Chapter I

General Introduction and Review of Literature

Divya et. al, *Biotech. & Bioengg, 2023, Vol 120, 116-136*

Divya et. al, *Journal of Ind. and Engg. Chemistry (revision submitted)*

1. Introduction

The introduction of biosensors has transformed diagnostics, environmental monitoring, and research through rapid, onsite, specific and sensitive detection of target analyte/biomolecules [1]. The fundamental operation of a biosensor relies on the interaction between the biorecognition component and the transducer, which transforms biological signals into quantifiable responses [2-3]. The effectiveness of these interactions has been significantly impacted by surface engineering techniques, which are essential for improving sensitivity, selectivity, and stability [4]. Surface engineering entails the modification of the sensing surface at the nanoscale or molecular level to maximize biomolecular binding, inhibit non-specific interactions, and augment signal amplification [5-6]. This modification can be performed through various different methods including nanomaterial coatings, chemical functionalization, self-assembled monolayers (SAMs), polymer functionalization, etc which helps in improving the quality/functionality of biosensors [7-8].

Different types of nanomaterials such as gold nanoparticles [9], carbon nanotubes [10], graphene [11], and quantum dots [12] have been used for surface modifications of the sensing probe. They provide increased surface for the immobilization of biomolecules, boosts electron transfer efficiency for electrochemical transduction system, also helps in plasmonic improvements that eventually enhances signal of optical biosensors [13-15]. Chemical functionalization is a crucial surface engineering technique that involves the introduction of certain chemical groups onto the biosensor surface to facilitate selective biomolecule adhesion [16]. In this, chemical reactions are used such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide -N-Hydroxy succinimide (EDC/NHS) coupling (for carboxyl groups) [17], Schiff base formation (for aldehydes) [18], and click chemistry (for azide-alkyne reactions) [19] that helps in formation of strong and stable interfaces. Further, the incorporation of specific functional groups including hydroxyl (-OH), amine ($-NH_2$), carboxyl (-COOH), thiol (-SH),

and epoxy (-O-) improves the anchoring of biomolecules on the sensing surface [20]. This approach enhances reaction efficiency, charge regulation, enabling excellent selectivity and reusability, and hydrophilicity adjustment. SAMs are meticulously structured molecular assemblies that spontaneously form on a solid surface through chemisorption. Common examples of SAMs include thiols on gold, silanes on silicon, and phosphonic acids on metal oxides. This enables regulation of surface characteristics (hydrophilicity, charge, and functionality), facilitates directed biomolecule immobilization, enhances binding efficiency, minimizes non-specific adsorption, and improves the signal-to-noise ratio. Polymer functionalization method includes the coating of different functional polymers e.g., polyaniline (PANI), polypyrrole (Ppy) and poly(3,4-ethylenedioxythiophene): poly(styrenesulfonate) (PEDOT: PSS) to enhance the surface properties of sensing probe [21-22]. This method improves the sensor interface by introducing functional groups required for immobilization of biomolecules, facilitates charge transfer, enhances biocompatibility and surface stability. These techniques not only strengthen the interface but also improves signal transduction, resulting in increased sensitivity and lower detection limits [23-24]. Due to their role in advancing sensing platforms, different techniques have been utilized for the development of opto-electrochemical sensing platform for biomolecule detection. Opto-electrochemical biosensors are defined as analytical devices which integrate both optical and electrochemical transduction techniques for highly sensitive and specific detection of the target molecule. Optical biosensors detect target analytes by measuring changes in optical properties such as absorption, fluorescence, luminescence, or plasmonic resonance [25-26]. These sensors are widely used due to their label-free detection capability, high sensitivity, and real-time monitoring ability [27-28]. Electrochemical biosensors detect biological interactions through electrical signals generated from oxidation-reduction reactions. They are widely used due to their high sensitivity, rapid response, and miniaturization potential [29-30].

Here, we have tried to discuss about the different techniques and methods of surface engineering used for developing the advance biosensing devices. The detailed discussion entails on the surface engineering techniques used for the improving the sensitivity, selectivity and the signal amplification. Different strategies that help in signal amplification along with their methods are discussed in detail in the upcoming section. Advantages, limitations, and types of surface engineering methods were also discussed in tabulated form for better and clear understanding. The implementation of these surface engineering methods for the development of optoelectrochemical sensors describing the fabrication through different methods have been discussed in detail for the detection of different categories of biomolecule. Along with the detailed discussion, we have also compared different globally developed optoelectrochemical biosensors in tabulated form discussing their fabrication, detection technique, analytical details, etc. This study focusses on how these different techniques can help in achieving the required signal amplification with great sensitivity and selectivity. It allows the readers to have an overview about the different surface functionalization methods and their implementation for the designing and fabrication of advanced biosensing platforms.

2. Signal Amplification of Optoelectrochemial Biosensors via Surface Engineering

Signal amplification is crucial in biosensors to attain higher sensitivity, specificity, and lower limits of detection. These improvements include techniques for enhancing sensor performance, such as nanomaterial integration, chemical functionalization, and biomolecule immobilization. By increasing surface area through nanomaterial coatings, surface engineering techniques improve signal transduction which improves sensitivity because of biomolecule binding. Electrons are transferred quickly in electrochemical biosensors utilizing nanomaterials have aided electron transfer through conductive nanomaterials to realize rapid charge transference. Plasmonic enhancement through noble metal nanoparticles increases raman and fluorescence signal amplification in optical based biosensors. Enzyme-functionalized surfaces can also

enhance signal amplification through enzyme catalyzed process that generate enhance colored or colorimetric signals for signal amplification. Multiplexing & selective binding allows functionalized surfaces allow simultaneous detection of multiple analytes while reducing background noise. Polymer coatings help in signal amplification through the use of conducting polymers that enhances charge transfer in electrochemical biosensors, hydrogel matrices improve biomolecule retention and reduce non-specific binding. Localized surface plasmon resonance using nanostructured surfaces enhances optical biosensor sensitivity by concentrating the electromagnetic field near the sensor surface. Photonic Crystals and Meta-Surfaces controls light propagation and enhance fluorescence or raman scattering signals. Electrochemical signal enhancement using redox-active nanomaterials increases charge transfer efficiency and improve sensitivity in amperometric. Enzyme and catalytic signal amplification utilizes enzyme-labelled nanoparticles to drive multiple redox reactions to amplify electrochemical signals. With the intention to maximize biosensor sensitivity, selectivity, and signal amplification, surface engineering approaches are needed. By way of chemical functionalization, nanotechnology, and interface design, biosensors can be developed to previously unattainable performance capabilities in environmental monitoring, food safety, and medical diagnostics. Future research on surface engineering, nanomaterials, and hybrid biofunctionalization will result in increasingly complex biosensors that can amplify signal response, yield higher sensitivity, and yield faster response times for more potential applications. One of the greatest hurdles in the design of biosensors is reaching high sensitivity, which is often achieved through surface engineering and signal amplification methods. Different methods of surface engineering methods and their role in fabrication of optoelectrochemical sensing devices have been represented in **Figure 1.1**.

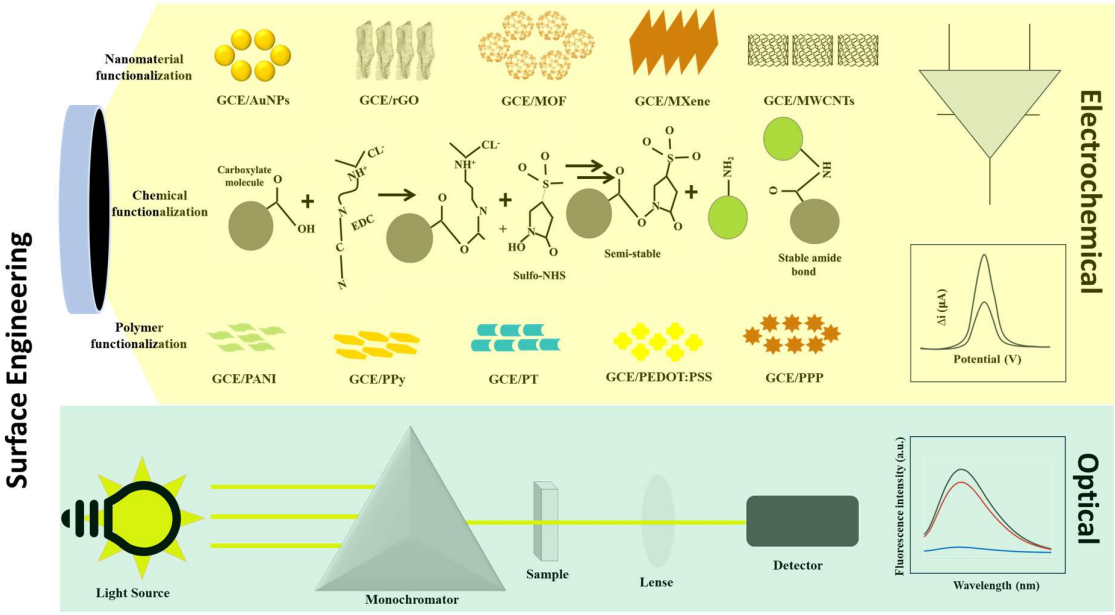

Figure 1.1: *Schematic representation of different types of surface engineering methods and their role in development of optoelectrochemical sensing devices.*

2.1 Opto-electrochemical Biosensors

Opto-electrochemical biosensors, combining optical and electrochemical transduction, constitute a powerful platform for the sensitive, targeted detection of biomolecules. The fitting of nanomaterials, chemical functionalization, and biomolecular immobilization as part of surface engineering to enhance signal amplification greatly improves these biosensors' effectiveness [3,8,15]. Pioneering approaches, including plasmonic nanostructures, electroactive materials and enzyme-assisted amplification, continue to push boundaries with respect to sensitivity, selectivity, and real-time detection [31-33]. Future developments in interface chemistry, signal transduction and nanotechnology will broaden the range of different applications of opto-electrochemical biosensors in areas such as environmental monitoring, and medical diagnostics [34-35]. Surface engineering plays a critical role in enhancing the performance of high-performance opto-electrochemical biosensors, as it has a significant impact on all relevant aspects, including stability, selectivity, and sensitivity. In surface engineering, modifying the biosensor's interface facilitates enhanced electron transport, optical

response, and biomolecule immobilization. Increasing sensor response (while amplifying signal) via chemical functionalization is one of the best methods. Chemical functionalization in sensor technology is defined by the use of - nanomaterials, biomolecules or other functional groups in sensor technology. Optical biosensors are valuable for recognizing the presence of a target analytes based on measurement of changes in the optical property of a sensor, which can relate to a number of phenomena including plasmonic resonance, fluorescence, luminescence and absorption. Surface Plasmon Resonance (SPR) are valuable for real-time biomolecular interactions, which detect binding events between analyte and biorecognition element, especially with high sensitivity and label-free. When surface plasmon waves are stimulated on a solid metal surface like gold alloy, there is a fundamental change in the surface electronic states, which enables measurement of these interactions. Fluorescence based biosensors utilize fluorophores or quantum dots, that emit fluorescence upon the interaction with target analyte that aid previous detections and used in medical diagnostics. Raman and Surface-Enhanced Raman scattering biosensors utilizes either metal nanostructures or raman scattering to amplify the vibrational signals of analyzing biomolecules for ultra-sensitive detection. These biosensors have been used to detect cancer biomarkers, monitor drugs and sense the environment. Colorimetric biosensors are an inexpensive form of biosensing that utilize color changes observable by the human eye resulting from interactions between biological systems and nanomaterial for point-of-care diagnostic [25,36-37].

Electrochemical biosensors detect biological interactions and are based on electrical signals produced by oxidation-reduction reactions. They are also used widely due to their sensitivity, reaction time, and potential for miniaturization. There are several types of electrochemical biosensors: amperometric biosensors measure current resulting from redox reactions when the analyte is present, and are widely employed for the measurement of glucose in diabetes monitoring. Potentiometric biosensors are used for neurotransmitter monitoring, ion-selective

detection and pH measurements. They provide a measure of the voltage change attributable to the ion concentration change. Impedimetric biosensors measure changes in impedance corresponding to the binding of analytes. They are also very sensitive biosensors for detecting infections, cancer markers, and DNA hybridization. Photoelectrochemical sensing systems utilize charge transfer reactions induced by light and have been used for pollution monitoring and biomedical diagnostics and are better than conventional sensors at signal detection [38-41].

2.2 Nanomaterial-Based Surface Modification

Nanomaterials enhance electron transfer, optical resonance, and analyte binding, leading to amplified sensor signals. Metallic Nanoparticles enhance SPR for optical biosensors, improve charge transfer for electrochemical biosensors. Carbon-Based Nanomaterials improve electrochemical sensing by enhancing electron conductivity, provide a large surface area for biomolecule attachment. Semiconductor Nanostructures used in photoelectrochemical biosensors for enhanced light absorption and charge separation. Effectiveness of the sensing setup depends on various parameters, including sensitivity, selectivity, stability, response time, linearity, and detection limit, that can be improved through surface engineering. Integration of nanomaterial onto the sensing probe through surface engineering facilitates electron transfer kinetics and improve the overall performance of the sensor. It can be done using different types of nanomaterial such as metallic nanoparticle, carbon-based nanomaterial, semiconductor nanostructure.

Metallic nanoparticles are the nanoscale zero-dimensional material derived from different metals such as gold, silver, and platinum. It offers tuneable electronic and optical properties depends on their size. It may also act as catalyst in array of chemical and electrochemical reaction and also facilitates the mechanical properties of the sensing device [42–44]. In a study by Sharma et al., proposed a novel electrochemical sensor using AuNPs for detection of

cortisol. Here, the gold nanoparticles were electrodeposited employing one step synthesis process directly on screen printed electrode. The sensor reported wide linear dynamic range (LDR) of 0.1 pg/mL to100 ng/mL with a limit of detection (LOD) of 0.25 pg/mL in buffer and 0.28 pg/mL in artificial saliva [45]. In another recent study by Zhou et al., a novel ecofriendly fabrication of silver nanoparticle over SPCE for sensing of aqueous bisphenol A. The nanoparticles were synthesized from fresh parsley foliage offering high catalytic activity for bisphenol A which showed a wide LDR of 0.05 - 5 μM and low LOD of 0.02 μM [46]. In a different study by Li et al. PtNPs based electrochemical sensor was reported for H_2O_2 sensing where the nanoparticle were synthesized using hydrothermal synthetic route showing high antibiotic susceptibility [47]. These studies helped in establishing the fact that nanoparticles were employed for developing sensing devices and different routes of synthesis were utilized. In another study by Sharifi et. al, a paper based optoelectrochemical sensing device was developed for the monitoring of pesticide concentrations (**Figure 1.2a**). Here, the prussian blue nanoparticles were incorporated on nanocellulose paper surface which enhances the colorimetric readout, thereby improving the selectivity and sensitivity of sensor. Along with nanoparticle, butyrylcholinestersase enzyme was combined which helped in developing sensing platform for detection. The developed platform was able to detect in a wide concentration range from 20 -100 ppb and shows the recovery of 98 to 107 % in wastewater samples [48]. In a recent study by Tomaşoğlu et. al, iridium oxide nanoparticles (IrO_2NPs) were integrated with bacterial cellulose (BC) for the monitoring of neurotransmitter (**Figure 1.2b**). IrO_2NPs possess a high density of hydroxyl groups on their surface which facilitates the adsorption of organic molecules. This adsorption induces optical changes in IrO_2NPs in the presence of biologically significant analyte. The IrO_2NPs-doped BC (IrO_2NPs@BC) was fabricated into circular spots to enhance handling convenience and subsequently applied for dopamine detection. The sensor exhibited a linear response to dopamine concentrations ranging

from 0.30 to 10 µM in solution, whereas the linear range was determined to be 0.60 mM to 2.5 mM in the paper-based platform. The LOD and limit of quantification (LOQ) were found to be 0.107 µM and 0.325 µM, respectively, in solution, while the values for the proposed paper-based optical sensor platform were 0.479 mM and 1.45 mM, respectively [49].

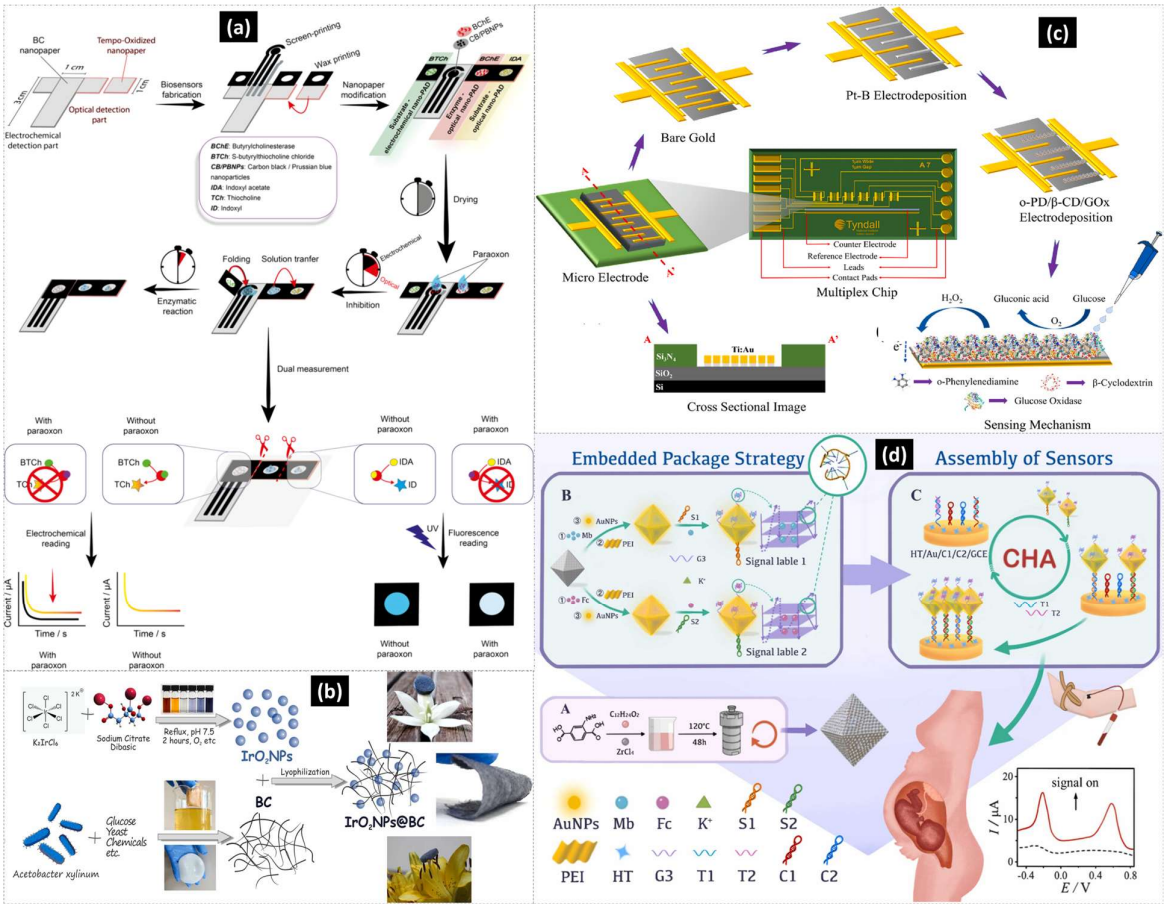

Figure 1.2: ***(a)*** *Step-by-step fabrication of paper based optoelectrochemical sensor through coating of nanoparticles and immobilization of enzymes for the detection of paraoxon (Reproduced with permission [48]),* ***(b)*** *Illustration of bacterial cellulose based biosensor fabrication through incorporation of IrO_2 nanoparticles for the detection of dopamine (Reproduced with permission [49]),* ***(c)*** *Schematic representation of microelectrode fabrication through electrodeposition of graphene oxide and immobilization of glucose oxidase enzyme for the detection of glucose (Reproduced with permission [50]),* ***(d)*** *Representation of MOF based electrochemical sensing platform immobilizing gold and aptamers for the detection of thalassemia (Reproduced with permission [51])*

These studies help in establishing a fact that nanoparticles were also integrated with paper surface using different methods which eventually helps in improving the signal for the optical and electrochemical monitoring of target analytes.

Carbon based nanoconfined materials such as graphene, carbon nanotube, and graphene oxide have emerged as powerful engineered material in biosensing due to their exceptionally high surface area, electrical conductivity, and biocompatibility. These carbon-based nanomaterial enables stable platform for biomolecule immobilization. Graphene is a uni-c sp_2-hybridized carbon atom, decorated as a honey comb like lattice and this unique structure provides mechanical stability with facilitated electron transfer kinetics. In a study by Nagaraja et al., demonstrated a novel microplotter printed graphene-based electrode for phosphate sensing employing electrochemical method. They have prepared ink nanocomposite using ethyl cellulose and graphene followed by their deployment in electrode printing. They reported a wide LDR of 1 - 600 μM and LOD of 2.2 μM [52]. Graphene oxide is a chemically engineered form of graphene functionalized with oxygen based functional groups such as -OH, -COOH, -O-, while reduced graphene oxide is reduced form of graphene oxide which enhance electrical conductivity, high dispersibility in water, and high functionalizability due to their functional group and unique structural nanoarchitecture [53]. They have been explored in different types of sensors including electrochemical, flexible, fluorescence owing their exceptional opto-electrochemical properties. In a recent study by Adib et. al, interdigitated microelectrodes were designed for the monitoring of glucose in artificial and calf saliva samples (**Figure 1.2c**). Here glucose oxidase and phenylenediamine and cyclodextrin was immobilized on platinum black modified microelectrodes through electrodeposition method. This enzyme-based method was used for sensitive and selective detection of target analyte. The developed device shows wide range of concentrations from 0.02 mM to 7 mM, with a LOD of 0.3 μM [50]. In another study by Qian et al., proposed a graphene oxide decorated sensing probe for electrochemical

detection of isoniazid. Modification of the electrode with graphene oxide offering very high surface area and exceptionally high sensitivity for analyte and reported LDR of 5 - 100 μM with a LOD of 0.4 μM and sensitivity of 4.02 $\mu A \mu M^{-1}$ [54]. In a study by Bunnasit et al., reported a very sensitive and portable electrochemical sensor employing graphene oxide as probe modifier for chloramphenicol detection. They used differential pulse voltammetry (DPV) to asses analytical performance of modified probe showing dramatically high cathodic peak current due facilitated electron transfer kinetics of rGO. They reported wide LDR of 30 - 100 μM and LOD of 0.7 μM [55]. CNTs are the one of the most important nano-allotropes of carbon showing cylindrical with sp_2 bonded carbon atom. After its discovery by Iijima in 1991 it attracts the material scientist due to their unique opto-electrochemical properties. It can be categorized into SWCNTs and MWCNTs based on the number of the rolled overlapping cylinder.

Table 1.1: *Description of globally developed electrochemical biosensors using surface engineering methods (NR: Not reported)*

S.No.	Designed Surface	Matrix	Target	Detection Technique	Dynamic Range	LOD	Real Sample	Ref.
1.	Prussian blue nanoparticles were combined with butyrylcholinestersase and incorporated on nanocellulose paper surface which enhances the colorimetric readout	Paper	Paraoxon	Colorimetric	20 - 100 ppb	12.2 ppb	Wastewater	[48]
2.	2D $Cs_2AgBiBr_6$ encapsulation with silk fibroin for the sensitive detection of cardiac troponin I	Silk Fibroin	cTnI	Optical	10 pg/mL - 100 ng/mL	10 pg/mL	NR	[56]
3.	Direct solid-phase immobilization of primers on silicon wafers	Silicon Wafers	Pathogen	Optical	10^1 - 10^7 CFU/mL	10 CFU/mL	NR	[57]
4.	SnO_2 metallic oxide thin film was functionalized on D-shaped fiber	Optical Fibre	IgG	Optical	0.5 - 10 µg/mL	0.12 µg/mL	NR	[58]
5.	ZnS nanoparticles were capped on schiff base using coprecipitation	Nanoparti-cles	Hg^{+2}	Optical	NR	1.49 nM	Tap water	[59]
6.	Iron oxide coated on chitosan and tungsten disulfide-based quantum dots immobilized on fiber	Fiber	*S. aureus*	Optical	1 - 10^8 CFU/mL	1.47 CFU/mL	Food sample	[60]
7.	AgNPs extracted from *Curcum Longa* along with enzyme used	Optical Fiber	Ascorbic Acid	Optical	110 - 400 µM	NR	NR	[61]

8.	Ni/Cu doped carbon doped functionalized with polyethyleneimine for fluorometric detection	-	Bilrubin	Fluorescence	10 - 280 µM	0.0907 µM	Serum sample	[62]
9.	Glucose oxidase enzyme was self-assembled using poly(allylamine hydrochloride)	Optical Fiber	Glucose	Optical	0.3 - 2.4 mg/mL	NR	NR	[63]
10.	Lanthanide-doped upconversion nanoparticles were conjugated with glial fibrillary acidic protein antibody	-	Glial fibrillary acidic protein	Photolumines-cence	5 -5000 pg/mL	0.082 pg/mL	NR	[64]

Semiconductor nanostructured recently explored as emerging material owing their unique opto-electrochemical properties and facilitated sensitivity and selectivity of the sensor. There is plethora of semiconductor nanostructured material of which we have briefly discussed the MXene quantum dots (MXene QDs), TiO_2 and MoS_2. MXene QDs are categorized among the zero-dimensional semiconductor nanostructured material owing size dependent opto-electronic properties due to quantum confinement effect. They typically composed of material such as titanium and vanadium etc. MAX phase (3-D bulk) can be changed into 2D MXene sheets via exfoliation and etching and further chemically chopped into QDs through hydrothermal cutting. Recently Lin et al., developed a novel MXene QDs based optical sensor for detection of Bacillus anthracis biomarker. They have synthesized this MXene QDs employing hydrothermal route and deployed for ratiometric fluorescence detection of dipicolinic acid which showed a LDR of 0 - 11 μM and LOD of 0.26 nM [65]. In another study by Ma et. al, a metal organic framework based electrochemical sensing device for the detection of multiple β-thalassemia mutation. In this work, methylene blue and ferrocene were embedded with metal organic framework and integrated with G-quadruplexes (**Figure 1.2d**). The developed probe shows superior stability and specificity for the target molecule. This shows wide dynamic range of 1.0 - 500.0 fM and such sensing platform can be employed for the detection of various mutations by altering the templates. In another study by Chen et al., proposed a TiO_2 based electrochemical sensing platform for phosphorpeptide detection. TiO_2 based nanowire offers unique one-dimensional stable nanoarchitecture with a very high aspect area to volume ratio and enhanced opto-electronic properties compared to their counterpart. While TiO_2 based nanoparticle offers enhanced photocatalytic activity and high electron mobility compared to their parent material owing nanoconfinement. They have developed TiO_2 semiconductor nanostructure through facile sol-gel method using titanium tetra-n-butanol. Following this TiO_2 was fabricated over the gold electrode and deployed for sensing which showed an LDR of 1

pg/mL to 1 mg/mL with a very low LOD of 0.24 pg/mL [66]. Recently Govea et al., proposed a MoS_2 nanostructure based amperometric sensor for non-invasive dopamine detection. They have synthesized MoS_2 nanostructure employing hydrothermal synthetic route and fabricated over carbon-based paper electrode and then further modified with enzyme. The developed probe (CPE/MoS_2/LacII) was deployed for sensing which showed an LDR of 0.1 - 5 μM with LOD of 10 nM which is lowest value reported in the literature employing laccase enzyme [66]. All these studies shows that the integration of different types of nanomaterials in the optoelectrochemical sensing platforms provides improved signal generation. These materials have also been explored by integrating with different types of matrices and hence helps in development of advanced sensing platforms with better sensitivity, specificity and lower detection limits. Additionally, some of the globally developed optical sensing devices utilizing different methods of surface engineering were analytically compared in **Table 1.1** to provide more clear representation.

2.3 Chemical Functionalization

A critical factor in the development of high-performance opto-electrochemical biosensors is surface engineering, which significantly impacts sensor sensitivity, selectivity, and stability. Surface engineering involves modifying the biosensor's interface to enhance biomolecule immobilization, improve electron transfer, and amplify optical responses. One of the most powerful strategies for signal amplification is chemical functionalization, where nanomaterials, biomolecules, and other functional groups are introduced to optimize sensor performance. It enabling the immobilization of molecule such as antibody, nucleic acid, aptamer, and enzymes over the sensor surface. Different surface engineering method have been explored for such chemical functionalization including self-assembled monolayer fabrication, functional group facilitated bioconjugation such as EDC/NHS coupling (**Figure 1.3a**) [48], for aldehydes (**Figure 1.3b**) [15], and click chemistry (**Figure 1.3c**) [49]. Chemical functionalization plays a

crucial role in improving analyte capture efficiency, reducing non-specific interactions, and enhancing signal transduction.

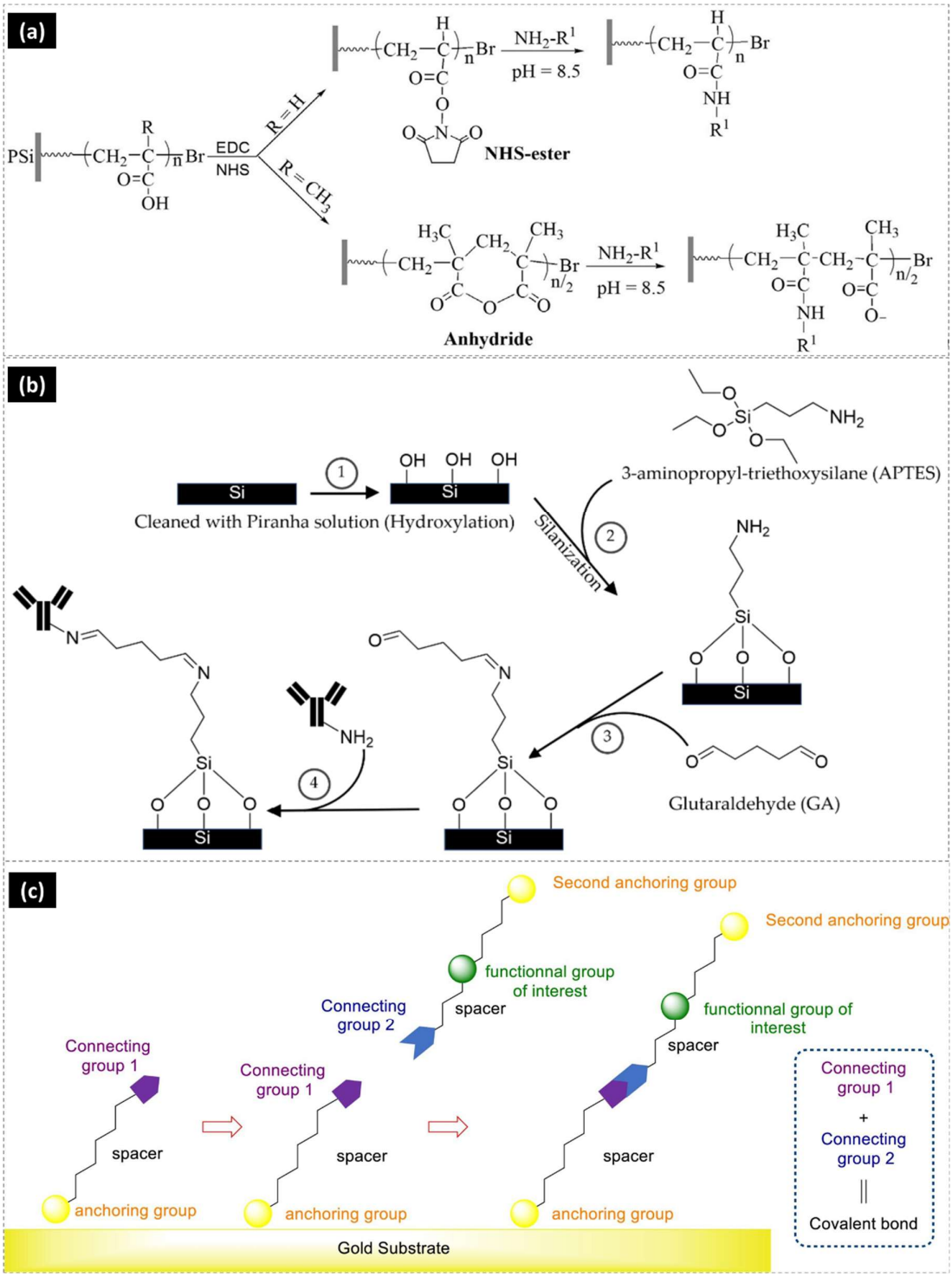

Figure 1.3: *Different methods of chemical functionalization for biomolecules conjugation through* ***(a)*** *EDC-NHS (Reproduced with permission [67]),* ***(b)*** *APTES-Glutaraldehyde reaction (Reproduced with permission [17]),* ***(c)*** *Click chemistry (Reproduced with permission [68])*

SAMs helps in organizing functional molecules on the sensor surface to control biointerface properties and enhance signal response, commonly used with gold and silver surfaces for SPR biosensors. SAM is highly ordered spontaneously arranged single layered nanoarchitecture over the matrix. It typically composed of three parts including head group (attached with sensor matrix), spacer (structural stability), and terminal functional group part (binding site for biomolecules). The terminal group of SAMs may covalently or non-covalently attached with the biomolecules, establishing a chemically tuneable interface for immobilization of biomolecules. This single layered nanostructured platform enhances the molecular affinity, reduces non-specific adsorption efficiency offering specificity to sensor probe. In a study by Patel et al., reported a novel self-assembled monolayer tailored sensor probe to enhance sensitivity and specificity of the sensor. They have fabricated thiolated (5'-end) ssDNA-SH over the gold coated glass electrode and deployed for sensing of genomic DNA, which showed a detection range of 100 - 500 ng/μL within 60 s of hybridization time [69].

Bioconjugation with functional groups is another method of chemical functionalization of biomolecules with sensor probe. Among the different bioconjugation method EDC-NHS is the most commonly explored and widely applied for covalent coupling of antibody and protein. It is a water soluble, noncytotoxic, and biocompatible material, offering carbodiimide-based coupling reaction for biomolecules linking with the exposed surface terminations facilitated stability and specificity to the sensor. EDC reacts with the -COOH group of sensor probe and form o-acylisourea intermediate (unstable), in the presence of NHS it forms a stable NHS-ester which preventing rapid hydrolysis for more controlled bioconjugation. Following this, primary amine group of antibody, protein, or enzyme reacts via displacement reaction and attached with sensor probe through a stable amide bond. In a study by Liu et. al, optical platform was developed using CRISPR/Cas12a to detect the target nucleic acids (**Figure 1.4a**). The probe was developed through functionalization of aptamer and DNAzyme using EDC-NHS method

of immobilization. The developed optical tweezer was connected with fluorescence imaging that produces stable signal and can be used for multiflux measurement [70]. In another study by Ullah et. al, a fluorescence-based sensing platform was developed for the iron-regulated surface determinant protein A detection. The sensor was designed using the mesoporous nanoparticles and the immobilization of aptamers on the surface using APTES glutaraldehyde method of functionalization for signal enhancement (**Figure 1.4b**). The probe was designed in such a way that aptamer molecules used as molecular recognition probes and gated molecules, and MSN-bound rhodamine 6G serving as the fluorescent signal. The probe shows a wide dynamic range from 2.5 to 100 nM with a LOD of 2.34 nM [71].

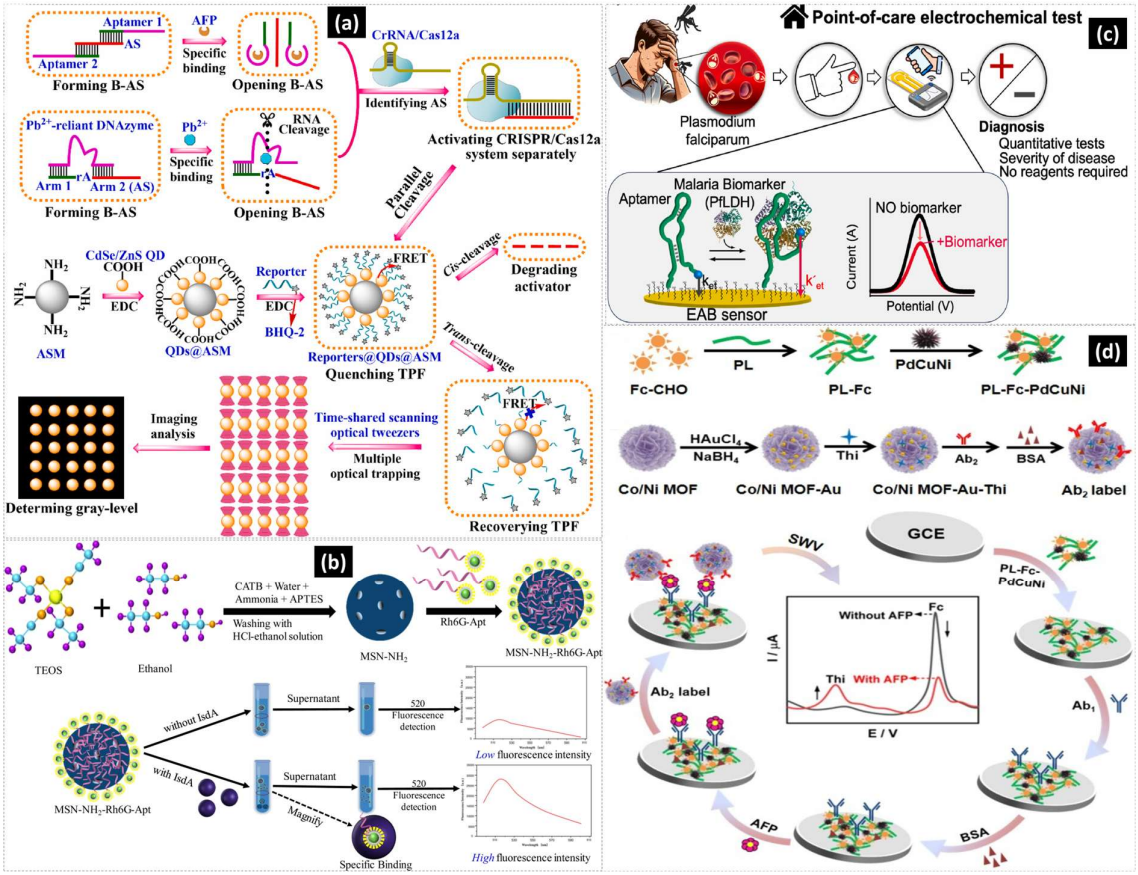

Figure 1.4: (a) *Illustration of optical sensing platform using CRISPR-Cas12a method and the immobilization of aptamer using EDC-NHS bioconjugation reaction (Reproduced with permission [70]),* ***(b)*** *Synthesis of optical platform for the monitoring of the iron-regulated surface determinant protein A using the immobilization of aptamer and DNAzymes using APTES (Reproduced with permission [71]),* ***(c)*** *Representation of aptamer based electrochemical diagnosis of malaria biomarker lactate dehydrogenase (Reproduced with permission [72]),* ***(d)*** *Schematic illustration of synthesis of Co/Ni MOF and immobilization of antibody on the electrode surface using the functionalization of chemical group (Reproduced with permission [73])*

Another study by Rabiei et al., proposed electrochemical aptasensor for detection of Staphylococcus aureus employing EDC-NHS chemistry. They fabricated the aptasensor over the modified GO/PDES/NiO through EDC-NHS reaction and then deployed for sensing of bacteria which showed LDR of 10^1 - 10^8 CFU/mL and LOD of 10 CPU/mL [74]. Further, in a recent study by Yang et. al, an electrochemical sensing platform was developed for the

diagnosis of malaria (**Figure 1.4c**). The sensor was developed through immobilization of aptamer specific to malaria biomarker plasmodium falciparum lactate dehydrogenase using thiol reaction over gold electrode. The developed system able to detect the target molecules within a concentration range from 6.28 to 51.7 nM and can be employed for detection in serum samples. In another study by Sun et. al, an electrochemical sensing platform was developed for the diagnosis of hepatocellular carcinoma by targeting alpha-fetoprotein. The sensing probe was designed using gold nanoparticles modified with Co/Ni metal organic framework which acted as probe capturing surface (**Figure 1.4d)**. The probe was enriched with thionine for the coupling of antibodies specific to target and for signal generation. The probe shows good linearity from 0.01 - 200 ng/mL with a low detection limit of 3.16 pg/mL and can be used for the carcinoma detection [73]. These examples help in concluding that chemical functionalization is one of the most preferred techniques for the development of optoelectrochemical sensing devices. These methods of chemical functionalization help in the conjugation of biomolecules which eventually leads to development of highly sensitive and specific detection. This method helps in ensuring stable and oriented biomolecule attachment, improving reaction efficiency, reducing steric hindrance, leading to higher target-binding sensitivity, enhancing catalytic efficiency when combined with nanomaterials.

2.4 Polymer Functionalization

Polymer coating is a unique approach of surface engineering employing the coating of polymer over the sensor probe which crucially enhances the sensitivity, and stability of the sensor [75]. It also provides functional interface to the sensor probe for further tunning of sensor for desired characteristics. There is a plethora of polymer that potentially applied over the sensor probe such as PANI, Ppy, and PEDOT: PSS, here we have briefly discussed the PANI. PANI is a conductive polymer gained a significant attention, owing their high electrical conductivity, biocompatibility, and stability [76,77]. It can be functionalized with different functional groups

such as -COOH, -NH_2 or -OH for further tunning of sensor probe enabling selectivity to sensor. In a recent report by Pal et. al, optical sensing device was developed using PANI for the detection of aflatoxin B1 in body fluids. PANI was deposited on optical fibre and the optical properties of PANI were explored on polymethylmethacrylate surface of plastic optical fibre (**Figure 1.5a**). The developed probe detects aflatoxin B1 in a wide dynamic range from 0.05 - 500 ppb with a LOD of 0.112 ppb in serum samples. This system also detects in different types of samples including nut, cereals and beverages [78]. In another study by Wu et al., polyaniline was employed for the electrochemical monitoring of cadmium in waste water. PANI-AuNPs were molecularly imprinted on electrode surface and then deployed it for voltametric quantification of cadmium which showed an LDR of 5 - 100 µg/L and LOD of 1.2 µg/L [79]. Another study by Jikamo et al., developed a polyaniline based non-enzymatic electrochemical sensor for malathion detection. They have developed the sensor probe using the sequential modification to obtain GCE/NiO-ZnO-PANI and deployed it for sensing which showed an LDR of 10 - 70 nM and LOD of 10 nM [80]. Plasmonic and photonic property enhancement is a very crucial step in advancing optical sensing technologies for desired characteristics. This property enhances the light matter interaction to facilitates the sensitivity and performance of the sensor. There are a range of optical signal enhancement mechanism of which localized surface plasmon resonance (LSPR), photonic crystal, and meta surface approach ones are briefly discussed here. LSPR is a phenomenon where light interaction enhances the oscillation of free electrons over the surface of nanostructure which in turn enhance magnetic field strength. It generates enhanced surface plasmon resonance over the nanostructured surface. In a study by Yang et al., proposed LSPR based rapid detection of corona virus employing silver nanotriangle nanostructured probe. They have functionalized the silver nanostructured surface with the human angiotensin-converting enzyme 2 protein to develop LSPR based sensor to detect SARS-CoV-2 spike RBD protein and CoV NL63 virus with exceptional sensitivity. The

performance of sensor was tested in spiked RBD protein, CoV NL63 in buffer and untreated saliva, which showed very LOD of 0.83 pM, 391 PFU/mL, and 625 PFU/mL respectively [81]. Photonic crystal sensor utilized the periodically structured dielectric material to control the propagation of light via photonic band gap phenomena. They allow to pass only the desired wavelength of light restricting the others. The interaction of the analyte with photonic crystal structure enhances the optical characteristics by changing the refractive index which enable highly sensitive and specific sensing. In a study by Nankali et al., developed a 3-D ZIF-8/PDA photonic crystal for sensing of blood component with enhanced sensitivity. They developed sensor probe by coating silicon substrate using polydopamine supported zeolitic imidazolate framework -8 (ZIF-8) nanoarray. It deployed for sensing of different blood components including biotin-streptavidin, bovine serum albumin (BSA), cytop, glucose, hemoglobin, blood plasma, sylgard 184, white blood component, urethane dimethacrylate, and polyacrylamide [82].

Another study by Shi et el, a super-antifouling electrochemical biosensor was developed for protein detection in complex biofluids based on PEGylated multifunctional peptide (**Figure 1.5b**). PEDOT: PSS was electrodeposited to form a conductive layer and the gold nanoparticles were deposited to form the final electrode surface. PEGylated multifunctional peptide was self-assembled onto the surface via Au-S bonding, forming PEG-MPEP/AuNPs/PEDOT sensing probe. The developed surface showed increased conductivity with PEDOT and AuNPs, while PEG-MPEP reduced electron transfer due to its antifouling nature. The developed biosensor able to detect the human immunoglobulin G in the range of 1 pg/mL to 1 μg/mL, with a low detection limit of 0.41 pg/mL in complex biological media [83]. Further in another study by Alam et. al, PANI was employed for the development of electrochemical sensing platform for the detection of SARS-CoV-2. The screen-printed electrodes were modified with PANI-titanium nanotubes and the surface was used for the immobilization of monoclonal antibody

specific to SARS-CoV-2(**Figure 1.5c**). The developed device able to detect in a wide dynamic range from 80 to 200 copies/μL with a LOD of 25.59 copies/μL .

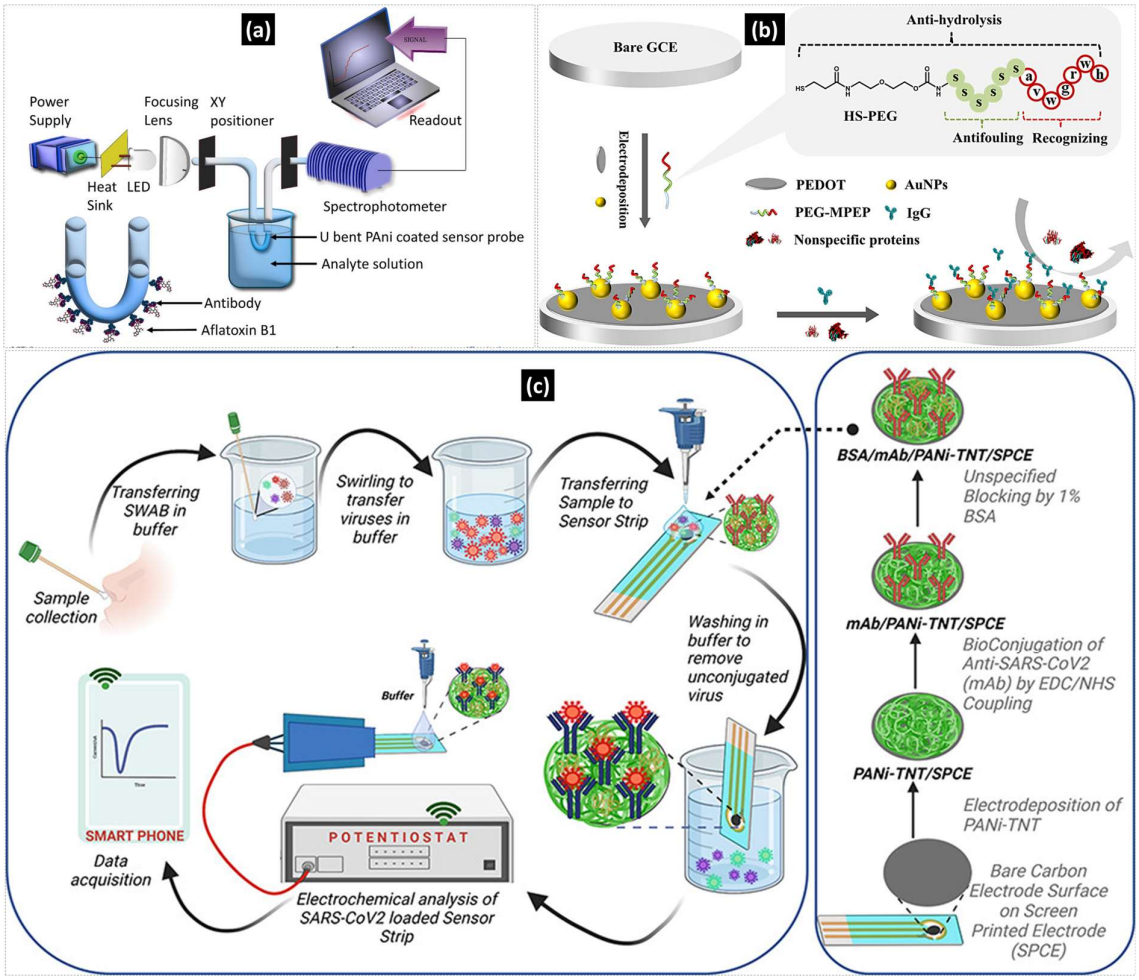

Figure 1.5: (a) *Illustration of optical biosensor for the detection of aflatoxin B1 through deposition of PANI on optical fibre and using the coated fibre for the incorporation of antibodies specific to the target molecule and the analytical signal was recorded using spectrophotometer (Reproduced with permission* [78]*),* ***(b)*** *Representation of fabrication of sensing probe using the polymer deposition on electrode surface for the detection of IgG (Reproduced with permission* [83]*),* ***(c)*** *Representation of smartphone based electrochemical sensing platform for the detection of SARS CoV-2 describing the detailed fabrication method utilizing functionalization of PANI and immobilization of SARS CoV-2 specific monoclonal antibody for the detection (Reproduced with permission* [84]*).*

This method of surface engineering helps in improving reaction efficiency, allowing high selectivity and reusability, charge control & hydrophilicity tuning, modifying the surface charge can influence biomolecule interactions, enhancing signal transduction. Additionally, we have also discussed the globally developed electrochemical sensing probe for the detection of various molecules using the different methods of surface engineering in **Table 1.2.**

Table 1.2: *Description of globally developed electrochemical biosensors using surface engineering methods (NR: Not reported)*

S. No.	Designed Surface	Matrix	Target	Detection Technique	Dynamic Range	LOD	Real Sample	Ref.
1.	Methylene blue treated Neuro-2a cells were immobilized on thin-film electrode and viability was checked after exposing them to ciguatoxins	Gold electrode	Ciguatoxins	DPV	0.5 - 32 pg/mL	NR	Fish extract	[85]
2.	PtNPs and MXene nanosheets electrochemically deposited and CA15-3 antibody immobilized	GCE	CA15-3	DPV	1 - 100 nU	1 nU	Serum sample	[86]
3.	Aptamer specific to plasmodium falciparum lactate dehydrogenase	Electrode	Lactate dehydrogenase	DPV	6.28 - 51.7 nM	NR	Blood sample	[72]
4.	ZIF-8 nanoparticles were conjugated with boronic acid and drop casted on electrode surface	SPCE	Glucose	SWV	100 - 450 mg/dL	NR	Blood sample	[87]
5.	Cu-PTC based MOF were incubated with metalloproteinase-2 peptide and the prepared solution was added to electrode	Electrode	Metalloprotein-ase-2	DPV	0.5 - 200 ng/mL	0.25 ng/mL	Serum sample	[88]
6.	Copper iodide nanoparticles were stabilized with chitosan drop casted on ITO and tyrosinase enzyme was immobilized on it	ITO	Dopamine	CV	7 - 80 µM	0.02 µM	Human cerebrosp inal fluid	[89]
7.	A laser induced ZnO/graphene photoelectrode was developed based on photocurrent-polarity-switching signal	ITO	Tobramycin	Photocurr -ent	20 nM - 1.0 µM	5.43 nM	Milk and Lake water	[90]

8.	Aptamer specific to ACE-2 immobilized on Ni doped ZnO nanorod	Electrode	SARS-CoV-2	EIS	200 - 1000 ng/mL	60.13 ng/ mL	Serum Sample	[91]
9.	Au film deposited on polylactic acid nanofibre sheet and specific aptamer was immobilized	Electrode	Cortisol	SWV	10 pg/mL - 10 µg/mL	1 pg/mL	Saliva and Sweat sample	[92]
10.	HER2 specific antibody immobilized on gold leaf electrode	Gold electrode	HER-2	EIS	0.1 - 1000 ng/mL	1 ng/mL	cell culture medium	[93]

3. Development of opto-electrochemical sensing platforms for the detection of chronic kidney disease

Chronic Kidney disease has emerged as one of the most common conditions in which an individual's kidney is unable to perform its function [94]. Chronic Kidney disease has affected more than 10% of the world's population which counts to more than 800 million people [95,96]. Over the last two decades, an exponential surge in the number of deaths has been recorded worldwide. According to Kidney International reports it will become 5th major cause of death worldwide by the year 2050 [97]. One of the major concerns relating to chronic kidney disease is that its cases are increasing in children which are becoming a major public health issue. In one of the studies, it is found that there is 30 times increase in mortality rate and severely affected life quality of children [98,99]. Most of the children require dialysis or kidney transplant which is not easily available in developing and under-developing countries. Other major issues with chronic kidney disease are the prevalence of other serious health conditions like cardiovascular diseases, depression, anxiety, and diabetes [100,101]. In a recent study, the risk associated with chronic kidney disease and outcomes after coronary artery bypass has been established [102]. These risk factors have increased social and psychological problems in society and thus affect the disease condition worldwide. High-income countries are already spending around 18% (costing 45.5 billion dollars) of their medical expenditure on chronic kidney illness and it tends to increase with an annual growth of 6-12% in expenditure [103,104]. But this high expenditure requirement especially in dialysis for end-stage renal disease patients cannot be fulfilled in underdeveloped and developing nations. Therefore, early-stage diagnosis of renal dysfunction in a simple and cost-effective manner is required. The divergence in prevalence and severity of chronic kidney disease is possibly due to the complex interaction between biological and non-biological risk factors [105–107]. The diagnosis of chronic kidney disease is usually done in laboratory settings and requires skilled personnel to

perform a test by targeting various biomarkers relating to it. Current methods of laboratory diagnosis are perceived as invasive, time-consuming, and expensive. The early diagnosis of kidney disease is also one of the major issues because the concentration of biomarkers is very less and the biomarker is related to other health conditions too. In such situations, biosensors have emerged as a savior for providing simple, easy-to-use, cost-effective point-of-care testing of various diseases. Biosensor is defined as an analytical device that produces a readable signal proportional to the concentration of the target analyte [1,14,108]. Point-of-care testing and monitoring of medical biomarkers is a promising approach towards rapid, cost-effective, reduces the complexity of analysis, also enable deliver of home healthcare services [109–111]. In cases, where high precision is required, many conventional clinical techniques for the analysis of biomarkers have some limitations in terms of cost, size, and integration into portable point-of-care medical systems. With advancements in technologies, emerging miniaturized sensing systems based on different readout methods such as optical (e.g., colorimetric, fluorescence, chemiluminescence, ionophore, etc.) and electrochemical (e.g., amperometric, voltammetric, conductometric) have the ability to provide real time data of the disease diagnosis and management though analysis of physiological fluids such as blood, urine, and sweat [11,112,113]. People who are suffering from chronic kidney disease i.e., end-stage renal infection, have limited mobility, and need to go to the hospital for diagnosis and care. The detection of specific biomarkers for this health condition has, however, not been straightforward. A series of biomarkers have been proposed such as abnormal levels of creatinine, albumin, and urea which are present in the blood as well as urine of individuals with chronic kidney disease, and since ages these are considered the major biomarkers for clinical diagnosis [114,115]. Many other biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL), N-acetyl-β-glucosaminidase (NAG), kidney injury molecule 1 (KIM-1), β-2-microglobulin, fatty acid binding proteins (FABP), and others comes under clinical validation

but their applicability in clinical settings have not been established [116,117]. These biomarkers and their stage in clinical level have been illustrated in **Figure 1.6a**. Therefore, finding the applicability of creatinine, albumin, and urea levels in the kidney patients are specifically used in clinical laboratories and trusted by medical personnel. Interestingly numerous biosensors have been developed for the detection of creatinine, albumin, urea levels for the renal disease and it has been observed that for the creatinine detection optical methods have been preferred and this data has been represented in **Figure 1.6b**.

Here, optical and electrochemical sensors designed for the diagnosis of chronic kidney disease by targeting various biomarkers are explained. The different biomarkers namely creatinine, albumin, cystatin C, albumin to creatinine ration, urea present either in serum or urine are targeted. We have reviewed in detail the different types of optical and electrochemical readout system that are integrated for the qualitative and quantitative detection of clinical biomarkers of chronic kidney disease. Detailed advantages and concerns related to various types of optical and electrochemical developed system are discussed in detail. We have also tried to provide the basic understanding of chronic kidney disease and various biomarkers, and their mechanism and mode of action. Also, detailed comparison table for different biomarkers comparing their sensing mechanism, analytical performance, sensor configuration, linear detection range, limit of detection and response time has been described. The overall aim of this article is to provide and give an insight of present situation of chronic kidney disease and the advancement in diagnosis methods with a special focus on optical and electrochemical readout mechanism.

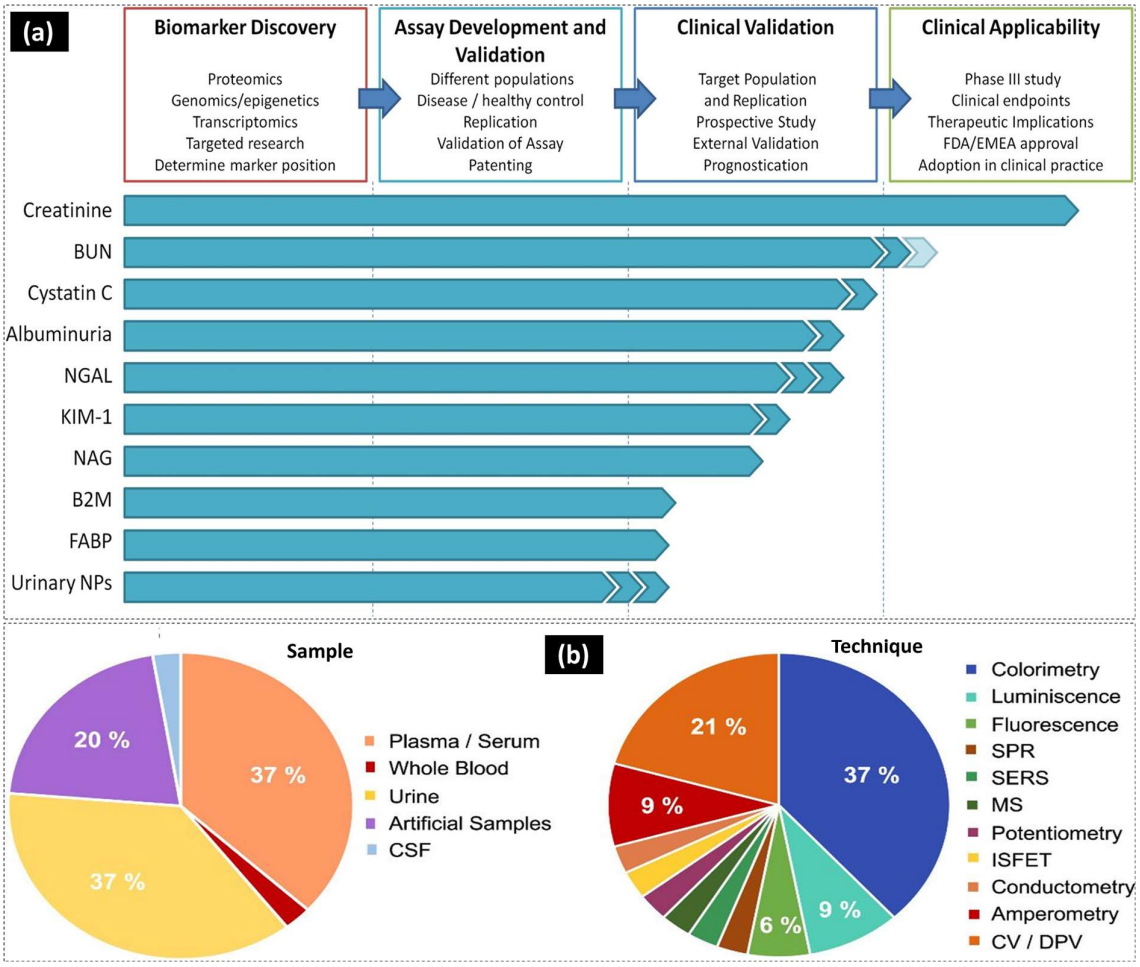

Figure 1.6: (a) *Representation of biomarkers relating to chronic kidney disease and their clinical validation and applicability (Reproduced with permission [117]),* ***(b)*** *Pie-charts representing the percentage of particular sample used and the technique applied for globally developed sensors for creatinine (Reproduced with permission [113])*

4. Different Biomarkers for Chronic Kidney Disease

CKD is a term that has undergone several definitions and classifications throughout the years. However, according to the most recent international standards, CKD is defined as reduced kidney function having a glomerular filtration rate (GFR) about less than 60 mL/min per 1.73 m^2 or kidney damage markers, sometimes both, for at least three months irrespective of the underlying condition [118–120]. However, the glomerulus, the kidney's specialised filtering organ, is mostly responsible for the clinical evaluation of renal disease [121]. The limitations

of this one-dimensional paradigm on renal disease diagnosis and therapy are immediately clear. Both acute and chronic kidney illnesses are becoming acknowledged as serious global health issues because they continue to progress clinical care [122,123]. Furthermore, because these abnormalities are discovered too late in the disease's progression, no viable treatments have been created to lessen kidney damage, change the course of the illness, or reduce the morbidity and mortality associated with it [124]. In order to overcome these constraints, research utilising cutting-edge techniques has concentrated on finding biological markers of kidney injury in the urine or in systemic circulation that are either created directly by the kidney or accumulate as a product of renal cells malfunction after kidney damage. The biomarkers of kidney health are related to the pathophysiology of renal injury and it may help with early diagnosis, localization of the injury, etiologic differentiation, and prognosis of renal injuries [125]. Some of the widely used clinical biomarkers for kidney injuries are discussed in the following sections:

4.1 Creatinine

Nonenzymatic dehydration of muscle creatine produces creatinine at a constant rate as a byproduct of regular cellular metabolism, which is then readily filtered by the glomerulus and released through the renal tubules without being reabsorbed [126,127]. One of the most reliable measures of kidney health is the glomerular filtration rate (GFR) [128]. The serum creatinine is the gold standard for estimating glomerular filtration rate (eGFR) in the clinic. An estimation of GFR can be made from the measured clearance of creatinine. Urine is collected for a period of 24 hours, or preferably 5 to 8 hours at a precise interval, as 24-hour sample collections are known to be problematic. Patients' creatinine levels, together with their ages, sexes, races, and weights, are all used in the GFR equation.

4.2 Albumin

There are a variety of crucial functions performed by serum albumin. Like a magnet in the blood, it helps direct fluid to where it needs to be. Numerous studies have demonstrated that

people with CKD have decreased serum albumin have a higher risk of progressing to renal failure than those who have normal blood albumin [129,130]. Researchers have been using studies to speculate on possible causes, but a full explanation remains elusive. A low serum albumin level is merely a marker (kind of sign in the blood), according to some experts, which indicates that the kidney has been damaged. In other words, decreased serum albumin levels never occur in isolation [131]. Some underlying issues causing kidney damage are: Undernourished bodies make less albumin and break it down more rapidly; and albuminuria, wherein albumin is lost in the urine due to renal damage. Possible causes of an abnormally high protein level in the urine include: Defects in the permselectivity of the glomerular filtration barrier to plasma proteins causing glomerular proteinuria (for example, glomerulonephritis or nephrotic syndrome), incomplete protein reabsorption in the kidney tubules leads to proteinuria, which is called tubular proteinuria (e.g., interstitial nephritis), abnormally high concentration of proteins in the blood plasma, causing overflow proteinuria (for instance, multiple myeloma-Bence Jones protein, myoglobinuria), and inflammation or malignancy of the urinary tract [132–134]. The extent of renal impairment can also be measured by performing an ACR (albumin to creatinine ratio). ACR measures the amount of albumin in the urine. There shouldn't be more than 30mg/g of albumin in your urine. Even if your GFR is at 60, a creatinine level of 30 mg/g or above may indicate renal disease [135,136]. The likelihood that a patient's renal illness would worsen is also evaluated with this test.

4.3 BUN (Blood Urea Nitrogen)

BUN is a serum byproduct of liver-produced protein metabolism that is transported to the kidneys for elimination via the blood. Additionally, BUN is used to assess renal function; rises in BUN levels are frequently, but not always, brought on by a decline in GFR. Gastrointestinal haemorrhage, corticosteroid medication, and a high-protein diet are a few other causes of a rise in urea level, and as a result, they limit the usefulness of BUN in determining kidney function.

The distribution volume, production, catabolic rate, and tubular reabsorption all have an impact on BUN concentration, so it is advised to carefully analyze this level before deciding whether to start dialysis.

4.4 Cystatin C

Cystatin C is a protein that is produced by the cells in the body. The optimum level of cystatin C in blood reports that kidneys are working fine without any problem. And when the level of cystatin C increases, it represents the problem occurring in kidney function. The GFR (glomerular filtration rate) is inversely related to the serum cystatin C level [137]. Even though both cystatin C as well as creatinine are readily filtered by glomeruli, cystatin C gets reabsorbed and digested via proximal renal tubules. When compared to creatinine, cystatin C provides a more reliable index of GFR, especially in early renal disease, and is unaffected by age, muscle mass, or nutrition [138,139]. But its clinical applicability has not been established yet so more studies need to be done to make it reliable and trusted method for kidney disease diagnosis. The concentration of these biomarkers is important for the proper diagnosis of the diseased condition. Therefore, for easy understanding, the clinical range and the physiological matrices of the clinical biomarkers have been discussed in **Table 1.3**.

Table 1.3: *List of different clinical biomarkers and their pathological ranges*

S.No.	Biomarker	Clinical Range	Sample Type	Ref.
1.	Creatinine	0.4 - 1.4 mg/dL 05 - 2 g/day	Serum, Urine	[140]
2.	Albumin	35 - 50 mg/mL	Serum	[121]
3.	BUN	5 - 20 mg/dL	Serum	[141]
4.	ACR	< 30 mg/g	Urine	[142]
5.	Cystatin C	0.6 - 1 mg/L	Serum	[143]

4.5 NAG (N-Acetyl-b-D-Glucosaminidase)

Chronic kidney illness often causes tubular dysfunction even before glomerular failure becomes noticeable. Researchers have found that N-acetyl—D-glucosaminidase (NAG) is a promising novel biomarker for tubular integrity [144]. The enzyme N-acetyl-/-glucosaminidase (NAG; EC 3.2.1.30) is a hydrolytic lysosomal enzyme with a high molecular weight (140 kDa) that is present in a wide variety of cells and tissues [145]. It is the most active of the glycosidases occurring in the lysosomes of the proximal renal tubule epithelial cells, but is present in many other tissues as well. If you have chronic kidney disease, you can find out how your glomerular system is functioning with the help of NAG in your urine. And because of its renal functional reserve, NAG is a much more sensitive biomarker of renal parenchymal damage [146]. It is recommended to measure urine NAG multiple times during the course of a patient and longitudinal follow-up, since this may improve its clinical value as a sign of continuous tubular injury [147,148].

4.6 NGAL (Neutrophil gelatinase-associated lipocalin)

NGAL (also known as lipocalin-2, oncogene 24p3, siderocalin, or uterocalin), a member of the lipocalin family, has a molecular weight of 25 kDa and is made up of 8 -strands that wind together to form a -barrel around a calyx. While NGAL was first discovered in neutrophils, it has since been found to be expressed in kidney, liver, as well as epithelial cells as a response to diseases like infection, inflammatory response, ischemia, intoxication, acute kidney injury, and neoplastic transformation. Pathways of NGAL-mediated kidney protection in acute injury may involve NGAL-induced processes like bacteriostasis, anti-apoptotic effects, and increased proliferation of renal tubules. Low but steady levels of NGAL expression are found in many different cell types. Therefore, in the systemic circulation of healthy people, low levels of NGAL are detectable. When filtered in the glomerulus, NGAL is reabsorbed easily in the proximal tubule via a megalin-dependent pathway. Directly after acute kidney injury, NGAL

is dramatically upregulated in the distal nephron, including the thick ascending limb of Henle's loop, the distal tubule, and the collecting duct. Increased urinary NGAL levels may be exacerbated by impaired proximal tubular reabsorption in the context of proximal tubular injury. The potential clinical value of NGAL in AKI has been the subject of a great deal of research.

4.7 Interleukin-18

Inflammatory conditions such as acute kidney injury (AKI) and chronic kidney disease (CKD) have been linked to IL-18, which has been implicated in inflammation in a wide range of animal models. Clinical applications for IL-18 in disease diagnosis and severity/outcome prediction are being actively pursued. Increased urinary IL-18 levels have been associated with acute tubular necrosis following kidney transplantation. Extensive research has been done on the urine level of IL-18 as a possible early diagnostic marker of acute kidney injury (AKI). After cardiac surgery, a meta-analysis of reports found that urinary IL-18 as a biomarker for the diagnosis of AKI had a sensitivity and specificity of 0.58 and 0.75, respectively. Urinary IL-18 levels predicted AKI with a 95% confidence interval (CI) of 0.70. Urine interleukin-18 (IL-18) is a biomarker of moderate value for detecting acute kidney injury. When compared to patients with urinary tract infections, pre-renal acute renal failure, chronic kidney disease, or nephrotic syndrome, those with acute tubular necrosis have significantly higher urinary IL-18 levels. It is important to note that urinary IL-18 is elevated in septic patients as well, so caution should be exercised when using this marker for the diagnosis of AKI.

Biomarkers such as NGAL, NAG, KIM-1, β-2-microglobulin, and others have been utilized for the development of biosensors [149–151]. But these biomarkers still not able to find their clinic presence, thus in this discussion we are mainly focussed on clinically relevant biomarkers for chronic kidney disease diagnosis. Specifically, the serum creatinine, a byproduct of creatine and phosphocreatine that is mainly readily filtered by the glomerulus, has been used as a

diagnostic tool for renal disease. However, as an indirect measure of kidney disease, serum creatinine has several well-recognized drawbacks, including delayed diagnosis of injury, it has remained the gold standard for almost a century due to its accessibility and affordability. Along with creatinine, albumin/creatinine ratio is prominently used technique to determine the chronic and acute kidney diseases.

5. Optical Sensors for detection of chronic kidney disease

Optical sensors are defined as the light-based sensors in which the change in signal or result can be observed easily. The signal transducer can be colorimetric, fluorescence, luminescence, or interferometric [152,153]. Optical sensor has emerged as one of the most reliable platforms for medical diagnosis [154]. In this section, we have reviewed globally developed optical sensors for the detection of clinical biomarkers for chronic kidney disease.

5.1 Creatinine Sensor

Recently in 2021, a colorimetric paper-based sensor device was developed by Izabela et. al. for detection of creatinine [155]. The sensor was developed by focusing on two different reactions for the creatinine measurement in urine sample. They have compared both the methods and establish a 3-D printing based automatic detection of coloured zone. The first method is the universally acceptable Jaffe method and an alternative method was developed by the group using 3,5-dinitrobenzoic acid. Both the methods are based on change in colour by channelling intensity of Hue from HSV color pattern and green channel from RGB color region was utilized as the measurable signal in Jaffe and 3,5-dinitrobenzoate method, respectively. They have utilized a solid ink printer (ColorQube 8570, Xerox, USA) for printing the required shape and form hydrophobic barriers in the paper surface. The designed paper was heated at 120 ◦C for 2 min to dried because this allowed the wax to melt and penetrate the pores in the paper. For the quantification of creatinine, the paper was put in a sampler such that lower part was dipped in the sample solution. Before this dipping method, the end part of the paper strip was cut off so

that liquid will flow in the sampler space (shown in **Figure 1.7a**). After this process, a proper incubation time was provided and finally, the sensor was kept in the smartphone case and the image was taken for analysis. The linear concentration range was observed for both kind of reactions shows significant result when tested in urine sample, shows precision with a RSD, below 5%. The developed sensor shows limit of quantification for both methods as 1.05 mmol/L and 0.82 mmol/L for Jaffe reaction and 3,5-dinitrobenzoate method, respectively. The developed sensor for selective creatinine detection showing great performance in undiluted urine using artificial urine samples and the observed recoveries were found to be in the range of 70 to 129 %. Another colorimetric sensor was developed for the detection of creatinine in urine samples by Ciou et. al [156]. In this for developing colorimetric device, redox chemical reaction between 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical cations ($ABTS^{\bullet+}$) was utilized for detection in urine sample. ABTS was entrapped into a chitosan film which further modified on the FTO substrate and this whole modification was layered with Nafion. After that the negative charged surface was utilized and further pretreatment was perfrom to form ABTS•+. The developed colorimetric sensor exhibits promising sensing characteristics, including high accuracy for the detection of creatinine in neat urine. The performance of the sensor was tested after incubating the FTO/CHIT$ABTS^{\bullet+}$/Nafion electrode into the 3.5 mL neat human urine sample placed in the cuvette. The absorbance at 419 nm was determined during 2.5 min incubation time and the amount was proportional to the concentration of creatinine using the calibration curve. The disappearance of UV-vis features characteristic to $ABTS^{\bullet+}$ at prolonged incubation duration suggests $ABTS^{\bullet+}$ was reduced by creatinine. The developed optical sensor fabricated using FTO/CHIT$ABTS^{\bullet+}$/Nafion electrode shows high sensitivity, broader linear detection range containing the normal concentration of urine creatinine, and high accuracy has been observed. Another colorimetric sensor was developed for the selective detection of creatinine by the group Unni et al. [157]. Copper

nanoparticles (L-cys-CuNPs) were stabilized using L-cysteine that provides high selectivity and sensitivity towards the creatinine. When creatinine was interacted with L-cys-CuNPs, shows a change in color from red to yellow along with a change in spectral profile of L-cys-CuNPs from blue-shifted band to red-shifted band and reduced intensity was noticed. The aggregation of L-cys-CuNPs in the solution was the possible reason of change in spectral profile of the modified nanoparticles. To confirm that the aggregation of particles observed only after reacting with creatinine TEM was performed. It was observed that change in colour after reaction of creatinine with L-cys-CuNPs can easily be observed through naked eyes as visible in **Figure 1.7b**. Additionally, the decrease in intensity was observed with increase in concentration of creatinine with L-cys-CuNPs probe. The developed sensor shows the linear concentration relationship in the range of 5.33×10^{-6} to 3.33×10^{-7} M. The detection limit and the quantification limit were calculated to be 4.54×10^{-10} M and 1.51×10^{-9} M, respectively. The developed sensor was checked in spiked artificial serum and urine samples, and the sensor shows significant result depending upon both direct naked eye detection and also colorimetric changes calculated. This kind of sensing device can further be used in clinical testing if further change can be done to make it more handy and clear observation can be done. In another study, a non-invasive colorimetric sensor has been developed by Zhang et. al. [158]. They have used the enzyme immobilization system for creatinine detection in sweat. The change in color was directly proportional to the concentration change of target analyte and the colour change and device was shown in **Figure 1.7c**. Researchers have tried to develop an adhesive prototype to measure the change in renal biomarkers through colour change. Such type of system proves to be promising devices and will definitely be applied in future for simple, non-invasive diagnosis of renal disease.

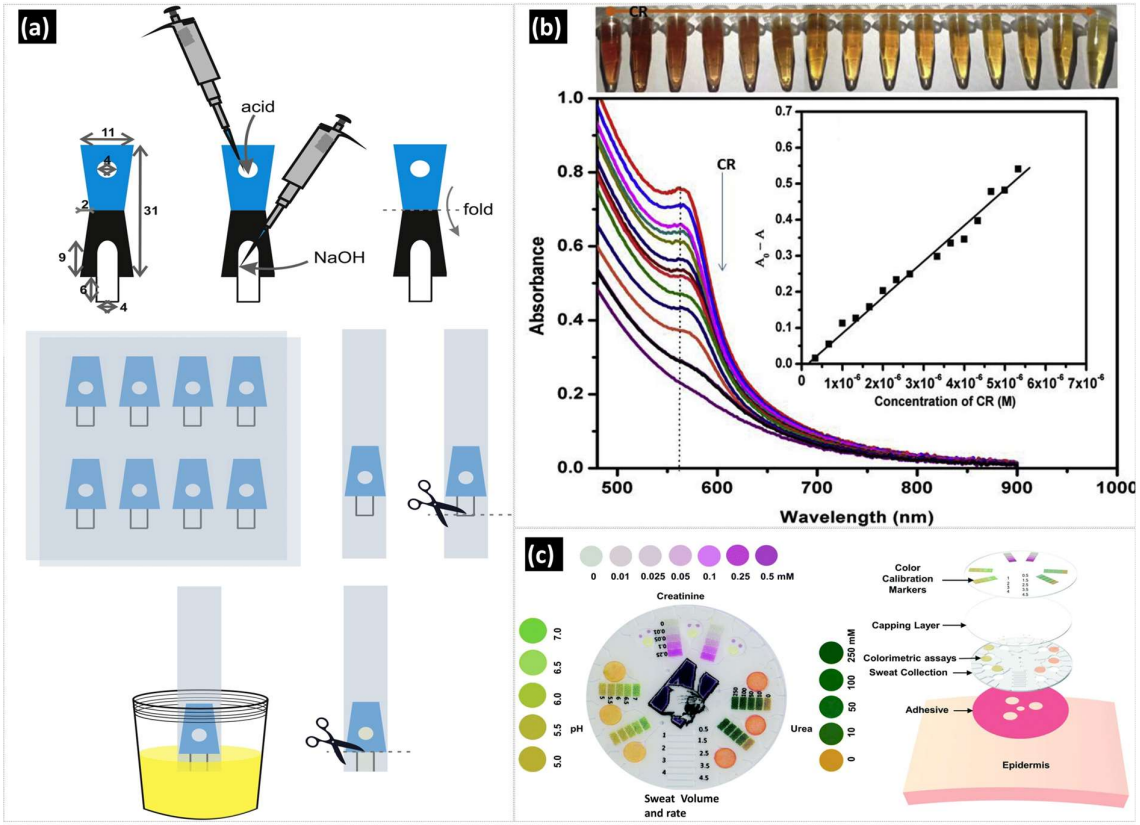

Figure 1.7: (a) *Step-by-step representation of optical sensor developed for creatinine by dipping in the solution (Reproduced with permission* [155]*), **(b)** Showing the change in colour and signal due to presence of creatinine using the developed sensing device (Reproduced with permission* [157]*), **(c)** Image of the developed optical device for the non-invasive detection of creatinine (Reproduced with permission* [158]*)*

5.2 Albumin Sensor

Other biomarkers apart from creatinine have also been explored and the globally developed sensors for another biomarker has been explored. Recently in 2022 a novel fluorescence-based sensor has been developed for the quantification of albumin by Ting et al [159]. This sensor is based on the highly selective molecular imprinting technology combining with the great enhancement of fluorescence using upconversion nanoparticles and metal–organic frameworks that provides high specific surface area. In the first step, the upconversion nanoparticles UCNPs ($NaYF_4$: Yb^{3+}, Er^{3+}) were synthesized through a solvothermal method in oleic acid and 1-octadene. The ligand exchange of oleic acid and polyacrylic acid were utilized to convert the

resulted nanoparticles into carboxyl modified form. Step-by-step core–shell UCNPs@MIL-100 were prepared for fabricating final sensing probe. The MIL-100 shell was grown on the polyacrylic-modified UCNPs surface which was initiated by the Fe-ions coordination with the carboxylate groups on the surface of UCNPs, with which then the finally form surface binds. After that the adsorption process was investigated by performing the kinetic studies. The kinetic studies shows that there is an increase in adsorption rate at 60 min and the equilibrium was achieved around 120 min. The reason for this increased adsorption was that the UCNPs@MIL-100 shows high specific surface area as support material which increases the mass transfer. The proposed system shows the linear range of 1.00 to 8.00 μM, and the detection limit was 0.59 μM. In another study, a photoluminescent based optical sensor was designed by Punuri et al. using a biocompatible carbon-gold (C-Au) nanocomposites for the detection of creatinine [160]. The strong photoluminescence emitting property of carbon nanodots (CNDs) can be utilized for the synthesis of strong photoluminescence exhibiting gold nanocomposites. The fabrication of photoluminescence C-Au NC was done through reduction of $HAuCl_4$ by CNDs which act as a reducing agent and these are also adsorbed onto the AuNPs during the synthesis process. Further, the synthesized C-Au NC are functionalized with bovine serum albumin to form a non-enzymatic sensing nanomaterial for detection of creatinine. The synthesized C-Au NC are biocompatible and exhibit wavelength dependant multicolor fluorescence property. The reducing and capping properties of CNDs derived from *Citrullus lanatus* for the synthesis of C-Au NC is determined. The developed device shows linear concentration range of creatinine, at low (0.2 - 1.5 mg/dL) and high (4 - 20 mg/dL) concentration. Recently in 2022, a fluorescence-based sensing device was designed to quantify human serum albumin [161]. They have used water-soluble donor-acceptor fluorophores for the selective and quantitative determination of human serum albumin. The probe was designed using a strong acceptor i.e., 3-ethyl-1,1,2-trimethyl-1H-benzo [e]indol-3-ium iodide which was

conjugated to from a fluorophore which shows π-pull when substituted with a phenol through an alkenyl bond. The developed fluorophore shows weak fluorescence in aqueous solution because of the formation of hydrogen bond and the high dielectric nature of the microenvironment. The limit of detection was found to be 95 nM at pH 7.4 in simulated urine samples. The developed sensor shows linear relationship between the fluorescence intensity and the concentration range of albumin even upto one unit of albumin. The linear relationship shows that the amount of albumin was determined in simulated urine samples with high percent recovery values. Moreover, no the developed sensor does not show any cross-reactivity in the target sample and the sensor shows good performance in the physiological pH range. Another colorimetric sensor was developed for the sensitive and selective determination of serum albumin using fluorophore by Nayim et. al. [162]. The two different types of berberine derivatives and treated them with various available biomolecules by means of various spectrophotometrical and spectrofluorimetrical techniques to find out whether they can sense the presence of any of them. From the two different derivatives, only the one 9-O derivative was showing the increase in the weak fluorescence intensity through the addition of serum albumin concentration. It was found that the detection limit of developed sensor was 6.8 nM for albumin. Recently a group of researchers in 2022 have developed a fluorescent probe for the determination of microalbumin and creatinine [163]. The fluorescent probe was designed using glutathione-capped copper nanoclusters (GSH-CuNCs) for targeting dual molecules. The developed sensing probe and change in signal in presence of albumin shows in **Figure 1.8a**. The developed sensor shows that creatinine was checked in acetate buffer at different pH form 4.0 - 5.0, and in phosphate buffer at pH 6.0 and 7. It was observed that the detection sensitivity increases as pH increases from 4.0 to 5.0 and the sensitivity decreases at higher pH. It was observed because the creatinine remains in neutral stage at pH 5.0. Under optimal conditions, the developed sensor system was checked in the presence of albumin in turn-on mode ranging

from 1.0 to 300 nM and in the turn-off mode, detecting creatinine concentrations from 10 to 3000 µM. The response to albumin concentration was linear in the range of 5.0 nM to 150 nM and to creatinine concentration in the range of 30 µM to 1000 µM. The detection limit was 1.510 ± 0.041 nM for albumin and 13.0 ± 1.0 µM for creatinine while the quantification limit was 5.04 ± 0.14 nM for albumin and 44.0 ± 3.0 µM for creatinine. Another group of researchers developed an optical immunosensor for the detection of albumin [164]. They have used CdSe/ZnS quantum dots as a fluorescent agent and developed a system with diode laser to measure the signal. APTES modified glass was utilized and immobilization of monoclonal anti-human serum albumin antibody for specific detection. The whole procedure of development was represented in **Figure 1.8b**. The developed device shows the detection limit of about 3.2 x 10^{-5} mg/mL. This type of sensor system can be used in future according to the requirement.

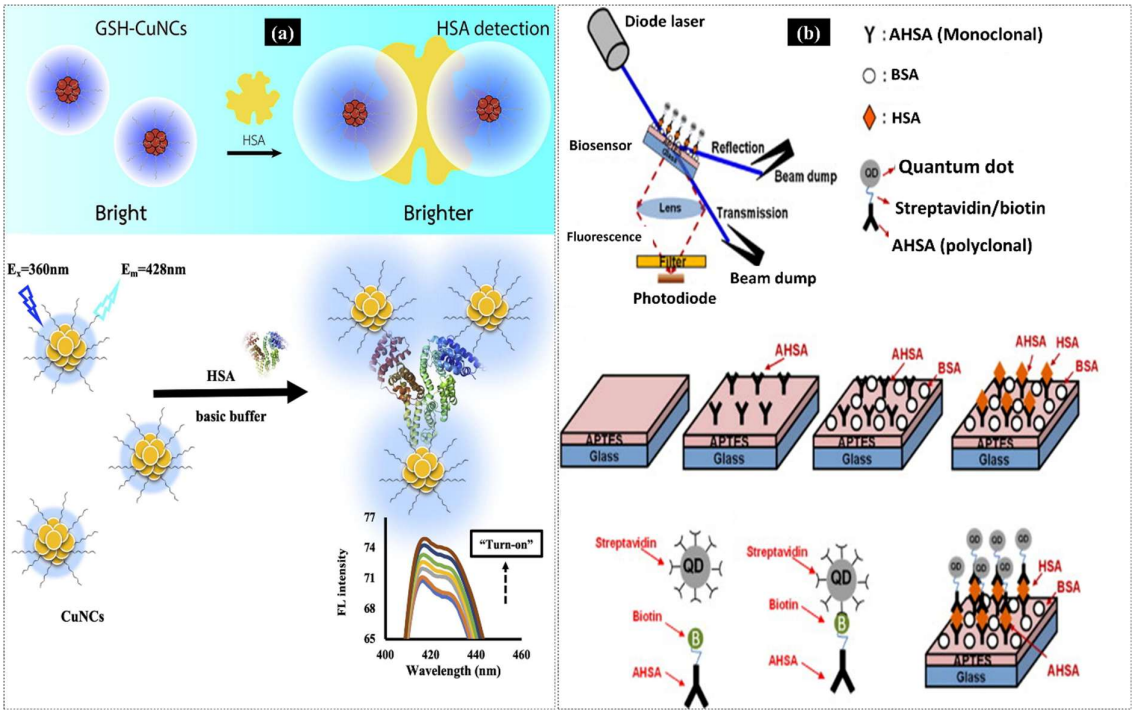

Figure 1.8: (a) *Representation of sensing probe and change in intensity in presence of albumin (Reproduced with permission* [163]*), **(b)** Illustration of step by step fabrication process and the whole developed system for the optical detection of albumin (Reproduced with permission* [164]*)*

Additionally, other globally developed optical sensors for the detection of chronic kidney disease clinical biomarkers are discussed and compared in **Table 1.4** along with their detection limit, linear dynamic range, response time and real sample analysis.

Optical sensors are already establishing their presence in medical field but for a greater impact on the society much more advancement is required. Advancement in quantitative detection of target analyte, specificity need to addressed as this is one of the major issues in optical systems, and along with that it should be cost effective. The emergence of COVID-19 has impacted the world in a brutal way and this also provide awareness to be prepared in advance to face any such circumstances for this the disease diagnosis system need to strengthened. This lack can be fulfilled with the advanced biosensors and providing much better space for early diagnosis.

Table 1.4: *Comparison table of globally developed optical biosensors for diagnosis of chronic kidney disease (NR: Not reported)*

S.No.	Biomarker	Transduction System	Sensor Configuration	Response Time	Real Sample	Dynamic Range	LOD	Ref.
1.	Creatinine	Colorimetric	CuNPs have been integrated with L-cysteine for selective and sensitive interaction with creatinine	20 min	Serum, Urine	5.33×10^{-6} - 3.33×10^{-7} M	4.54×10^{-10} M	[157]
2.	Creatinine	Colorimetric	ABTS was introduced and entrapped in fluorine-doped tin oxide modified chitosan film	12.5 min	Urine	0 - 21300 μM	400 μM	[156]
3.	Creatinine	Electrochemiluminescence (ECL)	Polyethyleneimine capped CdS quantum dots doped silica nanocomposite were used as ECL emitter	4 min	Serum and Urine	0.05 nM - 5 μM	0.02 nM	[165]
4.	Creatinine	Optical (Ionophore-Based)	Aryl-substituted calix pyrrole receptor was used as coextraction scheme	3 min	Urine	10^{-5} - 10^{-2} M	10^{-5} M	[166]

5.	Creatinine	Colorimetric	A 3D ‑ printed device was combined with a smartphone to detect Jaffe method using Hue channel intensity	6 min	Urine	1.05 - 20.0 mmol/L	0.35 mmol/L	[155]
6.	Creatinine	Colorimetric	3,5-dinitrobenzoate was used for quantification by targeting green channel intensity	6 min	Urine	0.82 - 10 mmol/L	0.27 mmol/L	[155]
7.	Creatinine	Fluorescence	Copper nanoclusters were designed based on glutathione, utilizing ascorbic acid to enable turn-on mode	15 min	Urine	30 - 1000 μM	13.0 μM	[163]
8.	Creatinine	Optical Sensor	C-gold nanocomposite was designed using carbon nanodots derived from *Citrullus lanatus* for photoluminescent imaging	10 min	NR	0.2 - 20 mg/dL	0.07 mg/dL	[160]
9.	Albumin	Fluorescence	Upconversion nanoparticles were synthesized with metal organic framework for gradual quenching	NR	Serum	1.00 - 8.00 μM	0.59 μM	[159]

10.	Albumin	Fluorescence	A substituted Phenol donor and cationic benzoindolium was developed for selective detection	NR	Urine	NR	95 nM	[161]
11.	Albumin	Fluorometric	Two different 9-O Berberine Derivatives were synthesized (N1 and N2)	NR	Serum	NR	17.8 nM (N1) 16.3 nM (N2)	[162]
12.	Albumin	Fluorescence	Glutathione based copper nanoclusters were designed using ascorbic acid for turn-off mode for the fluorescence-based detection of albumin	NR	Urine	5 - 500 nM	1.51 nM	[163]
13.	Albumin	Fluorescence	Dipyrrole intermediate based fluorophore probe (BDY-OH) was developed for enhanced signal intensity in presence of albumin	4.5 min	Urine	0 - 0.4 mg/mL	1.89 mg/mL	[167]

14.	Albumin	Fluorescence	Raspberry pi based portable system for measuring fluorescence intensity used for the detection of albumin	40 min	Urine	upto 25 mg/L	NR	[168]
15.	Albumin	Fluorescence	Poly (thymine) (Poly T)-Templated Copper Nanoparticles designed for quantitative detection	15 min	Serum	0.15 - 2.5 µM	8.2×10^{-8} mol/L	[169]
16.	Albumin	Optical Immunosensor	CdSe/ZnS Quantum Dots immobilized on glass surface modified using APTES for optical signal	NR	Mouse Source albumin	2×10^{-1} - 2×10^{-4} mg/mL	3.2×10^{-5} mg/mL	[164]
17.	Albumin	Fluorescence	Thieno[3,2-B] Pyridine-5(4h)-derivative was used for the enhancement of fluorescence signal	NR	Blood	50 nM - 5 µM	8 nM	[170]
18.	Cystatin C	Optical	SPR imaging was used for cystatin C concentration on biochip for its specific detection	10 min	Urine	0.01 - 5 mg/L	0.1 mg/L	[171]

19.	Cystatin C	Fluorescence	Anti-CysC antibody were immobilized for developing lateral flow kit for non-invasive detection of cystatin C	15 min	Urine	0.023 - 32 µg/mL	0.023 µg/mL	[172]
20.	Cystatin C	Electrochemilu-minescence	Rubrene functionalized graphene composite and immunorecognition-induced mechanism	60 min	Serum	1 fg/mL - 10 ng/mL	0.38 fg/mL	[173]
21.	Cystatin C	SPR	Paper based substrate coated with maltose and poly lactic glycolic acid was used for the sensitive detection of the target	30 min	Blood	NR	0.01 µg/mL	[174]
22.	Cystatin C	SERS	Fab 2 antibody of anti-cystatin C were immobilized onto conductive gold coated substrate was utilized for the detection	50 min	Blood	10^{-7} - 10^{-12} M	1 pM	[175]
23.	NAG	Fluorescence	New enzymatic probe was developed for selective and sensitive detection of the target molecule	1 hr	Blood	NR	NR	[176]

24.	Urea	Optical	LED based sensor with immobilization of urease and agarose to form composite which can be utilized for the detection of urea	5 min	Serum	0.025 - 10 mM	0.01 mM	[177]
25.	Urea	Optical	Artificial molecular imprinted polymer was integrated with optical fibre to form artificial receptor-based sensor	14 min	NR	10^{-11} - 10^{-4} M	17.1 fM	[178]
26.	Urea	Optical (Reflectance)	Calcium carbonate nanoparticles were functionalized with succinimide ester and further immobilized with urease for the sensitive detection	10 min	Urine	30 - 1000 mM	17.74 mM	[179]

6. Electrochemical Sensors for detection of chronic kidney disease

Electrochemical sensors are devices which detects the electrochemical reaction and measured by using electrode system [180]. The signal change used to measure quantitative and qualitative detection of target analyte and signal measurement based on amperometry, potentiometry, and conductometry [181,182]. This section is focussed on globally developed electrochemical sensors for the detection of clinical biomarkers relating to chronic kidney disease. The main focus of this discussion to know the fabrication pattern and their application in real sample. Also, the requirement to make them more effective and reliable need to be more studied and the advancement in technologies will help in future.

6.1 Creatinine Sensor

In one of the study, enzyme based amperometric sensor have been designed for the detection of creatinine by Do et. al. [183] The immobilization of enzymes to develop sensors have been utilized since years. The sensor was fabricated using transparent Nafion layer onto the Au/Al_2O_3 electrode using the thin film microfabrication method. Further the surface was electropolymerized with PANI to form the final surface i.e., Nafion-nsPANI/Au/Al_2O_3. Also, the final developed surface was washed several times with deionised water to remove any resides. Finally, the creatinine deiminase (CD) enzyme was immobilized on the electrode surface by drop casting to form CD/ Nafion-nsPANI/Au/Al_2O_3. The developed surface was used to detect creatinine by sensing ammonium which was produced through conversion of creatinine to N-methylhydantoin through CD. The obtained sensitivity of the sensor was 100 - 163 µA/mM. The sensitivity also varies with the concentration range and it lies linearly in the range of 0.005 - 0.1 and 0.1 - 0.4 mM. The observed sensitivity in this sensor have been significantly higher than the previously reported sensors. The sensor was also tested in human serum sample for checking its applicability in real world. In another study a paper based electrochemical sensor for the detection have been by Boobphahom et. al in 2019 [184]. The

sensor was designed using whatman filter paper and the three-electrode system was screen printed on its surface. The electrode was further modified with copper oxide-ionic liquid using a HP D300 dispenser. After that the electrochemically reduced graphene oxide layer was used as a stable film on the modified electrode surface. The detailed representation of the fabrication process and signal change shown in **Figure 1.9a.** The developed sensor was able to detect creatinine in a linear dynamic range of 0.01 - 2.0 mM with a limit of detection about 0.22 µM. In another study, non-enzymatic based disposable sensor for the detection of creatinine have been developed by Raveendran et. al. [185]. The electrodes were printed using carbon ink on the surface of polyethylene terephthalate and kept for 2 hours at 60 ºC. Electrode was modified through the electrodeposition of copper and electrochemical detection was performed through formation copper-creatinine complex. The overall detection process was presented in **Figure 1.9b**. The developed sensor was able to detect the creatinine in a linear range between 6 - 378 µM and a detection limit of 0.0746 µM. Further the sensor was tested in serum sample and shows the recovery between 95 - 98%. Another enzymatic electrochemical sensor for the detection of creatinine has been developed by Dasgupta et. al. [186] using Co modified electrode (**figure 1.9c(i)**). Creatinine deiminase was used for the conversion creatinine to N-methylhydantoin (shown in **figure 1.9c(ii)**) and the amount of 1-methylhydantoin utilized for indirect estimation of creatinine concentration. The electrode was modified with cobalt and the reaction of cobalt with 1-methylhydantoin was used for signal generation. The detailed rection mechanism and the effect of 1-methylhydantoin on the signal is represented in **Figure 1.9c(iii)**. The developed sensor shows the linear detection range of 0.8 - 4 mg/dL. The sensor was also tested in serum sample and thus can be applied in future with further testing. Recently in 2022, a disposable amperometric biosensor for the detection of creatinine have been developed for the detection of creatinine by Dong et. al. [187]. The sensor was designed by printing the three-electrode system onto the PET substrate and the electrodes were connected to potentiostat.

Further the layer of Prussian blue was deposited on the electrode surface to enhance the detection process. Further the surface was immobilized with three different enzymes namely creatinine amidohydrolase, creatine amidinohydrolase and sarcosine oxidase. After the action of these three enzymes the creatinine was converted to hydrogen peroxide and finally the sensor was used to detect the hydrogen peroxide. The maximum sensitivity of the sensor was observed after the optimization of all three different enzymes in a particular ration which indicates the sufficient conversion of creatinine. The developed sensor shows the linear detection range of creatinine concentration from 0.05 to 1.4 mM with the response time of about 3 min. The developed sensor shows comparable results when detection was performed in the serum samples and the results obtained shows the error of 17%. Therefore, it is expected that after advancement and automation in instrument will help in developing better performing device in future.

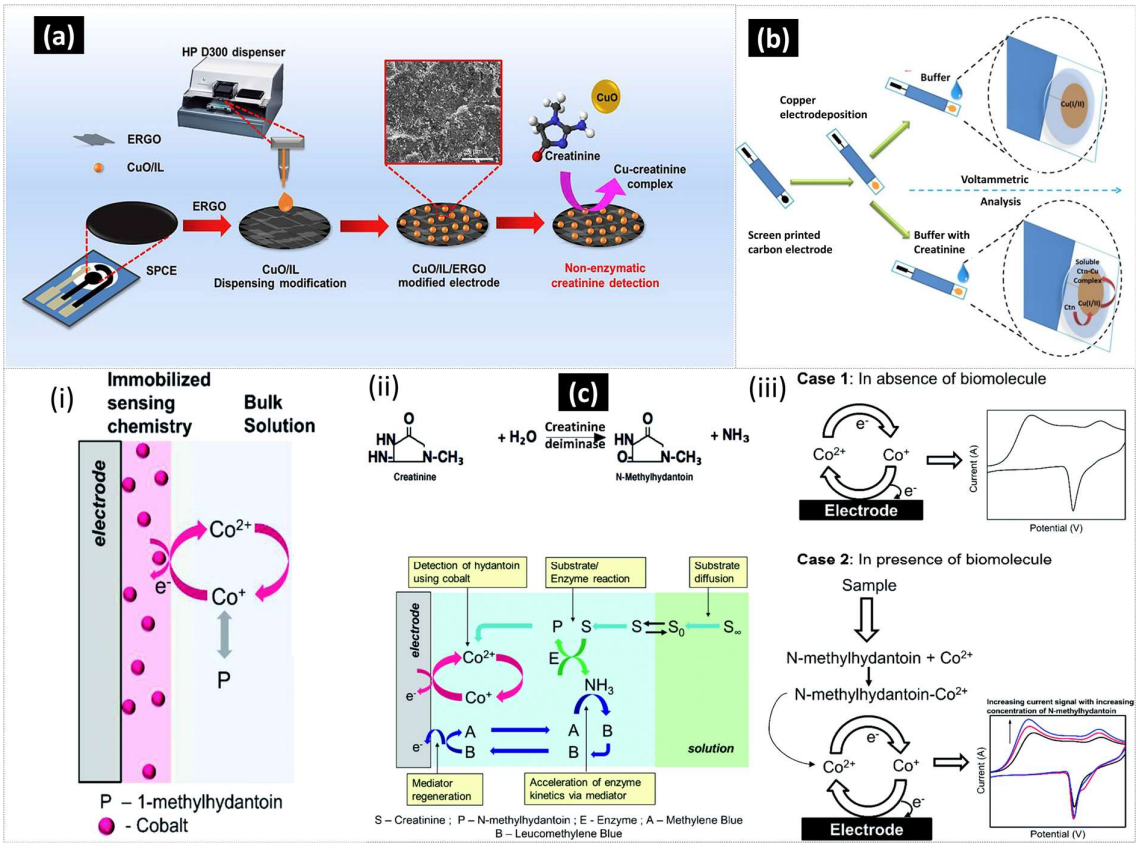

Figure 1.9: (a) *Schematic representation of fabrication using dispenser and detection process of electrochemical sensor for creatinine (Reproduced with permission* [184]*), **(b)** Illustration of both modified and unmodified electrode for the determination of creatinine **(**Reproduced with permission* [185]*), **(c) (i)** Representation of modified electrode and redox mechanism **(ii)** Showing conversion of creatinine with enzyme and the complete reaction mechanism in solution **(iii)** effect of presence and absence of target molecule on signal generation (Reproduced with permission* [186]*)*

6.2 Albumin Sensor

Apart from creatinine, other clinical biomarkers are also targeted and electrochemical sensor have been discussed in coming section. Recently in 2021, an electrochemical microchip-based biosensor was designed for the detection microalbuminuria by Tseng et. al. [188] The sensor was fabricated using filter paper and it was infiltrated in amaranth solution and the paper was cut into circular detection zone using a CO_2 laser. Amaranth is an electroactive substance which reacts with the microalbuminuria present in the urine sample. Further the three electrodes were

printed on a cardboard using a semi-automatic screen printer. After that the reagent paper and electrode were assembled onto the packaging film to form an electrochemical microchip biosensor. Also, the designed three electrode system was connected with electrochemical analyzer and the detection was performed. The detailed step by step fabrication of the system have been illustrated in **figure 1.10a.** Also, in the **figure 1.10b** detailed reaction mechanism on the surface of electrode has been represented. The developed sensor shows two different linear correlation in the concentration range of 0.1 – 10 mg/dL and 10 – 40 mg/dL. Also, the sensor was also tested with the chronic kidney patient samples and the results obtained shows the recovery rate from 95-109 % with a R^2 value of 0.988. Therefore, this type of sensor system can further be implemented if more studies were done to make this microchip-based system as a feasible system for common use. In a recent study, another electrochemical sensor for the detection of albumin have been developed by Saeed et. al 2022 [189]. They have designed the Cobalt Telluride (CoTe) nanorods through hydrothermal method using cobalt sulfate and hydrazine hydrate as the raw material. CoTe nanorods were deposited on the surface of glassy carbon electrode and all the measurements were performed in three-electrode system. The detection of albumin was done with increasing concentration from 0.2 nM to 1 nM and the obtained limit of detection of the sensor was 0.003 nM with optimized pH of 7.4. Further the sensor was also tested with the urine sample of chronic kidney patients and the obtained recoveries were 50 - 120 %. Such type of sensor needs further modification and advancement in future to find its applicability in real world. In addition to these other globally developed electrochemical sensor have been described in **Table 1.5** comparing their analytical performance, response time, configuration, and real sample test.

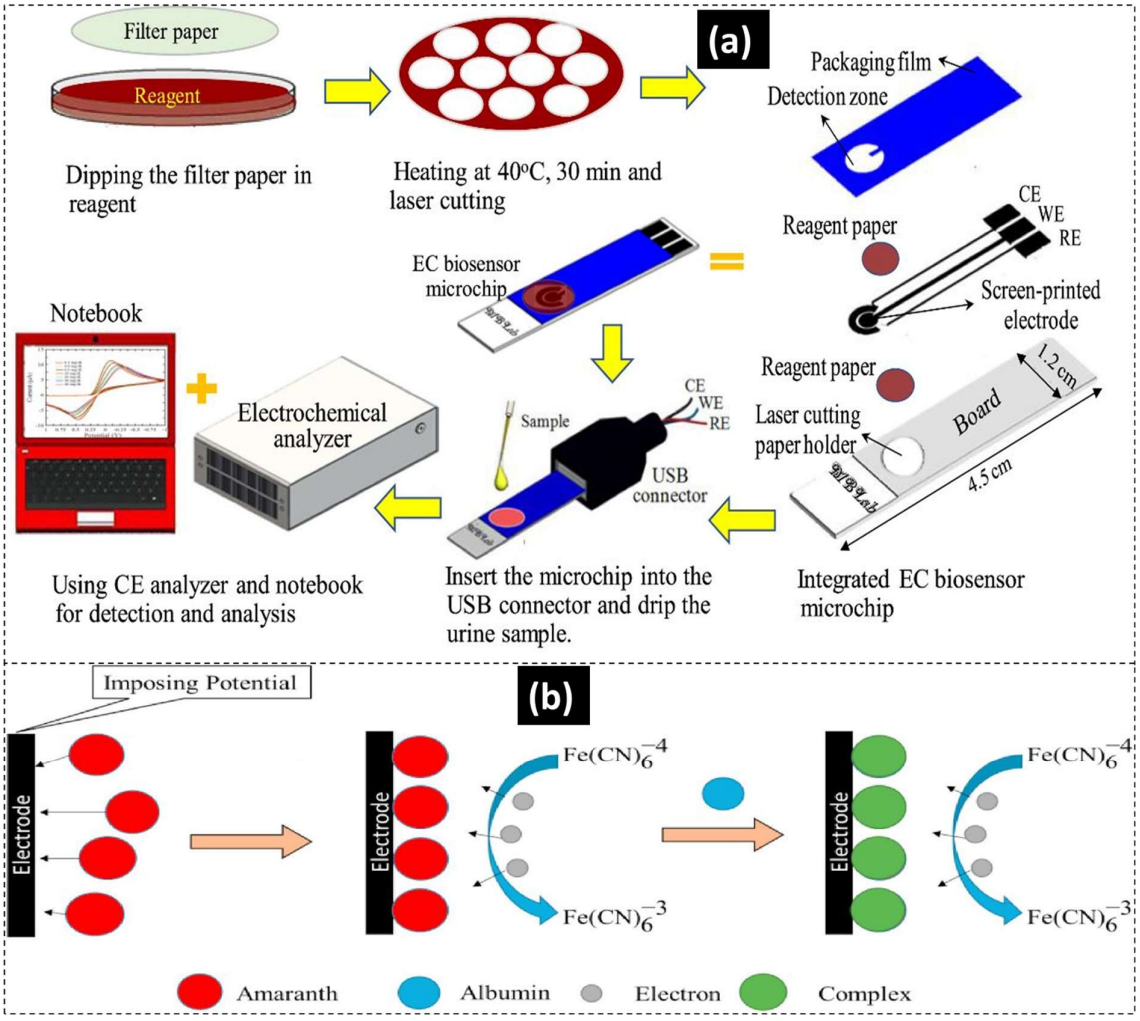

Figure 1.10: ***(a)*** *Step-by-step illustration of synthesis of paper based electrochemical sensor for the albumin detection,* ***(b)*** *Representation of reaction occurring on electrode surface (Reproduced with permission* [188]*)*

6.3 Other Biomarkers

Electrochemical sensors have also been explored for other biomarkers such as cystatin C, KIN-1, NAG and others for the chronic kidney disease. In this section we are going to discuss sensors which are developed for these biomarkers that in future will provide a way forward for medical diagnosis. An electrochemical biosensor for the detection of Cystatin C has been developed by Mi et. al. [190]. The sensor was developed by first preparing the nanobodies specific to Cystatin C and the affinity of the nanobodies was checked by SPR kinetic analysis.

Further, the thermal stability of the nanobodies was checked by testing them with indirect ELISA. The sensor was developed through the deposition of TiO_2 nanotube array on a titanium slice. The immobilization of nanobodies on TiO_2 nanotube array was done by using the APTES ethanol method. The detailed fabrication process has been illustrated in the **Figure 1.11a**. The developed sensor was able to detect the cystatin C in the linear concentration range of 0.72 pM to 3.6 nM with a detection limit of 0.14 pM. The sensor was also tested in the serum sample and the recovery % was determined to be 86 - 108 %. Such type of proposed system proves to be sensitive sensor system for detection of Cystatin C and promising sensor for renal infection. Recently in another study, electrochemical immunosensor for the detection of Cystatin C have been developed by Paula et. al. [191]. Gold nanostructure was electrodeposited on screen printed electrode and then the electrode was functionalized for immobilization of biotinylated Cystatin C specific antibody. The proper orientation and functionalization over the surface of screen-printed electrode are done in a sequential manner and shown in **Figure 1.11b**. The immunocomplexation reaction was confirmed by observing the strong covalent interaction between biotin and streptavidin. The developed system was able to detect Cystatin C in a linear concentration range of 10 - 100 ng/mL with a limit of detection of 6 ng/mL. Further the sensor was also tested in serum sample and the results obtained are acceptable as per standards. This type of study proves that immunosensors are providing way specially in medical field for the diagnosis of various diseases.

In a recent 2022 study, Boyacıoglu et al. developed an electrochemical immunosensor for the detection of acute kidney injury using kidney injury molecule-1 (KIM-1) as a biomarker [192]. The sensor was fabricated over a GCE modified using a composite of covalent organic frameworks (COFs) (based on 3,5-tris(4-aminophenyl)benzene and p- phthalaldehyde) and gold nanoparticles (AuNPs) which greatly enhanced the conductivity, specific surface area, and catalytic activity. Using the COFs-AuNPs/GCE as the platform, the anti-KIM-1-Ab1

antibody was immobilized due to affinity between amino groups and gold atoms, followed by incubation with BSA, which is used to block any of the non-specific binding sites, when the specific antigen KIM-1 is added. Finally, the porous $NiCo_2S_4$ @CeO_2 microspheres used for amplifying the signal were conjugated with secondary antibody anti-KIM-1-Ab2 due to strong electrostatic forces and were immobilized upon the KIM-1/BSA/anti-KIM-1-Ab1/CFs-AuNPs/GCE to obtain the biosensor. The microspheres showed incompact nanosheet porous structure after incorporation of CeO_2, which enhanced electron transfer due to increased specific surface area. This experiment used H_2O_2 as a redox probe because it easily oxidizes into O_2 for continuous monitoring. The experiments showed a linear range of 0.01 - 50.00 pg/mL with a minimum detectable concentration of target analyte KIM-1 as 2.00 fg/mL, in addition to the high stability (97.81% in 7 weeks at 25°C), high selectivity, and low response time of 20 sec. In another study by Yin et al. in 2022 devised an immunosensor for detecting the KIM-1 biomarker by utilizing black phosphorus (BP) and trimetallic PdPtCu nanosheets as the substrate [193]. The negatively charged BP nanosheets were dissolved in an aqueous solution of positively charged polyethyleneimine, followed by the dissolution of PdPtCu nanosheets. Bilayer nanosheets of PdPtCu@BP were collected after centrifugation of the prepared solution and made into a suspension in water. GCE was selected as the working electrode and modified using the PdPtCu@BP suspension to form a membrane by incubating it at 37°C. KIM-1 antibody was immobilized on the modified surface of GCE and treated with PBS and BSA solutions to prevent any non-specific interactions. For measurements, KIM-1 containing sample solution was added on the modified electrode, incubated for 60 min at 37°C, and washed with PBS. Amperometric readings obtained from the oxidation of H_2O_2 in the working solution were analyzed to give the target analyte concentration. Such activity, similar to the peroxidase enzyme, of the BNs of PdPtCu@BP is a result of the synergistic coupling between the BP and PdPtCu metals. This label-free POC biosensor exhibited a linear range

from 0.1 to 100 ng/mL and a LOD of 32 pg/mL. Due to the low non-specific adsorption behavior of the fabricated BNs and high antigen-antibody affinity, the prepared sensor showed high specificity along with high stability of 95.4% after 20 days at 4°C.

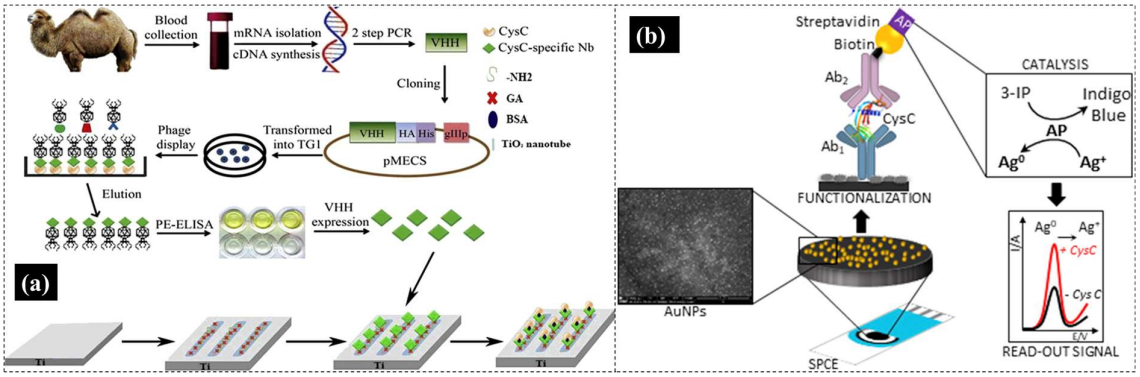

Figure 1.11: (a) *Schematic representation of step-by-step synthesis of Cystatin C specific nanobody using phage display method and layer by layer sensor designing on the surface of titanium slice (Reproduced with permission* [190]*), **(b)** Representation of modifications performed on the surface of electrode starting AuNPs deposition and further activation with primary and secondary antibody for Cystatin C detection, and the signal change (Reproduced with permission* [191]*)*

Table 1.5: *Comparison table of globally developed electrochemical biosensors for diagnosis of chronic kidney disease (NR: Not reported)*

S.No.	Biomarker	Transduction System	Sensor Configuration	Response Time	Real Sample	Dynamic Range	LOD	Ref.
1.	Creatinine	Electrochemical	Creatinine deiminase was immobilized on to a Nafion-nanostructured PANI composite film modified on to Au/Al_20_3 electrode	NR	NR	0.005 - 0.4 mM	NR	[183]
2.	Creatinine	Electrochemical	Creatinine deaminase was immobilized with a nanocomposite of copper-polyaniline to make it selective for detection	NR	Serum	1 - 125 µM	0.5 µM	[194]
3.	Creatinine	Electrochemical	Creatinine amidohydrolase, sarcosine oxidase was utilized to form a surface over Prussian blue – carbon graphite film using PET surface	3 min	Serum	0.05 - 1.4 mM	NR	[187]
4.	Creatinine	Electrochemical	Creatinine deaminase immobilized on a nanocomposite of polyvinyl alcohol, polyethleneimine and AuNPs	2 - 3 min	Serum	10 - 600 µM	2 µM	[195]
5.	Creatinine	Electrochemical	Three different enzymes (creatinine amidohydrolase, creatine amidinohydrolase	5 sec	Serum	10 - 750 µM	0.1 µM	[196]

			and sarcosine oxidase) were coimmobilized on carboxylated MWCNTs modified PANI surface					
6.	Creatinine	Electrochemical	Screen printed electrode were modified with copper oxide and reduced graphene oxide was electrodeposited	5 sec	Serum	0.01 - 2.0 mM	0.22 µM	[184]
7.	Creatinine	Electrochemical	Copper was electrodeposited to fabricate non enzymatic sensor for creatinine	NR	Serum	6 - 378 µM	0.0746 µM	[185]
8.	Creatinine	Electrochemical	Creatinine deiminase along with cobalt chloride and methylene blue was used for electrode modification and signal depends on the N-methylhydantoin production	1 min	Serum	0.8 - 4 mg/dL	NR	[186]
9.	Microalbuminuria	Electrochemical	Sensor was designed by depositing Amaranth on electrode and further modified with addition of potassium ferricyanide	NR	Urine	0.1 - 40 mg/dL	NR	[188]
10.	Albumin	Electrochemical	Cobalt telluride (CoTe) nanorods were designed and CoTe modified GCE was utilized for detection	NR	Urine	0.09 - 0.961 nM	0.003 nM	[189]

11.	Albumin	Electrochemical	Ferricyanide and methylene blue were combinedly used on the graphene modified electrochemical paper-based device	NR	Urine	1 - 500 mg/dL	0.0723 mg/dL	[197]
12.	Albumin	Electrochemical	Rhodamine-B was attached with 2-Hydroxyethyl disulfide on the ferricyanide modified electrode	NR	Serum	20 - 200 µM	NR	[198]
13.	Albumin	Electrochemical	Electrode were modified with polyaniline and gold nanocrystals	NR	Urine	3 - 300 µg/mL	NR	[199]
14.	Albumin	Electrochemical	Polythionine-methylene blue was caged on gold nanoparticle modified electrode	NR	Urine	10^{-10} - 10^{-4} g/L	3 x 10^{-11} g/L	[200]
15.	Cystatin C	Electrochemical	Polypyrrole/Carbon Nanotube based interdigited electrodes	20 min	Blood	0 - 300 ng/mL	28 ng/mL	[201]
16.	Cystatin C	Electrochemical	Functionalized multiwalled CNTs were immobilized with papain using EDC-NHS chemistry	10 min	Urine	NR	0.58 ng/L	[202]

17.	Cystatin C	Electrochemical	Anti-cystatin C antibody were immobilized on a graphene oxide - ferrocine modified nanofilm surface	NR	Serum	0.1 - 1000 ng/mL	0.03 ng/mL	[203]
18.	Cystatin C	Electrochemical	Screen printed electrodes were modified with gold nanoparticles and further antibody immobilization was performed	NR	Serum	10 - 100 ng/mL	6 ng/mL	[191]
19.	Cystatin C	Electrochemical	Anti-cystatin antibody immobilized on graphene oxide-chitosan nanocomposite	7 min	Serum	1 - 10 mg/L	0.0078 mg/L	[204]
20.	Urea	Electrochemical	Ammonium sensitive disposable electrode system was designed using poly (carbamoylsulfonate) as immobilizing agent	NR	Blood	72 - 2100 µmol/L	20 µmol/L	[205]
21.	Urea	Electrochemical	Two identical field effect transistors were designed through immobilization of urease on to the PVA/SbQ through photopolymerization	1-2 min	Serum	0.5 - 40 mM	NR	[206]
22.	Urea	Electrochemical	Urease was immobilized on a developed ammonium selective copper-polyaniline nanomaterial	NR	Serum	1 - 125 µM	o.5 µM	[194]

23.	KIM-1	Electrochemical	Porous $NiCo_2S_4$@CeO_2 microspheres were immobilized with organic frameworks containing AuNPs	NR	Plasma	NR	2.0 fg/mL	[192]
24.	NGAL	Electrochemical	3D graphene foam doped with nickel and modified further with the layer of AuNPs	NR	Urine	0.05 - 210 ng/mL	42 pg/mL	[207]

7. Challenges and Possible Solutions

In recent years, biosensors have emerged as one of the most useful devices in the medical field. Even after the advances in technologies, it was observed that a major part of the biosensor industry is based only on glucose sensors. This happens because there are even more challenges that need to be encountered to employ the biosensors for various disease diagnosis. In this section, we have tried to discuss the challenges and possible ways to make the biosensors including the CKD devices available for disease monitoring in both developed and under-developing countries.

Complexity of sample matrix: Complex matrices are present in many biological samples, including blood, urine, and saliva, which can affect the results of diagnostic tests. For instance, proteins, lipids, and other substances present in blood samples may bind to the surface of the biosensor and reduce its sensitivity and specificity. Not only this the matrix fluidity also affects the performance of such devices which has been observed greatly while testing the same molecule in diverse matrices. Thus, for precise detection of clinical molecules including CKD markers, sample preparation, stabilization, and purification are crucial. Hence, more focus and advances in sample processing are required [208,209].

Stability: Environmental conditions, such as temperature, humidity, and pH, can have an impact on various sensors. A compromised sensor activity and stability can result from changes in these variables. To provide precise and repeatable results, the stability of the sensing matrix must be carefully monitored and enhanced [210].

Calibration: The calibration system is extremely necessary to guarantee precise and trustworthy measurements of various molecules including different CKD biomarkers as discussed in previous sections. However, the calibration procedure can be laborious and may call for specialized tools and knowledge. Additionally, changes in the sample matrix and other analytical conditions may have an impact on the calibration curve causing measurement errors.

Hence, calibration should be done in a manner that is more precise and focused on the required clinical ranges [211].

Cost: Because of their high development and production costs, biosensors may not be as widely available in environments with scarce resources. Additionally, their price may be too high for regular clinical use and their implementation in a larger population. Therefore, to make it easily available, the matrix of the diagnostic device should be simple and cost effective [212].

Specificity: Biosensors have the potential to be extremely specialized for the target analyte, but they also have the risk of reacting with other substances in the sample matrix and producing erroneous results, either positive or negative. Therefore, it is crucial to carefully choose the sensor components and validate their specificity [213].

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