Development of Immunosensor Based on Self Assemble Monolayers of Polymer Metal Nanocomposites for Rapid Quantification of MSG

Thesis submitted in partial fulfilment

For the Award of Degree

Doctor of Philosophy

BY

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2022

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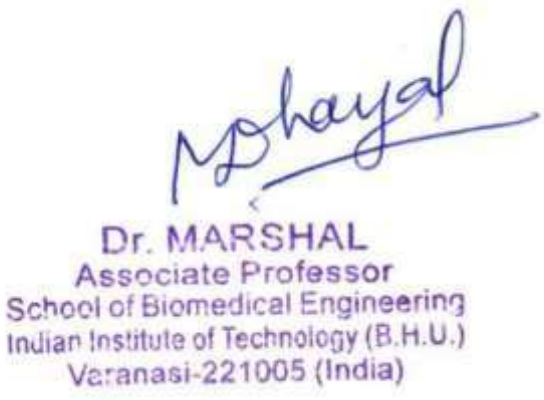

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ACKNOWLEDGMENT

I am thankful to my supervisor, Dr. Marshal, Associate professor, Nanocellular Medicine and Biophysics lab, School of Biomedical Engineering, India Institute of Technology (Banaras Hindu University) Varanasi 221005, UP, India, for his guidance and consistent support during my thesis. I am grateful to him for motivating me toward achieving high research standards and enforcing strict validations for each research result, thus teaching me how to do research. I express my deep and sincere gratitude to Dr. Raju Khan, Principal Scientist, CSIR-Advanced Materials and Processes Research Institute (CSIR-AMPRI), Bhopal, India, and Dr. Hemant Sankar Dutta, Senior Scientist, Analytical Chemistry Group, CSIR-North East Institute of Science and Technology, Jorhat, India, for their consistent support in utilizing their facilities and guidance while performing experiments at their labs.

I also extend my gratitude towards my Research Progress Evaluation Committee (RPEC) members (Dr. Sanjay Singh Rai, Associate Professor, School of Biomedical Engineering, India Institute of Technology (Banaras Hindu University), Varanasi, India, and Dr. Shreyansh Kumar Jain, Assistant Professor Department of Pharmaceutical Engineering and Technology, India Institute of Technology (Banaras Hindu University), Varanasi, India) for evaluating my work and providing useful feedback to improve it on time to time. I would also like to be thankful to the office staff school of biomedical engineering, who has been supportive in every way. I would also like to be special gratitude to Mr. Barat Lal, technical supervisor, and all non-teaching staff of the school for their help and cooperation extended for the successful completion of the work. My sincere gratitude to the School of Biomedical Engineering, India Institute of Technology (Banaras Hindu University), Varanasi, which provided a platform to carry out this research work.

I am wholeheartedly thankful to my dear lab mates Rasmita Devi at Analytical Chemistry Group, CSIR-North East Institute of Science and Technology, Jorhat, India, and Juhi Jaiswal, Shenlata

Yadav, Shubangi, Shailendra Kumar and Sanju Kumari with whom I shared the lab with many ups and downs.

Few notes of gratefulness to my grandparents, Lt. Dada Ji Shri Karta Ram, and my Lt. Dadi Smt. Vidyavati Devi, my parents, Shri Radhey Shyam and Santosh Devi, and elder Sisters Tripta Sharma, Kumkum Sharma, Rajni Sharma, and Deepika Sharma, whose blessings, love, support, and consistent encouragement have enabled me to glide through my troubles with effortless ease. Special Thanks to my nephew "Bargaav Sharma," whose simplicity and childhood have brought smiles to me at times of stress. Finally, my gratitude towards the almighty for giving me determination and showing me how to be what I am today.

Devdutt Sharma

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S. No.	Abbreviations	
1	MSG	Monosodium glutamate
2	HPLC	High-Performance Liquid Chromatography
3	GC	Gas Chromatography
4	CE	Capillary Electrophoresis
5	I_{pc}	Cathodic peak currents
6	I_{pa}	Anodic peak currents
7	$E_{p.c}$	Cathodic peak potential
8	$E_{p.a}$	Anodic peak potential
9	ITO	Indium Tin Oxide
10	PANI	Polyaniline
11	BSA	Bovine Serum Albumin
12	LB	Langmuir-Blodgett
13	CV	Cyclic voltammetry
14	DPV	Differential pulse voltammetry
15	EDC	N-(3 dimethylamino propyl)-N′-ethyl carbodiimide hydrochloride
16	NHS	N-hydroxysuccinimide
17	NMP	N-methyl-2-Pyrrolidone
18	EIS	Electrochemical impedance spectroscopy

PREFACE

This thesis is submitted for the degree of doctor of philosophy at Indian Institute of Technology (Banaras Hindu University), Varanasi. The research described herein was conducted under the supervision of Dr. Marshal in the school of biomedical engineering, Indian Institute of Technology (Banaras Hindu University), Varanasi, between July 2018 to July 2022.

This work is the best of my knowledge, original except where acknowledgment and reference are made to previous work. Neither this nor a substantially similar thesis has been or is being submitted for any other degree, diploma, or other qualification at any other university.

In 1908, Professor Kikunae Ikeda produced the first monosodium glutamate. Monosodium glutamate is frequently used in food processing units, restaurants, and packed food industry as a taste enhancer. It was produced commercially under the trade name Ajinomoto. Indian food Industries also use MSG, and it's consumption is estimated at 10510 tons per year. Food having MSG is labeled as code E-621. Glutamic acid has two optical isomer L- glutamic acid and D-glutamic acid. Only L- glutamic acid is used as a flavor enhancer in food. The taste of MSG is known as the UMAMI taste. Glutamate also plays a vital and many essential roles in metabolic processes. It is also used to transfer energy transport of amino acid across the cell membrane and remove ammonia by the urea cycle. Glutamate also acts as a neurotransmitter and its brain metabolic rate.

But in 1990 in the USA, a particular population reported headaches, nausea, and vomiting after eating food having MSG. These syndrome collectively is known as Chinese restaurant syndrome.

In 1995, FDA declared MSG as generally safe and decided its maximum permissible limit. India's Prevention of Food Adulteration Act defined the permissible MSG content in food as 1%.

Most recently, a controversy flared up when the food safety and drug administration, Utter Pradesh, found high level of MSG in Maggi noodles. The government issued a notice to Nastle to recall this product from the market. As per the FSSAI guidelines, MSG is not permitted in pasta and noodles. So, food industries should need sample, fast, highly selective, highly sensitive, efficient, and cost-effective methods of MSG quantification. The monitoring of glutamate is also essential for fermentation control during its production

The present thesis's objective was to detect monosodium glutamate's electrochemical detection by the self-assemble thin film of the polymer-metal nanocomposite. This thesis will be beneficial for the researcher/ academics / industrial working in the area of electrochemical quantification of monosodium glutamate biosensor

Part of this thesis published in the following publication

- **Devdutt Sharma**, Rashmita Devi, Juhi Jaiswal, Hemant Sankar Dutta, Raju Khan, and Marshal Dhayal. "A Highly Sensitive Immunosensor Based on In Situ Reduced Gold-Chitosan Nanocomposite for Detection of Monosodium L-glutamate." *Journal of Biosystems Engineering* (2022): 1-11. doi.org/10.1007/s42853-022-00127-z.
- **Devdutt Sharma**, Hemant Sankar Dutta, and Marshal Dhayal. "Langmuir-Blodgett Monolayer of Electrochemically Synthesized PANI-TiO2 Nanocomposites for MSG Biosensor. " *Journal of* Applied Surface Science Advances. doi.org/10.1016/j.apsadv.2022.100264

Part of this thesis communicated in the following publication

- **Devdutt Sharma**, Hemant Sankar Dutta, and Marshal Dhayal.“Biosensor Platform of Hydrophobic Nanoassemblies of Positively Charged Polyaniline-Gold on Piranha Treated ITO by Langmuir–Blodgett Film for Electrochemical Sensing of MSG” with Sensors and Actuators B.
- **Devdutt Sharma**, Marshal Dhayal. “Dual metal conjugate through polymer linkage to enhance conductivity and catalytic degradation of Evans Blue under visible light for environmental remediation”. With Colloid and Polymer Science.

ABSTRACT

This work focused on developing metal nanomaterials with hydrophobic characteristics to deposite through the Langmuir-Blodgett (LB) film process. It is expected that a monolayer of metallic nanomaterials with enhanced conductivity could provide a better choice for effective transportation of charge and improved biomolecule adsorption capabilities compared to other methods and materials used for developing immunosensor platforms. An immunosensor based on the monoclonal anti-glutamate antibodies immobilization onto a thin film of new nanocomposites of polymer-metallic nanoparticles, conductive polymer-metal oxide nanoparticles, and conductive polymer-metallic nanoparticles through a self-assembly process developed. New platforms developed in this thesis concurrently manage functional groups' availability for antibody immobilization with enhanced electrical conductivity. An antibodies-based immunosensor is ideal for industrial applications since it allows for detecting MSG at concentrations ranging from nano-molar to micromolar on a single platform. A hypothesis for low concentration detection is proposed by considering the following facts;

In-situ reduced chitosan gold nanoparticles matrix provides both higher sensitivity and extended limit of the detection range of MSG. It is expected that impregnation of the in-situ reduced gold nanoparticles in the chitosan network provides better charge transportation and enhanced selective functionalization capability and biocompatibility. The proposed in-situ reduced gold nanoparticles may have better chemical coordination and homogeneous distribution in the chitosan network. Thus, this reduction process has an advantage over the methods that adopt the physical mixing of nanoparticles with polymeric materials. An enhancement of four-fold in current has been achieved by incorporating GNP in chitosan. The formulation also has the advantage of stable and adhesive coatings on working electrodes to develop a platform based on monoclonal anti-glutamate antibodies immobilization, Coating of these nanomaterials allowed to achieve the highest detection

sensitivity with the lowest detection limit of 0.1 nM. Electrochemical characteristics of chitosan-gold nanoparticles were studied by measuring relative change in redox current compared to standard working electrodes. Antibodies' interaction with the CS-GNP platform was confirmed through a reduction in electrochemical current.

The second method uses the titanium dioxide (TiO_2) nanoparticles in the PANI matrix and optimizes conditions for preparing LB films of the polyaniline-TiO_2 nanocomposite. A unique strategy was adopted in which electrochemical polymerization of aniline in the presence of TiO_2 nanoparticles was performed on ITO substrate, dissolving it in a solution of NMP and isopropanol, and LB film deposition of contamination-free subphase of PANI-TiO_2. Thus, the work achieved a higher degree of polymerization through the electrochemical process and subsequently used this polymerized material to make LB film. The antibodies immobilized electrodes were successively used to quantify MSG ranging from 1 nM to 500 μM in the standard electrolyte. A linear relationship was obtained between the current change and the MSG concentration.

In the third method, the chemical synthesized of surfactant-free stable ultra-small gold nanoparticles (< 10 nm diameter) in the PANI matrix were used. Electrochemical polymerization of aniline monomers with these gold nanoparticles was performed on ITO substrate. This unique method enabled the synthesis of polyaniline-GNP nanocomposite with hydrophobic nature. Further, this PANI-GNP nanocomposite was dissolved in a solution of NMP and isopropanol, and the conditions were optimized for preparing LB films of the polyaniline-GNP nanocomposite. The developed thin film shows a very high conductivity with improved capability for the adsorption of biomoles. The developed electrochemical biosensor shows stability over a wide range of pH values. Antibodies immobilized immunosensor were sequentially used to quantify MSG ranging from 1 nM to 10 mM in the standard electrolyte and 1 μM to 1 mM in tomato sauce. A linear relationship was obtained between the current change and the MSG concentration.

The electrochemical properties of all the immunosensors mentioned above were investigated by measuring the relative change in redox current compared to standard working electrodes. To improve measurement sensitivity, monoclonal anti-glutamate antibodies were bound to the amine-functionalized spots on the working electrode using the carbodiimide coupling technique. Since monoclonal anti-glutamate antibodies were used due to their ability to bind the uniquely designed substrate and arrange reactive groups in a manner that promotes a specific reaction transition state, they give these antibodies their selectivity. Monoclonal anti-glutamate antibodies show stereoselective properties (e.g., the monoclonal anti-glutamate antibodies used in work reported in this thesis are specific for the L-isomer of glutamate). The developed immunosensors can quantify the concentration of MSG in the nano-molar range with higher stability and reproducibility.

This thesis work will be beneficial for the researcher/ academics / industrial working in the area of electrochemical quantification of monosodium glutamate biosensor