

Chapter VI

Overall Summary and Future Perspective

The increase in number of cases of CKD and the unavailability of donor and costly dialysis process has led to the need for rapid, affordable methods for kidney disease diagnosis. The early diagnosis of kidney diseases is a major challenge because the concentration of biomarkers remains quite low in sera fluids and also them being related to other health conditions as well. In such cases, biosensors emerge as a savior for providing simple, easy-to-use, cost-effective, point-of-care testing of these diseases. Hence, we have developed opto-electrochemical sensing platforms by targeting two different biomarkers using effective surface engineering methods for kidney disease diagnosis. In **Chapter I**, we have elaborately discussed the types of surface engineering methods and the different biomarkers which can be possible targets for CKD diagnosis. After discussing about the role of biomarkers and their clinical applicability, we have developed a paper-based optical sensor device for point-of-care quantitative monitoring of creatinine in serum sample in **Chapter II**. The detection was based on a modified chemical reaction to enhance selectivity and sensitivity. As per our knowledge, this is a first-of-its-kind attempt to fabricate an action specific sensor for creatinine by engineering a probe that includes an Anti-Cr antibody. The developed system readout was simplified in a way that the creatinine concentration was directly proportional to the change in color, and the image processing was performed based on an optical analysis approach. It was noted that under optimal conditions, the developed system exhibited a good linear range of 5- 400 μM , including the clinical range of Creatinine in humans with a limit of detection of 0.0146 μM . Additionally, the developed device showed a highly specific and accurate analytical performance when tested in the serum sample. Further, the probe showed excellent selectivity toward Creatinine in the presence of other proteins and ions present in the serum sample. The calculated selectivity of the developed sensor was 94.8 % and it exhibited a recovery of 82-98 % in serum samples proving its exceptional potential in the field of medical diagnosis.

Chapter VI

After developing paper-based optical platform for creatinine, we thought of targeting another biomarker and developed another paper-based micro device for the monitoring of human serum albumin (ALB), as incorporated in **Chapter III**. The paper micro-device was developed in two components where one was a paper chip and another being a 3D printed cascade for image analysis. The paper chip was developed in a simplified manner through antibody-immobilization for quantification of ALB in resource constraint areas. The bioengineered paper reaction zone was characterized using FTIR, SPM, XPS, and optical parameters. With strong consistency and linearity, the optical methodology employing parameters showed the best promise for the ALB quantification. The developed device showed linear calibration in the range of 1 – 60 mg/mL for ALB concentrations with a detection limit of 0.049 mg/mL and signal generation time of 10 sec which saturated around 40 sec. In multianalyte environment, the biosensor also showed low rates of cross-reactivity with a k_{sel} value of less than 1. The developed device could also determine the ALB concentration in serum samples effectively, with a recovery percentage of 88.14 - 97.70%. It was recommended to use the bioengineered paper within 30 days for more precise and accurate results as its activity was gradually decreased to 86.4 % after four weeks. The final hand-held device was designed with an in-built constant light source, requiring a smaller volume of sample, easy assembly process, and lower production costs than the conventional approach. We anticipate that the designed paper sensor integrated with 3-D printed device has a great deal of potential for accurate and accessible ALB detection. This platform has a great deal of potential to aid the identification of kidney disease, boosting the likelihood of early detection because it would facilitate routine testing more accessible across the primary healthcare centres. This could eventually lead to improved medicines and a longer life expectancy for kidney patients. After developing optical sensing platforms for the detection of creatinine and albumin for the diagnosis of kidney disease, we

thought of studying the electrochemical platforms for these two biomarkers and how the change in transduction system affect the monitoring of kidney disease.

In **Chapter IV**, we developed a smartphone-integrated electrochemical immunosensor for the detection of creatinine in physiological fluid. The immunosensor was fabricated using the step-by-step procedure to form the final GCE/AuNP/BMX/PANI/Anti-Cret sensing surface. The fabricated sensing surface after every modification over electrode surface was characterized using SEM, EDS, SPM, FTIR, and electrochemical techniques. The analytical performance was analysed using different concentrations of creatinine in buffer and serum samples. The developed sensor covered a wide dynamic range (10 nM - 0.1 M) including the clinically relevant concentrations, thus proving its application in the real-world analysis. We also integrated a software to simplify the process of data analysis that can possibly help in its applicability in health care facilities of remote areas. The software is able to calculate the creatinine concentration in the sample directly by providing the analytical signals after the immune-complexation reaction. Therefore, the ability to detect creatinine in clinically relevant ranges and the incorporation of software would facilitate the application of this technology in real-world analysis.

After that in the final **Chapter V**, we designed an electrochemical immunosensor to track ALB in human serum samples. The immunosensing probe was designed by functionalizing the Co-MOFs-c-MWCNT nanocomposite with anti-ALB. After each sequential modification, the electrode surface was characterized using numerous techniques, including SEM, EDS, XPS, FTIR, and electrochemical methods. Following extensive characterizations, the analytical efficacy of the probe towards ALB was evaluated in both buffer and serum samples. This nanocomposite provided the enhanced surface area for the functionalization of anti-ALB molecules on the electrode, and thus the fabricated probe was used for ALB detection. The probe detected ALB in a linear range of 0.1 to 60 mg/mL, covering its clinically pertinent

concentrations. The developed sensor is intriguing because of its facile fabrication procedure, wide LDR, low LOD, and excellent selectivity for the analyte. This was the first study of its kind to deploy a Co-MOFs-c-MWCNT nanohybrid immunosensing platform for ALB sensing.

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the future, it could potentially serve as a valuable platform for personalized sensing and thus be employed in the healthcare sector for the diagnosis of kidney diseases.

The limitations of developed platforms include the single-use nature of the optoelectrochemical chip/probe and the approximately 30-day lifespan of the matrix. This happens because the activity of the biorecognition element immobilized on paper or electrode decreases after four to six weeks.

Table 6.1: Comparison of different Optoelectrochemical platforms developed for the detection of Creatinine and Albumin

Target Analyte	Detection Method	Matrix	LDR	LOD	Estimated Cost
Serum Creatinine	Optical	C/DZ/EDC-NHS/Anti-Cr	5 - 400 μM	0.0146 μM	60 - 70 INR/test
	Electrochemical	GCE/AuNP/BMX/PANI/Anti-Cret	0.01 - 10^5 μM	0.0017 μM	100 - 120 INR/test
Human Serum Albumin	Optical	P/DS/EDC-NHS/Anti-ALB	1 - 60 mg/mL	0.049 mg/mL	60 - 70 INR/test
	Electrochemical	GCE/Co-MOF/c-MWCNT/Anti-ALB	0.1 - 60 mg/mL	0.02 mg/mL	90 - 100 INR/test

Further advancement in surface engineering methods and the emergence of different biorecognition elements such as nanobodies, aptamers, and others can also be used for the development of specific biosensors. The next step in the development process will involve moving from proof-of-concept-type studies towards clear translational pathways toward the

end user. Each translational pathway will involve carrying out studies with comprehensive validation and then undergoing subsequently designed preclinical and clinical studies in order to be able to declare safety, reliability, and accuracy. After that comes the approval from regulatory bodies post successful demonstration of the validation procedures, reproducible systems, and evaluation in real-world scenarios. In addition, there will be levels of development in parallel to identify solutions for manufacture at scale, consideration of the cost, simplicity, and the end-user design of the device. An additional useful area of research is moving towards the expansion of multiplexed sensing capabilities. This will provide a complete diagnostic picture from a single sample, since many disease diagnoses consist of multiple connected biomarkers. The potential benefits of such a system include better accuracy of test results, smaller sample volume, reduced assay time, and ultimately a cost-effective health care solution. Further integration of sensors with digital technologies such as smartphone-based readouts and cloud-enabled data analytics provides improved data quality.

The future of optoelectrochemical biosensors will be heavily influenced by how laboratory innovation is linked to clinical applications, particularly in terms of clinical validation, regulatory procedures, and commercialization. Furthermore, the focus should be on developing technology that may be easily integrated into basic healthcare facilities throughout the Indian healthcare system. These technologies may give cost-effective and simple solutions for improving diagnosis and encouraging improved health among the Indian population.