimer's diagnosis. The proposed biosensor has the potential to offer a range of crucial advantages, including non-invasiveness, user-friendliness, cost-effectiveness, label-free detection, rapid results, exceptional sensitivity, and remarkable selectivity. This holistic approach holds the promise of enabling early-stage diagnosis of Alzheimer's, a critical factor in improving patient outcomes and quality of life. Overall, the potential impact of Mr. Sopan Nangare's research is substantial, and his innovative contributions have the potential to significantly advance the field of Alzheimer's diagnostics. His diligent efforts in developing this SPR biosensor hold the promise of addressing a pressing medical need and contributing to the broader landscape of healthcare innovation.

Yours Sincerely,

OFFICE SEAL

Prof(Dr). Pravin Onkar Patil
(Ph.D. Supervisor)
Department of Pharmaceutical Chemistry,
H. R. Patel Institute of Pharmaceutical Education and Research,
Shirpur 425405, Maharashtra state, India