

REVIEW

Recent advancements in urea biosensors for biomedical applications

Saravjeet Singh¹  | Minakshi Sharma² | Geeta Singh¹

¹Department of Biomedical Engineering, Deenbandhu Chhotu Ram University of Science and Technology, Murthal, Sonapat, India

²Department of Zoology, Maharishi Dayanand University, Rohtak, Haryana, India

Correspondence

Geeta Singh, Biomedical Engineering Department, Deenbandhu Chhotu Ram University of Science and Technology, Murthal, Sonapat, India.
Email: gectasingh.bme@dcrustm.org

Abstract

The quick progress in health care technology as a recurrent measurement of biochemical factors such as blood components leads to advance development and growth in biosensor technology necessary for effectual patient concern. The review work of authors present a concise information and brief discussion on the development made in the progress of potentiometric, field effect transistor, graphene, electrochemical, optical, polymeric, nanoparticles and nanocomposites based urea biosensors in the past two decades. The work of authors is also centred on different procedures/methods for detection of urea by using amperometric, potentiometric, conductometric and optical processes, where graphene, polymer etc. are utilised as an immobilised material for the fabrication of biosensors. Further, a comparative revision has been accomplished on various procedures of urea analysis using different materials-based biosensors, and it discloses that electrochemical and potentiometric biosensor is the most promise one among all, in terms of rapid response time, extensive shelf life and resourceful design.

1 | INTRODUCTION

Urea is an organic nitrogenous compound and it is present in nature as well as in living organisms in a significant level. The significant amount of urea in human plasma is 15–40 mg/dl [1]. Higher levels of urea in the body provoke a cascade of symptoms, called uremic neuropathy or nerve damage. High risk side effects are urinary tract obstruction, gastrointestinal bleeding and renal failure etc. Lower urea levels contribute to cachexia, nephritic syndrome and liver failure [2, 3]. From the natural milk cream, butter and fat are separated from the cream separator machine. And to make this left over milk again creamy and foggy, urea is mixed to prepare synthetic milk. This synthetic milk is highly toxic and causes eye swelling and liver and kidney complications. In fact, synthetic milk is fatal to expectant mothers and people with hypertension and heart problems [4, 5]. Over the last twenty years, the usage of urea as a synthetic fertiliser has risen dramatically. Today, this is the most effective manure in farming worldwide. Soil urease induces environmental (soil, water, air) pollution due to continued enzymatic hydrolysis of urea to carbon dioxide and ammonia [6]. The urea-nitrogen volatilisation as ammonia often causes damage to crops, seedlings and germinating seeds [7]. Urea also plays a vital part in the aquatic nitrogen

cycle, like invertebrates and fish excretion outlets, and the bacterial decomposition of nitrogen and coastal drainage [8, 9]. Therefore, the measurement of urea is important in all fields such as innovative therapeutics, agriculture and dairy farm, manure fields, and ecological monitoring for good health and the environment. The kidney function test result play a significant role in the early phase treatment of renal diseases. Multiple tests like urinalysis, urinary composition, creatinine clearance, glomerular filtration rate, plasma creatinine, blood urea nitrogen are typically described in kidney function tests that involve both urine and blood samples [10]. The most significant of such measures are blood urea nitrogen and serum creatinine that are commonly used for monitoring renal function in every clinical laboratory. Such studies often involve an integral component of imaging technique in computerised X-ray imaging and magnetic resonance imaging (MRI) for the delivery of effective and desired radio-contrast [11] agents to avoid concerns like contrast-induced nephropathy and nephrogenic fibrosing dermopathy. The amount of urea in physiological fluids as well as in environmental samples [12] has been quantitatively evaluated using various methods, such as solid phase extraction chromatography (ion and gas chromatography, liquid chromatography, chemiluminometric, colourimetric, spectrophotometric (ultraviolet

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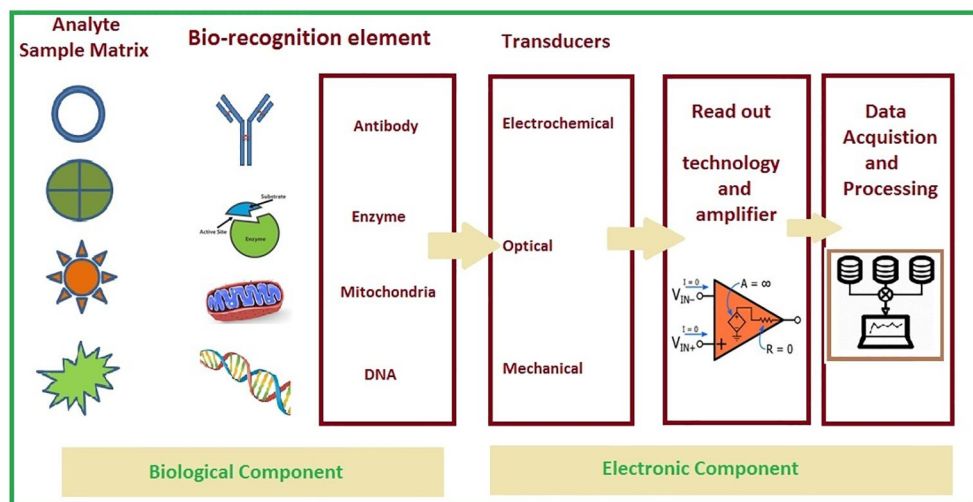


FIGURE 1 Schematic illustration of biosensor with its three components: (a) detector (b) transducer and (c) output System

visible spectroscopy, infra-red [IR] spectroscopy), mass spectrometry, fluorimetric and electrochemical methods [13–15]. Nevertheless, techniques such as colourimetric analysis and ion chromatography suffered from a relatively lengthy time-consuming processing of specimens, high cost of equipment, the operational need of qualified persons and long analytical time. The colourimetric approaches comprise nesslerization, Berthelot's reagent and the diacetylmonoxime–glucuronolactone reagent. Blood urea nitrogen analysis is the most widely employed method for determining blood urea rates and the procedure is often paired with a serum creatinine check for azotaemia [16]. Blood Urea Nitrogen determines how much nitrogen is available in a patient's blood. The major downside of this treatment is that it involves the retrieval of blood from the vein which is uncomfortable and unpleasant for the person performing the operation. The use of concurrent bodily fluids for the evaluation of urea is quite necessary. Due to the patient's ease of processing and availability, saliva is best obtained among non-invasive sample [17]. Several researchers have also used conventional methods to build a positive association between salivary and urea levels in the blood. The techniques of biosensing address the above disadvantages and boosts responsiveness and selectivity responses. A biosensor is an analytical instrument used to detect a chemical that combines a biological component with a physicochemical detector. Biosensor technology was prompted by the need to identify biologically relevant analyte responsively, reliably, inexpensively and rapidly [18]. After the sixth revolution nanotechnology, the utilisation of nanorange materials such as carbon nanotubes (CNTs; both single-walled [SWCNTs] and multi-walled [MWCNTs]), quantum dots, enzyme nanoparticles (ENPs), nano thin films, metallic and oxide NPs, graphene (bare and doped) and semiconducting NPs have acquired wide appeal over a decade for the development of specialised urea-detection biosensors. It was attributed to their extreme chemical stability, peculiar composition and very high surface-to-volume ratio of nanorange materials [19]. Such characteristics are the factors why such nanorange materials are increasingly attractive for chemical sensors development.

Recently, nanoporous silica powder, metal-oxide green NPs, natural polymers and nanoclusters are self-assembled to combine the characteristics of various high-quality components with a cooperatively higher performance in the manufacture of biosensors [20]. Today, though, attempts are made to produce a low-cost biosensor of urea as well as to enhance its specificity and stability.

The current review illustrates the wide range of urea biosensor methods, including the use of a variety of materials like metallic particles, natural polymers, nanorange thin film, zero dimension quantum dots, sol-gels, porous functionalised nanomaterials, zeolites and green metallic NPs [21, 22]. This review also highlighted the latest and prospect trends in the utilisation of nanorange materials (metallic NPs, SWCNTs, MWCNTs, thin film nanostructure, functionalised nano-entity, quantum dot, and bionanocomposites) for the manufacture of more perceptive and reliable urea sensors [23]. Note that the materials integrated on the use of nano-biocomposite materials updated with different nanomaterials to obtain economic sensors are also accessible to less developed nations.

2 | BASIC CONCEPT OF A BIOSENSOR

In 1918–2005, Professor Leland C Clark Jr. introduced the world about the biosensor. Biosensors are analytical devices that are used to detect an analyte according to the occurrence and concentration. Biosensors consist of three components: an element that recognises the analyte and generates a signal, a transducer and a reader tool. Figure 1 is showing schematic illustration of biosensor with its three main components: (a) detector, (b) transducer and (c) output system.

A nano-biosensor is a biosensor that works on the nano-scale range. Biosensors are commonly used in the clinical, biomedical, quality assurance, farming, livestock, agricultural, bacterial and virus diagnostics, drug production, industrial pollution management, forestry, military and defence sectors

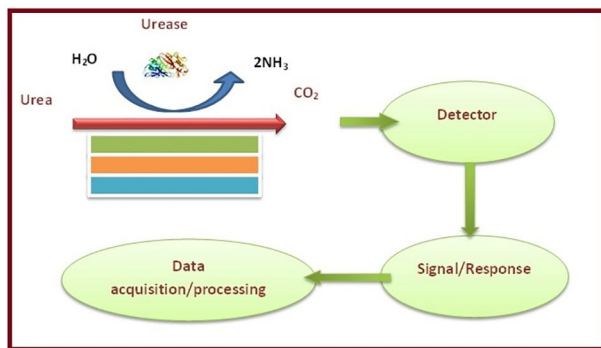


FIGURE 2 Schematic diagram of urea hydrolysis

[24–26]. The electrochemical biosensors integrate the benefit of electrochemical approaches with strong enzyme substratum sensitivity, rapid response time, and ease of operation. Currently, electrochemical biosensors have enhanced their analytical efficiency by utilising nanorange-materials.

The metal oxide nanostructure is known to provide an exceptional potential to develop accelerated kinetics of electron propagation between certain electrodes and the target enzyme's specific location. During the last few years, several modern nanomaterials have been designed with the advancement of nanotechnology and their unique characteristics were slowly found and applied to achieve enhanced biosensors. For the measurement of urea amount in different biological materials, a great deal of attention has been given over the last decade to various biosensors.

3 | UREA BIOSENSORS

In 1969 Guibault et al. developed their first urea biosensor. The study of this earlier urea biosensor was merely about its ability in ammonium ion identification of a cation-selective glass electrode, provided by urease that was directly proportional to the concentration of urea. Note that two forms of urea biosensors are common enzymatic [27, 28] and non-enzymatic. Both enzymatic and non-enzymatic biosensors have their own advantages and certain disadvantages. Urease enzyme based urea biosensors hydrolyse the analyte urea into NH_4^+ and HCO_3^- ions. Figure 2 is showing schematic diagram of mechanism of urease enzyme that catalyses the hydrolysis of urea. During the reaction, the urea is measured after the development of generated ions that lead to a change in pH. In fact, it is easy to detect the ammonium ion (NH_4^+) in traces by using a different transducer, aligned with the concentration of urea. A variety of urea electrodes have been developed by now, often utilising ion-responsive field effect transistor, CNT, and gold/silver/zinc-oxide/iron-oxide based transducer. Accomplishments were made to improve the biosensor based on the enzyme coating, leading to greater enzyme stability and lower limits on urea determination. Nevertheless, enzymatic biosensors have drawbacks, because they include costly enzymes, which include small lifetimes and expense.

Non-enzymatic biosensors utilising nanosize bare and functionalised metals and metal oxides to replace the enzymes were developed because of the high cost of biosensors.

Apart from cost savings, nanosize bare and functionalized metals and metal oxides are strong candidates to be used in non-enzymatic biosensors because they are highly sensitive and stable [29]. These have been widely used for the electrocatalytic oxidation of urea, owing to wide range and strong catalytic efficiency.

4 | CLASSIFICATION OF UREA BIOSENSORS

The performance of different types of urea biosensor depends on a variety of parameters such as bio-receptors, transducers (type of transduction element such as traditional/nanorange material) and various types of physicochemical reaction may be used [30]. We will illustrate many examples of recent biosensors of urea here (covering the period 2010–2020). We have classified urea biosensors broadly under 10 categories as shown in Figure 3.

4.1 | Potentiometric urea biosensors

Potentiometric measurement is an empirical technique which determines the potential difference among two electrodes. This variability in potential differences is attributed to urea hydrolysis and might be recorded offering quantitative evaluation of urea. The primary product ammonium ion, carbon dioxide, ammonia gas, or pH altered after enzymatic hydrolysis of the analyte urea is produced in conjunction with the urea concentration in the analyte. Many potentiometric urea biosensors are easily available and operated on the principle of medium pH shift, however a major issue for pH-sensitive electrodes is that the sensor reaction is highly dependent on the sample solution's buffer ability. Here, a potentiometric urea biosensor based on highly stable grafted fullerene was developed for the quantitative detection of urea. Fullerenes were used as matrix for the urease enzyme immobilisation. The surface of fullerene was grafted by carboxylic group by post impregnation method. Urease enzyme was covalently binding with carboxylic group of fullerene in the presence of $(\text{C}_6\text{H}_{11}\text{N})_2\text{C}$ and EDC hydrochloride. For the confirmation of immobilisation process of urease enzyme and grafting of carboxylic group was characterised by Fourier-transform infrared spectroscopy (F.T.I.R.). Urease enzyme based fullerene was deposited on screen printed pH sensitive electrode. Acrylic membrane was coated on the electrode which provides good stability and quick response for the quantitative analysis of urea. Figure 4 is showing schematic representation of reaction mechanism between modified fullerene and urease.

This potentiometric biosensor showed a linear range of urea concentration of 0.12–0.042 mM [31]. A lab on a chip based green urea biosensor was synthesised to determine the urea level in the synthetic urine. Urea was assessed by the

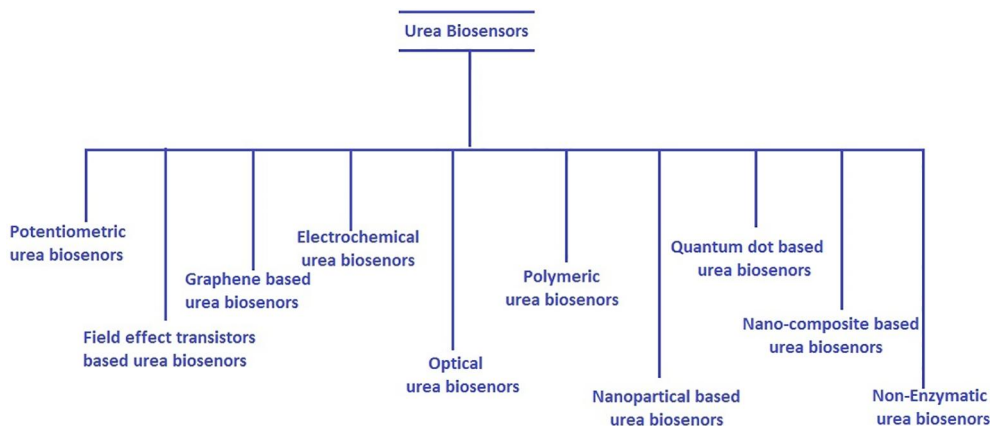


FIGURE 3 Flow chart for the classification of urea biosensors

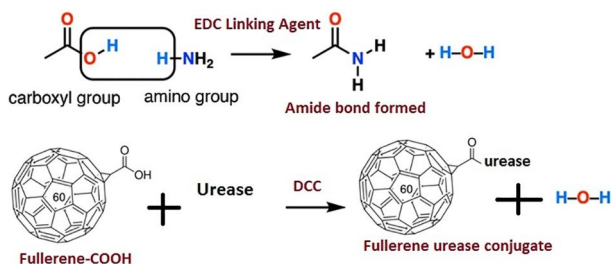


FIGURE 4 Schematic reaction mechanism between modified fullerene and urease enzyme (Adopted and reproduced from Ref. [31] Sensors, MDPI Publications)

electrochemical oxidation of ammonia released from the *Proteus vulgaris* bacteria's surface membrane. The technique of optical lithography was used for the manufacture of platinum electrodes and was used for whole-cell microbial sensors. Furthermore, *P. vulgaris* was used on the surface of electrode of platinum to convert urea into ammonia which prevents blockage on the surface of the platinum or also prevents its oxidation reaction capability. The proposed research serves as a medium for the sensing, tracking, in vivo urine and point-of-care urea detection of waste water [32].

4.2 | Field effect transistor based urea biosensors

Since semi-conductor technology is advancing, field-effect transistor-based biosensors are a promising tool in biomedical applications. The *trans*-conductance compatible biosensors for differentiated read out electronics were contrasted against the solid state reference system. Using nafion as a supportive matrix not only enables low primary load transition resistance but also reference field-effect transistors (REFET) and enzyme field-effect transistors (EnFET) manufacturing. The biosensor membrane was mounted in a conductive polymer-based matrix with urease immobilised. The device structure of polymer-based REFET and enzyme-based EnFET has been adjusted by modifying the photoresist/

Nafion™ to provide the differential pair *trans*-conductance curve that offers a broad dynamic measurement scale. This system can be rendered in miniature on one chip [33]. Similar to the potentiometric transducer, an ion selective field effect transistor-dependent urea biosensor has been described. Various types of zeolites were synthesised (silicalite, nano beta zeolite, and zeolite L). Electrode surface was modified with mucosal by spin coater and synthesised zeolites were mounted with direct attachment to the modified electrode. Urease enzyme was adsorbed onto the ion selective field effect transistor utilising monolayer of different zeolite forms. The urease biosensor activity is dependent on the proton-consuming urea cleavage reaction to NH_4^+ ions. This interaction results in a pH shift within the specific membrane that is captured by pH-sensitive field effect transistors. Glutaraldehyde vapour has been used as a control. The enzyme adsorption on silicalite and nanozeolite L monolayer has been shown to lead in linear range increase of up to 01.50 mM. Figure 5 show illustration of ion selective field effect transistor used for urea biosensor.

It has been observed that using monolayer of similar zeolites as a matrix for the enzyme adsorption to manufacture potentiometric biosensors may lead to an increase in linear range of their activity, a reduction in the minimum urea determination cap, improved reproducibility and inter-reproducibility of the reaction and a decrease in analysis time [34].

Fenoy et al. have developed an innovative approach for synthesis of acetylcholine sensors and immobilisation of Acetylcholinesterase enzyme on graphene based field-effect transistors. In this method polymer layer having amino moiety was synthesised electronically on the graphene channel. This copolymer base, that is poly (3-aminobenzylamine-co-aniline) offers the stable electrostatic charging condition for urease immobilisation and also increases the pH (40.80 to 56.30 $\mu\text{A}/\text{pH}$ unit). The designed biosensors thus displayed a limit of detection (LOD) of 2.3 μM and were able to track Ach in a flow configuration within the range of 5 to 1000 μM . In addition, they showed a sensitivity of $-26.6 \pm 0.7 \mu\text{A}/\text{Ach}$ decade and relative standard deviation of 2.6%, which indicated strong reproducibility of the biosensor [35].

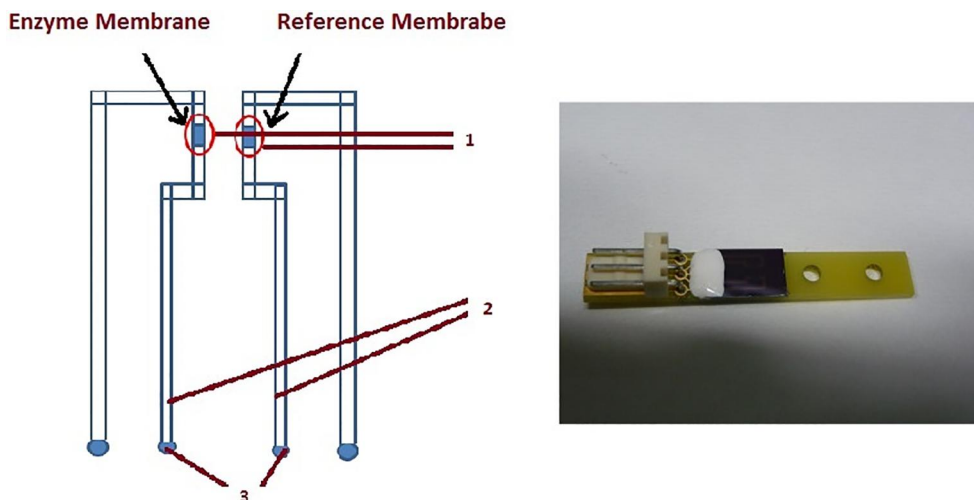


FIGURE 5 Schematic and general views of components of ISFET elements. 1, gate areas; 2, $p \pm$ diffusion buses from source and drain of each transistor; 3, aluminium contacts (Adopted and Reproduced from ref. [34] *Nanoscale research letters* Springer Publications)

4.3 | Graphene-based urea biosensor

Note that 2-D graphene fibre is the best candidate for urea biosensor manufacturing because of its prominent flexibility, high electron mobility, structural stability and ease of grafting. Today, these sensors are suffering from poor electrical conductivity, which greatly affects their sensing efficiency. A significant improvement was achieved by fibre grafting with various entities such as nitrogen, silver, zinc and gold NPs. The functionalised multi-layered graphene (MLG) is a new advancement in many techniques due to its interesting physical urea biosensing properties. The MLG is synthesised by an uncomplicated, harmless and replicable method. It was formed by MWCNTs treated with concentrated H_2SO_3/HNO_3 and further used for fabrication of urea biosensors. Functional MLG is a promising tool for urea biosensors and can be used for many other therapeutic bio-analytes as well [36]. Nitrogen doped graphene based urea biosensor has been developed due to its high catalytic activity towards the oxygen reduction reaction. The electrocatalytic property of nitrogen doped graphene based urea biosensor towards the oxygen reduction reaction has been measured by using rotating disk electrode. This graphene nano-sensor exhibited good detection limit as well as linear range [37]. A photo electrochemical urea biosensor (PUB) has been developed by using graphene fibre with broad linear range from 00.01 to 01500 micro-molar and a very low detection limit of one nano-molar [38]. A representation drawing of the manufacture procedure of stretchy PUB and recognition contrivance of its photoelectric reaction is exhibited in Figure 6. Precisely, graphene oxide fibre was manufactured consuming graphene oxide (GO) dispersal through a distinctive wet-spinning equipment with a congealing bath solution.

4.4 | Electrochemical urea biosensor

Electrochemical methods were also studied for providing an easier, more affordable and more efficient urea detection system in biosensor. Redox reactions may be monitored in electrochemical detection to detect the presence of urea in the enzymatic or non-enzymatic form. A temperature-dependent urea sensor has been fabricated by immobilising the urease on gold electrode. Zeolite was synthesised by the hydrothermal technique and its crystalline and amorphous nature was confirmed by X-ray diffraction technique. Gold electrode was used as a transducer and by using bovine serum albumin, synthesised zeolite was deposited on the transducer surface, then the urease enzyme was immobilised on modified electrode. The cyclic voltammetry technique was used to monitor the molar conductance catalytic activity of urea and urease. This zeolite based urea biosensor showed good stability and specificity [39]. For the identification of urea in blood and urine, an amperometric polymer-based urea biosensor was produced. A pre-cleaned pencil shape graphite electrode was electro-chemically modified with oxygenated functional group. The aniline monomer was used to generate a SNS aniline coated film. The amino group present on coated film was used to attach the DAFc. Finally urease enzyme was bind with amino group present on the DAFc. Urease-DAFc-SNS aniline-based biosensing electrode was prepared for detection of urea [40]. Figure 7 is showing schematic representation of preparation of urease immobilised biosensor for urea detection.

Here, a mobile urea sensor has been manufactured which monitors the concentration of urea under flow conditions. This is used in electrochemical system that tracks the concentration of urea in samples which flow in real time at a constant flow rate. A silk fibre layer which is porous in nature with urease already immobilised was installed in a sensor of polydimethylsiloxane

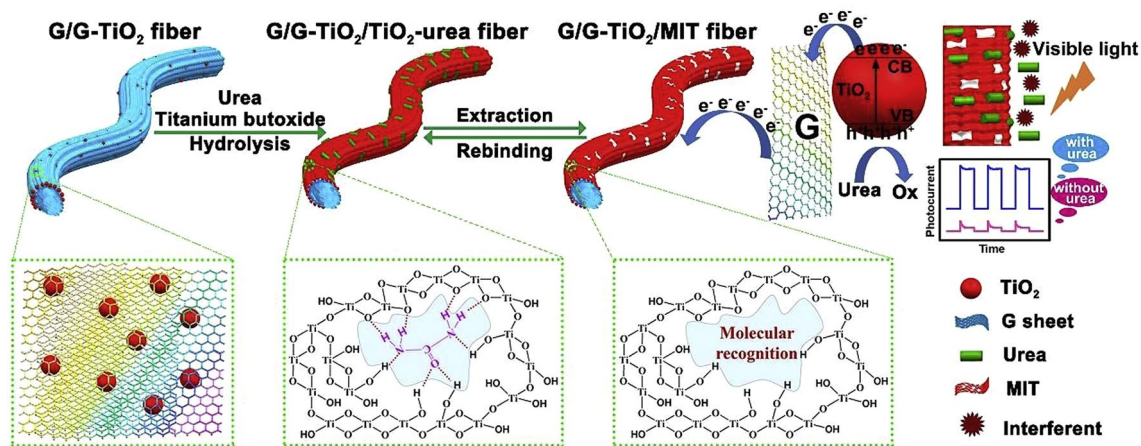


FIGURE 6 Graphene fibre based urea biosensor and its photo electrochemical detection process (Adopted and Reproduced from Ref. [38] Carbon, Copyright Elsevier Publications)

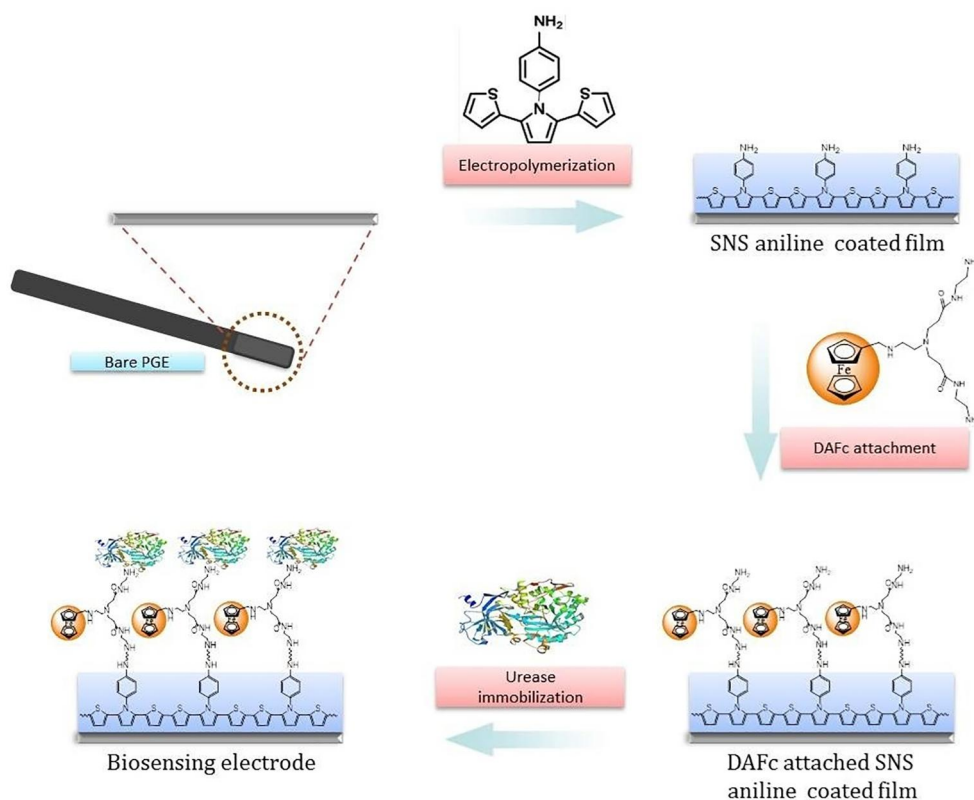


FIGURE 7 Schematic representation of preparation of urease immobilised biosensor electrode (Adopted and reproduced from Ref. [40], Enzyme and Microbial Technology, Elsevier Publication)

housing for electrochemical sensing. The manufactured urea sensor based on peritoneal dialysis evoked characteristics of linear current versus concentration within the concentration range (0.1–20 mM) which is significant for clinical purpose in different flow conditions. The synthesised urea biosensor might be one of the main components of a portable synthetic kidney system for monitoring urea in peritoneal dialysate of end-stage renal disease patients [41]. Kogularasua et al. has synthesised ZnO micro particles through the rapid microwave method. The

morphologies of the micro particles were engineered for the enhancement of its biosensing properties by altering the ratios of urea. ZnO was also examined morphologically for dopamine (DA) and uric acid (UA) sensing by varying the stoichiometric ratios of urease; ZnO has strong sensitivity towards DA and UA. The flower-like ZnO-b microstructures composed of orderly arranged ZnO nanosheets with adequate cavities has been formed due to positive morphological evolution that intensify the surface area. Due to this flower like structure electro-

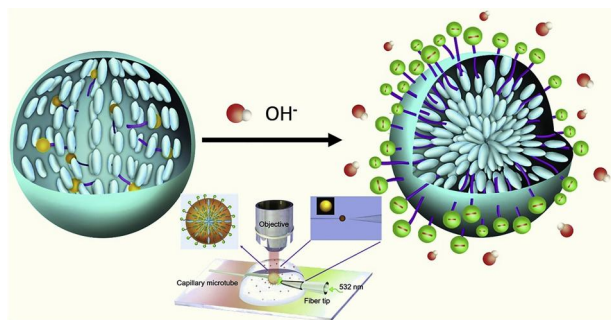


FIGURE 8 Schematic illustration of the structural transition of stearic acid-doped 5CB microdroplet from planar anchoring (a) to homeotropic anchoring (b) (Adopted and reproduced from Ref. [48], Talanta, Elsevier Publication)

catalytic and the bio-sensing properties of ZnO enhanced towards DA and UA. This sensor is helpful in recognition of DA and UA levels in the samples of human urine, meat and wheat flour [42]. Chanam et al. developed a simple flow analysis (FIA) method that is cost-effective for determination of two biomarkers of renal function in human urine namely creatinine and urea. This (FIA system consists of a light emitting diode (LED), two internal detectors and a contactless detector for conductivity. The FIA-GD-C4D/PEDD method successfully calculated the simultaneous determination of these biomarkers of renal function in human urinary system without any different preparatory work of samples. This system also demonstrated creatinine and urea detection limits of 0.9 mg L⁻¹ and 9.0 mg L⁻¹, respectively, and good linearity in the range of 30–240 mg L⁻¹ ($r^2 > 0.99$) and 10–500 mg L⁻¹ ranges. In addition, the results of interferences from large urine matrices are negligible [43]. A portable urea sensor was manufactured using porous polytetrafluoroethylene (PTFE) membranes covered with parylene-A for monitoring the concentration of urea in the fast flow condition. The urea-hydrolysing enzyme, urease was immobilised to generate a specific electrochemical signal on the amine functionalised parylene-A-coated PTFE (AP) membranes via glutaraldehyde cross linking. The urease-immobilized AP membranes were loaded into a polydimethylsiloxane fluidic chamber for the manufacturing of biosensors. Samples of urea were tracked at various flow rates under optimised conditions. The manufactured urea biosensor has been verified and found suitable for monitoring urea at flow rates in real time ranging from 0.5 to 10 ml/min. From these results, it has been confirmed that the monitoring of urea is capable in fast flow conditions such as 10 ml/min. Under the optimised conditions, urea samples were monitored at various flow rates. The designed urea sensor is deemed suitable for (portable) applications, such as artificial renal systems and portable dialysis systems [44].

4.5 | Optical urea biosensor

Furthermore, the use of optical urea sensors as an effective means of measuring urea has drawn greater attention. Optical fibre biosensors are important in determining optical properties,

heat, stress and reflectivity because of their low cost and efficient processing. The optical fibres consists of an inner core and outer cladding however, due to thick cladding of the optical field, show a sensing disadvantage. For sensing applications, the refractive core index should be high, so different mechanisms such as surface plasma interference, tapering and supported grafting are used to increase the transient field, or multi-mode interference. An optical biosensor for detecting urea in urine was developed. Acrylic micro range sphere was synthesised by photo polymerisation of 2-2-Dimethoxy-2-phenylacetophenone and 1, 6-hexanadiol diacrylate. The morphology and creation of functional group and micro sphere was analysed by SEM and FTIR. The *N*-acryloylsuccinimide functional groups present on the micro sphere act as a matrix for the immobilisation of urease enzyme. [45]. Moreover, an optical urea biosensor was successfully developed using Kappa Carrageenan bio-membrane doped with Nile blue chromoionophore. On the surface of bio-membrane, the immobilised urease enzyme has catalysed the hydrolysis of urea into ammonium hydroxide. Due to change in pH after the hydrolytic conversion, colour changed from blue to violet and detected by reflectance spectrophotometer based on fibre optic. Biosensor reported urea concentrations of 0.001–100.0 mM with an overall detection limit of 0.001 and response duration of 10 min, with no noticeable difference [46]. A silica based optical biosensor has been developed for urea detection. An aniline monomer was used to synthesise the PANI film and amorphosity of film was confirmed by X-ray diffraction technique. UV–visible spectroscopy gave two major peaks at 227 and 280 nm which confirmed that the processed PANI structure is in perfect form. Amorphous form of PANI layered on the optical fibre centre consisting of porous surface and allows for urease enzyme immobilisation. Assay urea has been used in approaches with doses from 100 to 100 mM limits to study the sensing response of biosensor. The processing time with each urea solution concentration was as high as 120 s. Because of its lower detection limit with broad range, precise selectivity, rapid response and good sensitivity, the optimised biosensor can thus be used as an innovative tool for the biomedical, forestry, fishery and milk industries for accurate and detailed urea determination [47]. Figure 8 is showing schematic illustration of the structural transition of stearic acid-doped 5CB microdroplet from planar anchoring (a) to homeotropic anchoring (b). Further, an acid-doped 5CB microdroplet biosensor has been developed to detect urea by whispering gallery mode (WGM) lasing technology. This real-time sensitive detector for efficient measurement of urea with WGM lasing was firstly reported by said work. The 5CB microdroplets were self-assembled and rendered using simple techniques. The 5CB microdroplet doped with stearic acid was treated both as a resonator (optical) and a reactor.

Due to double acceleration of 5CB molecules along with WGM resonance, the biological reaction signals at the interface have been significantly improved. The sensor proposed can detect urea minimal up to 0.1 mM concentration, which is superior to the current urea measurement limit for LC sensors. Further, it can detect urea form real human urine samples [48].

In the proposed model, a high sensitive optical urea biosensor has been developed. The single-mode coreless

TABLE 1 Comparative study of all urea based biosensor

Material Used	Method of urease immobilisation	Linear range	Detection limit	Response time	Sensitivity	Reference
Fullerene (potentiometric)	Covalent	2.31×10^{-3} M to 8.28×10^{-5} M.	0.1 mM	–	59.67 ± 0.91 mV/decade	[31]
Graphene(FET)	Electrostatic	5 to 1000 μ M	2.3 μ M	130 s	-26.6 ± 0.7 μ A/Ach decade	[35]
Graphene fibre (photo electrochemical)	-	0.01 to 1500 μ M	1 nM.	–	high	[38]
Multi-layer garphene (amperimetric)	Covalent	10_100 mg dl ₁	3.9 mg dl ₁	10 s	5.43 μ A mg ₁ dl _{cm_2}	[36]
Ferrocene (electrochemical)	Crosslinked by glutaraldehyde	0.12 to 8.5 mM	12 μ M	2 s	0.54 μ A/mM	[40]
Zeolitic imidazolate framework (optical)	Encapsulation	1 to 10 mM	0.1 mM	–	0.8 mM/RIU	[21]
NileBlue-chromoionophore-doped kappa-carrageenan (optical)	Physical	0.001–100 mM	0.001 mM	10 min	–	[46]
Succinimide-modified acrylic microspheres (optical)	Covalent	0.01 to 1000 mM	9.97 μ M	–	–	[45]
Polyaniline (optical)	Crosslinked by glutaraldehyde	100 nM–100 mM	100 nM.	28 days	High	[47]
Polypyrrole/polyethylenimine hybrid film (potentiometric)	Covalent	5.0×10^{-1} to 10.0 mM	–	30 s	56.15 mV/pUrea	[58]
polydiacetylene vesicles (colourimetric)	Cross-linking by phosphate buffer saline	20 pU/ml-0.2 mU/ml	70 pU/ml	15 min	–	[62]
poly(N-glycidylpyrrole-co-pyrrole) (Amperimetric)	Covalently immobilised	0.1 to 0.7 mM	–	4 s.	4.5 mA/mM	[54]
4-Cyano-4'-pentylbiphenyl (microfluidic based)	Covalently immobilised	–	3 mM	180 s	High	[53]
ZnO functionalised poly amide and polypyrrole nanofibre (impedimetric)	Physical	0.1 to 250 mg/dl	0.011 mg/dl	–	High	[85]
Nanocomposite of polyaniline and graphite nanodiamond (amperimetric)	Immobilisation EDC-NHS linker	0.1 to 0.9 mg ml ⁻¹	0.05 mg ml ⁻¹	20 s	381.5 μ A	[55]
Fe ₃ O ₄ -chitosan nanobiocomposite. (potentiometric)	Electrostatic	0.1–80 mM	0.5 mg/dL	12 s	42 mV/decade	[113]
Hybrid polyaniline nanofibres (electrochemical)	Entrapment by Nafion	Up to 20 mM	1.0 μ M	–	–	[67]
Ytterbium oxide (electrochemical)	Physical	0.05–19 mM	~2 μ M	~3 s	$124.84 \mu\text{A} \text{mM}^{-1} \text{cm}^{-2}$	[65]
Nickel oxide (amperimetric)	Non-enzymatic	100 –1200 μ M	8 μ M	–	High	[128]
Silica oxide nanoparticle (optical)	Immobilisation by glutaric acid linker	50–500 mM	10 mM	9 min	Good	[74]
Zinc oxide-iron oxide nanocomposite (electrochemical)	Electrostatic	5–150 mg dL ⁻¹	5 mg dL ⁻¹ .	Fast	High	[91]
Urease nanoparticle (potentiometric)	Immobilisation by glutaric acid linker	2–80 μ M/L	1 μ M/L	10 s	23 mV/decade	[89]
Polyamidoamine grafted multi-walled carbon nanotube dendrimers (amperometric)	Covalent	1–20 mM	0.4 mM	3 s	6.6 nA/mM	[84]
Nanoporous alumina membrane (piezoelectric)	-	0.08 μ M –1 mM	0.05 μ M	12 s	-	[71]

structure was synthesised by first splicing and then placing between the middle of two the simple mode fibres by CO₂ laser splicing machine. To embed urease enzyme zeolitic imidazolate framework was synthesised by aqueous amalgamation method. The online detection of urea was carried out on zeolitic imidazolate framework, when the substrate urea specifically binds with enzyme urease. Basically this coreless optical biosensor works on the principle of sensing the refractive index; the refractive index changed due to formation of enzyme-substrate complex and finally there is shift in wavelength of coreless fibre based biosensor. This optical fibre was label free biosensor which can detect the urea at very low concentration in the range of 1–10 mM. Its shows very good sensitivity 0.8 mM/RIU and detection limit 0.1 mM, if it operates in the range 1525–1590 nm. The main advantages of this optical urea biosensor for determination of urea were compact size, label free detection and very fast response. In addition, a micro fluidic chip has been integrated with the biosensor to conquer with the signal instability problem [49].

4.6 | Polymeric urea biosensor

One field that has created significant potential in the manufacture of urea biosensors is the use of polymer conductive polymers and non-conductive polymers due to their strong stability, eco-friendly and economical character. Specific conductive polymers such as polyaniline (PANi), polyPyrrole (PPy) and polythiophene have been used in the development of biosensors. And the design of urea biosensor uses various non-conductive polymers such as sodium alginate, poly(vinyl ferrocenium), polyvinyl alcohol, polyethylenimine, polyvinyl chloride and chitosan. An electro-synthesised poly-phenylene-diamine has been used to develop a fast and unique potentiometric biosensor for urea measurement. The poly-phenylene-diamine film was deposited on the glass carbon electrode and further used for urease enzyme immobilisation. Due to the excellent buffering capability of poly-phenylene-diamine film, the response of this synthesised sensor is linear with urea concentration in the range of 10 M–1 mM. The Nernstian response of 28.0 mV/decade is found at higher amount of urea. Further, this sensor is sensitive enough for realistic determinations, quick response and long-term durability [50]. An amperometric urease enzyme based urea biosensor has been developed. The polymer film of Poly (N-glycidyl-pyrrole-co-pyrrole) was electrochemically synthesised on gold electrode with the help of N-glycidylpyrrole monomer. Urease enzyme was covalently immobilised on gold electrode with the help of pyrrole functional group. Conducting pyrrole polymer based urea biosensor showed excellent sensitivity of 0.45 $\mu\text{A}/\text{mM}$ and a quick response time of 4s [51]. Further, biosensors of urea have been developed using transducer based on macro porous PPy. Nanoscale PPy have been developed using 460 nm sized monodispersed spheres of polystyrene as a template. After immobilisation of urease on PPy which is macroporous, nanostructured urea biosensors were arranged using two processes: covalent attachment or deposition layer over layer-sized monodispersed spheres of polystyrene as a template. After immobilisation of

urease on PPy which is macroporous, nanostructured urea biosensors were arranged using two processes: covalent attachment or deposition layer over layer-sized monodispersed spheres of polystyrene as a template. After immobilisation of urease on PPy which is macroporous, nanostructured urea biosensors were arranged using two processes: covalent attachment or deposition layer over layer. Results indicate that deposition of urease on PPy in layer over layer gives rise to biosensors with enhanced kinetics of detection and switchable sensitivities. When compared with high volume biosensors of urea, it shows that the use of PPy as a sensing system enhances the analytical performance of biosensors, especially because more activated sites are available for enzyme immobilisation [52]. For easy visual identification of urea in human samples, a modern and versatile micro-fluidics-based urea biosensor has been created. Poly-dimethyl-siloxane was processed by carefully combining the cross-linker and prepolymer to remove the excess air bubbles. Upon separating it from the formed silicon wafer, a silicon wafer mould was put on the final product and treated in a microwave. With the help of oxygen plasma treatment, patterned piece of polydimethylsiloxane was attached with micro-slide glass. The dispersed phase in the micro-fluidic system consists of 5 ascorbic acid and poly (acrylicacid-b-4-cyanobiphenyl-4-oxyundecylacrylate) continuous phases. The urease enzyme was immobilised on poly (acrylicacid-b-4-cyanobiphenyl-4-oxyundecylacrylate) chain. Functionalised 5 ascorbic acid senses urea by means of a polarised optical microscope owing to the bipolar-to-radial orientation transformation property of structure. The detection maximum was as high as 3.0 mM, with duration of 180 s [53]. A modified amperometric urea-biosensor based on graphite was developed to analyse the urea. Micro particles of polyurethane urea from polyvinyl alcohol and hexamethylene diisocyanate were synthesised in dimethyl sulfoxide/water solution by single step chemical process. In standard buffer solution, the immobilisation of urease on micro particles of polyurethane urea was carried out. Modified graphite particles (MG) have been formulated from bare graphite through oxidizing it in alkaline media, using potassium ferricyanide K₃[Fe(CN)₆]. Modified graphite particles powder was combined with the polyvinyl dichloride pasting liquid in acetone and used for electrode construction. Running urease-MG electrode urea biosensor has been developed by mechanically connecting to the surface of modified graphite particles the membrane comprising polymeric carriers with immobilised urease. The response of the biosensor to substrate attachment was tested under potentiostatic conditions at 0.2 V in a stirred buffer solution. Standard buffer solution, composed of 1 M of urea or dialysate, has been used as a substratum. Reliable data compatibility with accredited system has shown that the proposed analyser can be used to detect urea in dialysate quickly and easily [54]. Using intermolecular polymerisation techniques, the fast and non-intermediate urea biosensor was prepared by a composite of PANi and graphitised nanodiamond. The urea amperometric sensing was assessed due to ion generation following a catalytic reaction of urea with PANi and GND/PANI composite. This demonstrated strong linearity across concentrations ranging from 0.1 to 0.9 mg mL⁻¹ with 0.07 and 0.05 mg mL⁻¹ detection limits for PANi and

GND/PANi respectively. The urea sensitivities were 140.50 and 381.50 $\mu\text{A} (\text{mg mL}^{-1})^{-1} \text{cm}^{-2}$, respectively, with a latency of 15.0 and 20.0 s [55]. Semiconducting thiophene Copolymer based potentiometric urea biosensor has been developed. After esterification of 3-thiopheneacetic acid, a conducting copolymer, poly(3-hexylthiophene-co-3-thiopheneacetic acid) (P (3HT-co-3TAA) was formulated. Next, hydrolysis of poly(3-hexylthiophene-co-methyl-2-(thiophene-3-yl) acetate) was conducted. Urease enzyme was covalently bonded to synthesised thiophene copolymer carboxylate groups by the amine specificity in the amino acids, through carbodiimide linking. And spectrophotometric analysis studies were conducted for the urease behaviour. This electrode could routinely calculate the urea content in aqueous solutions up to a total of close to 5 mM [56]. A portable urea biosensor has been developed especially for point of care application. Eudragit polymer pH responsive membrane was designed and sandwiched between two copper strips. And this membrane was further immobilised with urease enzyme. An appropriate mixture of different reactive Eudragit polymer and enzymes (amidohydrolases and carbon-oxygen lyases) were used. As a result either ammonia was produced after the combination with reactive Eudragit membrane, or hydrogen peroxide was produced with different oxidoreductases enzymes. This BioPoC device was used as a qualitative diagnostic test such as in detection of *Helicobacter pylori* in biopsy sample of upper gastric antrum [57]. A potentiometric urea biosensor has been developed for urea sensing by using PPy/polyethylenimine hybrid film. A PPy film was synthesised by cyclic voltammetry scanning using a potentiostat. Further a PPy/polyethylenimine hybrid film was developed by electro polymerisation of PPy and plasma polymerisation of polyethylenimine. Creation of increase number of free amino (NH_3) functional group on exterior of PPy and there incorporation of PEI was confirmed by CV and XPS. Urease enzyme was immobilised by covalent bonding of free amino group and glutaraldehyde. This PPy/polyethylenimine hybrid film showed a 30 s response time with an outstanding potentiometric susceptibility response of 56.15 mV/pUrea over urea concentrations varying from $0.50 \times 10^{-1} \text{ mM}$ –10.00 mM [58]. The urease enzyme was immobilised on polyethylene glycol-aldehyde by non-covalent bonding. The activity of urease enzyme on PEG-aldehyde complex was determined by Berthelot assay. This fast and efficient non-covalent, immobilised urea biosensor complex is beneficial in enzyme technology [59]. A mobile sensor of urea for use under rapid flow conditions was manufactured by vapour deposition using PTFE which is a porous membranes covered with parylene functionalised with amine and parylene-A. Using glutaraldehyde, the urease enzyme hydrolysed with urea was immobilised on the PTFE membranes covered with parylene-A. Using glutaraldehyde, the urease enzyme hydrolysed with urea was immobilised on the PTFE membranes covered with parylene-A. The membranes immobilised with urease were mounted in a polydimethylsiloxane fluidic chamber, and the electrochemical measurements were based on a three-electrode screen-printed carbon instrument. It was determined that the optimum urease concentration for parylene-A-coated PTFE immobilisation on the membranes was 48 mg/mL, and that the optimal number of membranes in the

polydimethylsiloxane fluidic chamber was 8. Using these optimised conditions, the urea biosensor was manufactured and monitored using chronoamperometry at different flow rates from 0.5 to 10 mL/min. The sensor's applicability to physiological samples was checked using it to track the urea concentration of a patient with chronic renal failure in the waste peritoneal dialysis at 0.5 mL/min flow rate. The established urea biosensor is considered appropriate for the applications like artificial kidney systems and mobile dialysis systems [60]. A test strip for determination of urea has been developed using urease, hydrogel based on polyethylene glycol and pH-indicator paper which shows semi quantitative, fast, simple detection of urea and colourimetric detection at onsite. Hydrogel's swelling behaviour showed the urea's ability to absorb into the matrix and allow the test strip to react over a short period of time. This strip also showed a good linearity in the range of 20 to 200 mg/d. The LOD with naked eye was 20 mg/dL and light analyser 3.41 mg/dL, too. There is strong reproducibility to the assays. Relative standard deviation values were seen within the range of 3.37%–12.95% and 1.68%–9.54%, respectively for accuracy and precision assays. Preliminary results show that the urea strip established for the measurement of urea in saliva and urine samples was feasible [61]. Also, a colourimetric urea biosensor has been developed to detect the urea by using polydiacetylene vesicles. Polydiacetylene vesicles terminal are functionalised with the carboxylic group and bind with urease enzyme. These vesicles detect the urea by catalytic reaction between urea and urease. The detection limit for the urease sensor developed was 70.0 pU/mL after 10 min of incubation with a linear range of 20.0 pU/mL–0.20 mU/mL. The procedure was also used to evaluate the urease-inhibitor acetohydroxamic acid. Using the pH responsiveness of polydiacetylene vesicles and urea hydrolysis catalysed by urease, a colourimetric sensor network has been developed that is highly sensitive and inexpensive to physically test urease [62]. A portable urea biosensor has been fabricated by using porous PTFE membranes. The membrane was further functionalised with parylene group with the help amino entities. The urease enzyme was immobilised on functionalised membrane and assembled in a fluidic chamber. For the evaluation of urea electrochemical method was used by using screen printed carbon three electrode system. This biosensor sensor showed good results to detect urea in patient with chronic renal failure wastes peritoneal dialysate [63]. A new, usable colourimetric composite gauze test strip was formulated utilising non-conductive alginate polymer. Co-encapsulated urease with artificial tricyanofuranhydrazone receptor sites, sponsored on cotton gauze assay. A bridge-linked calcium alginate microcapsule consisting molecules of tricyanofuranhydrazone and urease enzyme has been synthesised. Cotton fibres were also used as a matrix to create a colourimetric strip of cotton that changes its colour after urea was determined. The strip replied linearly, depending on urea concentration in the range 00.1–250.0 ppm [64]. A hydro gel (acrylic acid-co-dimethylaminoethyl methacrylate) based urea biosensor has been developed in which the urease enzyme has physically entrapped during polymerisation. The specific reaction takes place between the urease enzyme and urea and resulted I change in pH which leads to swelling of pH sensitive hydro gel.

The swelling of hydro gel is totally depending on concentration of urea. This hydro gel based urea biosensors showed a high sensitivity in a range of 1 to 20 mmol/L. The main advantages of these biosensors are simple, cost effective and long-term stability [65].

4.7 | Nanoparticles based urea biosensor

For the identification of urea a biocompatible zirconia-based electrochemical biosensor was developed nanothin film of zirconia was deposited on gold layered glass electrode and further used for the immobilisation of mixture of urease and glutamate dehydrogenase enzyme. Zirconia nano-electrode has linearity of up to 100.0 mg dL^{-1} of urea with good sensitivity of $0.071 \text{ } \mu\text{A}/(\text{mM cm}^{-2})$ ($K_m = 0.5 \text{ mM}$) [66]. Electro spinning technique was used to produce polyacrylonitrilenanofibres. The received polyacrylonitrilenanofibres have been used as a frame work for PANi deposition. Synthesised PANi-nanofibres behaved as a conducting matrix for the deposition of platinum NPs. Platinum doped PANi-nanofibres membrane was used as a substrate for urease enzyme immobilisation. The flow injection analysis system was used for detection of urea. The nanocomposite had a wide range of application due to the presence of conducting polymer. It was used in a flow detection analysis showing a wide linear scale (i.e. 20 mM), a better detection limit ($10 \text{ } \mu\text{M}$) [67]. An eco-friendly gold nanoparticle based colourimetric urea biosensor has been developed. Gold NPs were synthesised from *Syzygium aromaticum* extract. The urea biosensor was formed by the co-immobilised urease enzyme with gold nanoparticle and phenol red indicator on a gelatin matrix. The sensor was especially utilised for the determination of milk quality. The green biosensor can discern safe and unsafe samples of milk quantitatively [68]. A new conductometric biosensor was established for the identification of enzymatic thin films utilising gold NPs. Gold NPs were used by the authors to immobilise enzymes using Layer by Layer technique. A high amount of enzyme will then be immobilised due to its high surface-to-volume ratio. They can behave like nanoelectrodes because of their electric conductivity. The detection threshold of 2.0 mM and the linear range of 0–3 mM were gained with urease-poly(allamine) gold-coated hydrochloride NPs [69]. A new strategy for urea determination was developed, where a nanorange thin layer of silicalite and zeolite beta dispersed on a conductometric biosensor's gold electrodes was used as a matrix to immobilise the enzyme. In comparison, it could be said that the conductometric responses for nanoelectrodes improved with an improvement in the silicalite and zeolite beta ratios. The absence of glutaraldehyde during immobilisation of enzyme leads to a stronger interaction between urease enzyme and zeolitic thin film. This zeolitic nanoelectrode can be applied to all types of electrochemical biosensors because it is easy to manufacture and these sensors, also exhibit excellent storage and stability [70].

Piezoelectric detection is yet another transduction technique which has achieved little interest in biosensing of urea. In addition, an acoustic system can be used to monitor the transition in

mechanical properties attributable to catalytically reaction on an electrode is piezoelectric sensing. For the identification of urea a highly stable and effective single-enzyme piezoelectric urea biosensor was established. A nano-porous alumina membrane was synthesised by electric anodisation method. Magnetic single-enzyme NPs encapsulated in an inorganic/organic polymer composite framework were produced by surface functionalisation and in situ aqueous polymerisation of specific enzyme molecule. Magnetic single-enzyme NPs were immobilised on the nanoporous alumina membrane and subsequently coated with the chitosan. Urea was detected by the electrode separated piezoelectric sensor on the basis of measurement of shift in frequency due to the change in conduction of the respective solution. This piezoelectric urea sensor showed fast response time (12 s), broad linear range from $0.08 \text{ } \mu\text{M}$ to 1 Mm with very low limit of detection of 0.05 mM [71]. A potentiometric iron oxide NPs based urea biosensor has been developed. Iron oxide NPs synthesised by chemical method under reflux condition. Under inert condition the telomerisation of poly(glycidylmethacrylate) (PGMA) was carried out. The grafting of iron oxide NPs was carried out by salinisation. The creation of silanol group was used to adsorb the urease enzyme. The gold electrode was coated with cysteamine and used as a matrix to integrate iron oxide NPs that have been modified with silanol. The creation of silanol on iron oxide NPs and telomerisation of polyglycidylmethacrylate was confirmed by FTIR and NMR. The thermal stability of enzyme on electrode was evaluated by TGA. The phase investigation of sensor was characterised by XRD. The potentiometric reaction of enzyme electrode was worked on enzymatic decomposition of urea in CO_2 and NH_3 . A wide linear range, excellent sensitivity and quick response of urea detection were the main features of urea biosensor [72]. A highly reliable urea sensor was discovered by growing Zinc oxide nanorods on a silver-glass electrode directly. This fabrication of urea sensor showed a great sensitivity of $41.64 \text{ } \mu\text{A}/\text{mM-cm}^2$ and a broad range of linearity. The direct synthesis of nanorods on silver electrode raised demonstratively its attachment and surface area. That enables greater loading of enzymes and greatly increases the performance of the sensor. The sensor revealed outstanding anti-interference capability versus electroactive species [73]. The optical urea biosensor based on silicon NPs was established to assess concentration of urea in urine samples. The optical urea biosensor based on silicon NPs was established to assess concentration of urea in urine samples. On the basis of the Stober process, silicon oxide NPs were synthesised by sol-gel technique. In the presence of glutaric acid cross-linker, the surfaces of silicon oxide is grafted with amine functional groups for the immobilisation of urease enzyme. The pH-sensitive chromoionophore dye was chemically adsorbed onto the grafted silicone oxide nanoparticle that serves as a pH transducer. Immobilised urease estimated the concentration of urea by reflectometric approach based on enzymatic urea hydrolysis as a consequence of the immobilised chromoionophore's colour change calorimetric detection was observed to interact with urea concentrations over a 50–500 mM linear response spectrum and 10 mM urea detection maximum and latency was 9 min. The silica oxide NPs based reflectometric urea biosensor revealed better vital linear response against other NPs

based ocular urea biosensors [74]. To develop a urea biosensor, nanorange conical shaped nickel Oxide rods (Ni-NiO) were synthesised under glancing angle deposition (GLAD) framework through radio frequency sputtering technique. For the production of an accurate and reagent less amperometric urea biosensor, high well order series of nickel oxide nanorods is produced on the Indium-Tin Oxide glass substrate. The urease was safely immobilised by a physical adsorption technique on the surface of the conical tip of nickel oxide nanorods. Urease-nickel oxide nanorods based Indium Tin Oxide-coated glass substrate was characterised by SEM, FTIR and XRD. The developed nano-bio-electrode has increased linearity within a wide range of detection (0.83–16.65 mM), a wide dynamic range of approximately $48.0 \mu\text{A}/(\text{mM})$, a strong observable enzyme activity of $5.1101 \text{ U}/\text{cm}^2$, a quick response time of 5 s and a long life period of 20 weeks. Due to low K_m value of 0.47 mM showed a strong affinity of the immobilised urease to nano-bio-electrode with urea. The bio-nano-electrode gives an adequate active surface with its own redox pair for intensified urease immobilisation, desirable conformation and rapid electron transfer that catalyses urea electrochemical oxidation [75]. A PPy grafted chitosan based optical urea biosensor has been developed. The self-assembly technique was employed to synthesise core shell nanoparticle of chitosan and PPy. By using the membrane dialysis technique, PPy grafted chitosan micelles were grown on the PPy core-shell structure. With the help of linker (ethyl carbodiimide hydrochloride and N-hydroxysuccinimide) the urease enzyme was covalently immobilised at the end of PPy grafted chitosan micelles. A nanorange Urease-PPy-chitosan micelle structure detect the urea, $\text{NH}_2\text{Hg}_2\text{I}_3$, a compound formed on the basis of reaction between Nessler's reagent and ammonia formed after urea enzymatic hydrolysis at maximum wavelength 385 nm, has been calculated to evaluate urease kinetics. The copolymerised nanorange micellar PPy grafted hybrid system gave a high volume of enzyme immobilisation, resulting in highly stable Urease-PolyPyrrole-chitosan nanomicelles. This self-reporting optical urea biosensor demonstrated a linear reaction to concentrations of urea varying from 0.01 to 30 mM and a sensitivity of $0.25 \mu\text{M}$ with a reaction time of 12 s [76]. For identification of urea a highly sensitive nanorange multi-layered porous nickel oxide urea biosensor was developed. The synthetic process of multi-layered polymers with nickel-based coordination consists of two phases. Multi-layered nickel nanostructure was formed from the dimethyl-formamide reaction of $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, with the aid of acetic acid and pyridine. Finally, highly porous multi-layered nickel-oxide nanostructures were synthesised by calcinations at high temperature. The processed porous nano nickel oxide was efficiently layered onto an indium tin oxide that works as an appropriate substrate for urease enzyme immobilisation. The formulated modulated porous nickel electrode was used with cyclic voltammeter for urea sensing. The formulated modulated porous nickel electrode was used with cyclic voltammetry for urea sensing [77]. For the immobilisation of the urease enzyme, a copolymer (acrylonitrile-methylmethacrylate- sodium vinylsulfonate) membrane was first integrated with chitosan and further with rhodium NPs. A grafted copolymer membrane for the development of an

amperometric urea biosensor was synthesised. A plot of calibration for concentration of urea ranging from 1.60 to 23.0 mM was obtained. A linear range was observed from 1.60 to 8.20 mM on the calibration range. The urea detection limit was measured at a signal-to-noise ratio of 3.0–0.5 mM [78]. To detect the ammonia, a simple, wearable, and highly sensitive ammonia sensor was developed. This sensor is unique in its eco-friendly and low power consumption features. Hybrid PANi and tin oxide were synthesised and mounted on a polyethylene terephthalate substrate. This hybrid sensor has an effective sensitivity and a strong linear response to NH_3 at a standard 21°C temperature [79]. Using the gold nanoparticle a colourimetric urea biosensor dependent on urease inhibitor has been synthesised. Gold NPs were synthesised by the one-step refluxing technique and mixed with phosphate buffer solution. For the Detection of urease, the urea was added to different urease concentrations and the solution was incubated, and then added hydrogen peroxide, 3, 3', 5, 5'-tetramethylbenzidine and gold NPs to the pre-synthesised solution. The TMB- H_2O_2 reporting system catalysed with gold nanoparticle was used as a high-sensitive colourimetric pH marker. The absorbance in the catalytic interaction of the yellow-colour product at 450 nm (A_{450}) reveals a longitudinal trend over the pH range of 6.40–6.60. The newly built colourimetric urea sensor is supersensitive (between ON/OFF conditions, i.e. pH 0.2) and, more specifically, within the biochemical measuring region, the pH-responsive limit showed incredible potential for urea, urease, and urease inhibitor [80]. Using an easy liquid exfoliation and method of heat treatment, a delaminated Ti_3C_2 doped with nitrogen and aided with urea has been prepared. Here urea plays a major role in synthesising N-d- Ti_3C_2 , which functions as a delamination intercalant and as a source of nitrogen for the purpose of doping. Because of the synergistic impact of the layered system, wider broad surface area, suitable pores distribution, suitable nitrogen doping stage, the electrode, that is nitrogen doped- Ti_3C_2 demonstrates a strong specific capacity of 266.5 F g^{-1} with redox reactions, at 5 mV s^{-1} scanning rate; flexibility and excellent cycling stability in 6 M aqueous solution of KOH [81]. The hydrothermal method has been used to develop two-dimensional (2D) rectangular ytterbium oxide (Yb_2O_3) nanodisks. Urea biosensor has been fabricated with clean glassy carbon based electrode which was modified with Yb_2O_3 . Modified carbon electrode was used as a matrix for the immobilisation of urease enzyme which was further covered by Nafion membrane. The biosensor was manufactured with a sensitivity of $124.84 \mu\text{AmM}^{-1}\text{cm}^{-2}$, an extensive linear range of 00.05–19 mM, a limit of detection $\sim 2 \mu\text{M}$, and a fast response time of $\sim 3 \text{ s}$ [82]. Nanoporous of Nano-ZnO/ TiO_2 having 3D hierarchy has been engineered and synthesised using the technique of sputtering and doctor-blade and used as an efficient transmitter for production of urea biosensor. The TiO_2 exist as a part of the hierarchy of heterostructure and has crucial impact on the efficiency of the biosensor by promoting electron transfer between Zinc Oxide and fluorinated-tin oxide substrate, and form a layer of electrostatic repulsion to reduce interference of anions in media by transferring high electronic density to the surface of biosensor. Due to porous structure of Zinc Oxide nanomaterials, a surface

area having large size is available for immobilisation of enzyme and decrease the distance of diffusion for the substrate to connect with immobilised enzyme, which promotes faster detection of kinetics once the ammonia, that is electrochemically detectable species is produced closer to the transducer. Thus zinc oxide reduces resistance to diffusion, increase sensitivity and decrease response times. As a consequence, the biosensor in the presence of urea has a similar sensing and a faster response time than other biosensors, which is most anticipated due to the development of heterojunction of Tin Oxide-Zinc Oxide [83]. For the measurement of urea, an effective amperometric fluorescence probe was made. A MWCNT -based nanobiocomposite film was synthesised to develop a consistent biosensor. An amine functional gold electrode was prepared by emerging the gold plated electrode in cysteamine solution. MWCNT was first crafted with amino group and further functionalised with polyamidoamine dendrimer and with glutaraldehyde. This dendrimer based MWCNT was used to covalently bind the urease enzyme. Finally a urea biosensing CNT based gold electrode was synthesised. A fifth generation of polyamidoamine dendrimer is formed using a contrasting method on the amine-functionalised MWCNT substratum. Compared to other polyamidoamine generation, the fifth generation of polyamidoamine played a remarkable gain in urea biosensor performance due to the higher generation's enzyme immobilisation capacity. The engineered urea biosensor ensures excellent analytical properties, like low concentration limit, wide dynamic range, durability and reproducible [84]. A biosensing system for the electrochemical identification of urea was built to assess urea contamination in dairy products to ensure product safety against contaminants. This biosensing device was fabricated with polyamide six which was in polymeric electro spun nanofibres form and PPy coated on tin oxide electrodes doped with fluorine. After which the device was modified with NPs of zinc oxide. The prepared biosensing system exhibited outstanding properties for urease immobilisation, showing high sensitivity to the modified urease electrode for urea measurement concentration in the range of 0.1–250 mg d L⁻¹ with a limit of 0.011 mg dl⁻¹ [85]. A urea biosensor was developed using the poly (propylene-coimidazole)/gold NPs electrode immobilised with urease. The urease which is adsorbed on the polymeric film hydrolyses urea into ammonium and ions that is bicarbonate. Using differential pulse voltammetry, ammonium was electro-oxidised on the gold electrode after this. The polymer quantity at pH eight is 1 µg, indicating optimum reaction. The electrode measured the concentration of urea in municipal wastewater with a recovery value of 106%. The prepared nanocomposite film produced a mild microenvironment for urease detection in urea because of the biosensor's strong operating and storage stability [86]. A study was carried out to detect the urea by developed urea biosensor with the help of magnetic biosensors. The important functional parts of this biosensor are sets of connecting wire and a reference/standard electrode. The screen printing technology was employed for developing an insulation layer on the PET substrate and with the help of radio frequency sputtering a NiO film was deposited as a matrix. Then on the surface of NiO film, graphene oxide and magnetic beads were titrated. The urease–magnetic bead

composite solution is immobilised with Go-NiO film. Urease–magnetic bead-nickel oxide composite film exhibited the minimum load transfer resistance among the various sensors, and displayed outstanding load transition capacities. The response time of biosensors for urease–magnetic bead-nickel oxide composite film was 29 s, the measurement liner was 1.338 mg/dL and the drift effect was 1.551 mV/h [87]. In another study a calorimetric urea biosensor was fabricated by developed a nanocomposite film sensor. The work has highlighted advantages of using natural rice straw for developed a spectroscopy probe. Cellulose nanowhiskers were prepared and further incorporated with tricyanofuran-hydrazone (TCFH). Then a pH film sensor was produce in which cellulose nanowhiskers acts as a matrix and TCFH and urease enzyme were introduced in to the matrix. This sensor worked on colouration measurement which changed its colour after the reaction between Tricyanofuran-hydrazone, Cellulose nanowhiskers and enzyme. The urease reaction was confirmed by observing the colourimetric shift due to change the pH of urea solution because of urea conversion into the ammonia. This TCFH based biosensor showed a detection limit in range of 50–1100 ppm. The film can be used as a naked-eye sensing mark or test strip, since the film's colour shift (yellow to pink) provides a simple and practical urea monitoring technique. The sensor at the dipstick displayed a very high sensitivity. Such urea detector tool can be used to monitor and indicate urea in stored foods and other urea-containing products because of their efficiency and simplicity [88]. Jakhar et al. have prepared the industrial urease aggregates which are NPs in size from jack beans scientifically known as *Canavalia ensiformis*. Such NPs were prepared by dissolving and cross linking glutaraldehyde, after which cysteamine dihydrochloride was used to functionalise it. The size of NPs of urease were 51.2 nm (average) in the range of 18–100 nm as shown in TEM images and these NPs were more prominent and stable than native enzyme particles, with a longer lifespan. The NPs were immobilised onto nitrocellulose activated with chitosan membrane by the method of glutaraldehyde coupling with free urease NPs having retention of 32.22% of initial activity and amount of 1.63 mg/cm² in conjugation. The nitrocellulose was placed with an O-ring at the lower end which is more sensitive, of the selective electrode for ammonium ion, and then linked to pH metre to build a biosensor potentiometrically for urea. At pH 5.5, this biosensor showed maximum response at 40°C within 10 s. This biosensor demonstrated a concentration limit of 1 µM/L having functioning range of 2–80 µM/L and 23 mV/decade sensitivity. The biochemical production was 106.33% in serum with added urea. The variance coefficient of the present biosensor within batches was 0.18% and between batches was 0.32%. A strong correlation obtained in the values of sera urea measurement with the present biosensor and reference method ($r = 0.99$). The biosensor had moderate interaction with ions which is resolved by special selective ion electrodes [89]. A glassy carbon of diameter (3 mm) was modified mechanically and used as a working electrode for urea biosensor. Ultrafine nanostructured-NiO is developed over a glassy carbon surface using an electro-deposition technique. This NPs based catalyst provides a framework for urea oxidation in the alkaline based

medium and has exhibited high current during anodic urea oxidation phase. High-performance NiO NPs prepared as a catalyst for urea electro oxidation. So, their support for the remediation of urea-rich wastewater and its use in fuel cells and for the manufacture of hydrogen is thus proven [90]. A sensitive electrochemical urea biosensor has been developed by fabricated a nanocomposite of iron oxide (Fe_3O_4) and zinc oxide (ZnO) nanoparticle. Iron oxide and zinc oxide were synthesised by coprecipitation method. A high control degree of Fe_3O_4 -ZnO/ SnO_2 composite electrode was synthesised by deep coating technique. Urease enzyme was adsorbed electro statically on composite electrode. Thin film composite based urea sensor showed a detection range of 5–150 mg/dL [91]. A urea biosensor has been developed by using cotton fibres. A high content nanocellulose dialdehyde was synthesised from cotton derived micro cellulose and this micro cellulose was acid hydrolysed to form nanocellulose. After the development of aldehyde group on nanocellulose the urease enzyme was covalently immobilised by amino acid and CHO group. And green urease biosensor had the application to detect the urea in blood serum and aqueous sample [92]. Surface-enhanced Raman Scattering paired with silver dendrites was used first time to detect limited urea concentration. Replacement reaction on the indium doped tin oxide surface was used for immobilisation of dendritic silver micro particles and the creation mechanism could be related respectively to deposition which is electroless and aggregation limited to diffusion. The average Raman intensity plot calculated at 1004 cm^{-1} with respect to concentration of urea with $R^2 = 0.999$ indicates that silver dendrites display a satisfactory and consistent output with an enhancement of the analytical urea. The results indicate that dendritic silver micro particles are able to detect urea well within the normal physiological concentrations, and also the potential for utilisation in urea biosensor. Further, the manufacture of dendritic silver micro particles on the substrates can be appealing as a surface-enhanced Raman Scattering platform for residue chemicals and toxicants detection. The detection limit for urea could exceed the physiological urea range [93]. The nano $\gamma\text{-Fe}_2\text{O}_3$ @microcellulose and nano $\gamma\text{-Fe}_2\text{O}_3$ @nanocellulose has been prepared from MC and NC by their magnetisation and oxidation followed by chemically immobilisation of urease with Schiff-base. Due to this immobilisation reusability, stability, pH resistance and urease temperature were improved, although there is no change in enzyme activity. In addition, 100% selectivity of both the designed immobilised urease-catalysed Hantzsch and Biginelli reaction was observed for Biginelli [94]. A surface-enhanced Raman spectroscopy (SERS) pH sensor has been developed that functions in the buffered solution and used to track specific analyte targeted by acid-producing enzymatic reactions. This SERS-pH sensor can be used to determine urea concentration. In addition, a hydrogel consisting of a SERS-sensitive pH reporter has been synthesised which contains polyelectrolyte multi-layer microcapsules, for example, silver NPs capped with 4-mercapopyridine and modified with bovine serum albumin. This material shows response in a wide range of pH between 6.5 and 9.7. This sensor will estimate concentration of urea 0, 0.1, 1 and 10 mM, if urease is incorporated in the hydrogel matrix [95]. Zinc Oxide micro

range particles were formulated using the quick microwave technique, and morphologies were designed through varying urea concentrations to improve its biosensing capabilities. This sensor appears to have been inventive in recognising concentrations of dopamine and uric acid in the urine of human, edible wheat flour and meat [96]. Here, the researcher had compared the two urea biosensors and found that nickel oxide based urea biosensor showed good sensitivity when compared to titanium oxide based urea biosensor. In both, urea sensor polyethylene terephthalate was applied as a substrate, on which paste of silver was printed by serigraph printing. The patterned substrate was used as a cluster of high-quality conductor and reference/standard electrode. In the first type of urea biosensor titanium oxide was used as a sensing element and in second type nickel oxide was used. The sensing matrices were obtained by radio frequency sputtering technique. Sensing matrices was covered by an insulating layer of epoxy resin. The graphene oxide was adsorb on insulating layer, and with the help of mixing of N-Ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride and magnetic beads, hydroxyl and carboxyl group were generated on it. The urease enzyme was covalently bonded with the functional group present on graphene oxide. The urease is act as an enzyme electrode. Both biosensors were incorporated with wireless and micros fluidic determine system. The principle of sensing was the catalysis and hydrolysis of urea by urease enzyme, resulting in production of different ions like hydroxide, ammonium and bicarbonate [97]. The NPs have a unique property of high surface to volume ratio. The poly (propylene imine) (PPI) dendrimer has gained a remarkable attention as a biosensor due to its large surface area and presence of different functional groups in its end. This nanosize dendrimer act as a mediator and used for the synthesis of electrochemical biosensors. The functional groups which were present at the end of dendrimer were used for immobilisation of biological particle. These dendrimer based biosensors detect the analyte by electrochemical technique such as amperometric, potentiometric, electrochemiluminescence and impedimetric. The DNA and enzyme biosensor were the commercial dendrimer based biosensor [98]. A new calibration circuit (NCC) has been developed to reduce the drift rate effect of the urea biosensor based on technique of voltage regulation. The structure of this NCC is simple and also suitable to use on different biosensors to reduce their non-ideal effect. The calibration of drift rate of NCC was tested with a ruthenium oxide (RuO_2) urea biosensor which was fabricated and measured by the system of voltage-time (V-T) measurement. The results showed that RuO_2 urea biosensor had a linearity of 0.999 and sensitivity of $1.860\text{ mV}/(\text{mg}/\text{dl})$ (average). Further, the NCC developed here decreased the drift rate of RuO_2 urea biosensor to $0.02\text{ mV}/\text{hr}$. Moreover, the drift rate measured by the NCC was 98.77% lower compared to that of conventional V-T measurement system. The calibration characteristics of the drift rate of the proposed new calibration circuit were tested using a RuO_2 based urea biosensor. This biosensor introduced a new calibration circuit aimed at reducing the urea biosensor's drift rate influence. The findings revealed a mean specificity of $01.860\text{ mV}/(\text{mg}/\text{dL})$ and a repetitiveness of 00.999 for the RuO_2 based urea biosensor [99].

4.8 | Quantum dot-based urea biosensor

The 0-D quantum dots are amongst the most popular and exciting nanomaterials. Quantum dot provides a large surface to volume ratio that makes them much more robust than organic molecules. High efficiency of transforming absorbed light into emitted light allows quantum dots to be more effective in sensing use. The absorption band of quantum dot varies from 10 to several 100 nm with a narrow spectrum of emissions. The optical properties are also extremely resistive to certain physico-chemical environments.

Here, a doped chitosan polymer based electrochemical urea biosensor has been developed and an innovative electrochemical sensor was designed which combines the imprinted technique with quantum dot NPs for detection of urea. At a standard potential, pre-treated gold electrode was immersed in the mixture of molecular imprinting based polymer cadmium sulphide quantum dot and urea. Potentiostat/galvanostat was used to measure the electrochemical activity of the quantum dot doped gold electrode. Due the zero dimensional structure of quantum dot, this sensor exhibited very excellent sensitivity [100]. An accurate fluorescence probe has been synthesised for the measurement of urea. Hydrophilic CuInS₂ quantum dot were coated with 3-mercaptopropionic acid by immersed aqueous solution technique. Then it was attached to dopamine to develop the dopamine-functionalised CuInS₂ quantum dot fluorescence probe. The fluorescence of the dopamine functionalised quantum dot was quenched by analyte urea in the concentration (0.2 to 6.0 mmol/L), in which the urease enzyme was used as a catalyst. The developed probe has been successfully implemented with satisfactory results for detecting urea well into the human serum specimen [101]. Researcher has illustrated the development of a fluorescent urea sensing scheme based on NPs. It is based on the observation that graphene quantum dots exhibit pH-sensitive green fluorescence when photo excited at 460 nm. Upon hydrolysis of urea by urease enzyme, fluorescence is slowly quenched as the local pH value increases. The analysis was used to evaluate urea at a concentration range of 0.1–100.0 mM, with a 0.01 mM maximum for detection. The method is simple, efficient and as such promises to be a resource for the sensing of blood urea [102]. For efficient estimation of urea, a fluorescence system was developed using pH-sensitive DAP that acts as a pH indicator and pH-stable MQDs that function as a reference fluorescent signal. This fluorescence ratiometric system showed good linearity between the fluorescence intensity of the ratiometric I568/I420. It exhibited good pH reversibility with a difference of pH 0.2, in the range of 3.8–6.0. This high sensitivity sensor detects urea concentrations in the 5.0 to 700.0 mM range [103].

4.9 | Nanocomposite based urea biosensor

New advances in nanotechnology and material science have allowed the development of bio nanocomposite with special chemical and physical properties. Along with these extraordinary physico-chemical properties, another benefit is that they can be conveniently processed in large quantities. In fact, they are ideally

adapted for sensing applications due to the presence of plentiful functional groups on their surface and strong biocompatibility. The operating traits of biosensors based on various zeolite/enzyme nanocomposites have been tested in comparison with those of totally urease-based biosensors. Note that different nanobiocomposite have been used by the authors in which Beta polymorph A (BEA) nanobiocomposite display better sensitivity to urea as compared with biosensors based on Linde type A (LTA) nanobiocomposite. Both zeolites were synthesised hydrothermally by gel solutions. The results showed 1.5% concentration of zeolites BEA in the bioselective elements. The sensitivity decrease of biosensors shows more stability in urea measurement in real field environmental samples [104]. A new electrochemical biosensor for urea has been prepared by immobilisation of urease on an improved platinum electrode. Amperometric co-polymerisation of the relative monomers in the existence of activated CNTs and a number of add-ons resulted in a nanocomposite film. These nanocomposite sheets on which urease are immobilised showed an extraordinary amperometric response with the scaling down of urea. The enzymatic nanostructures amperometric urea biosensor's response was extremely sensitive, over a broad linear scale and at smaller detection limitation [105]. A potentiometric biosensor of urea based on chitosan-iron oxide nanobiocomposite was constructed by immobilisation of urease enzyme and the structural purity and crystallinity of the iron oxide nanoparticle was confirmed by X-rays diffraction technique. The potentiometric output of ~42 mV per decade has been measured over the 0.1 to 80 mM broad exponential concentration range. The sensor demonstrated good sensitivity, flexibility, repeatability and efficacy performance due to a satisfactory stable biosensor output response of around 12 s [106]. Further, an amperometric modified graphene based urea biosensors has been developed. Graphene oxide was synthesised and graphene oxide sulphonation was achieved through covalent association between the graphene oxide carboxylic groups and the 2-ethane-amino sulfonic acid amine group. A nanocomposite was developed by electrochemical deposited the PANi on sulphonated graphene. Nanoelectrode was developed by deposition of the polyaniline-sulphonated-graphene nanocomposite on the indium tin oxide matrix. Then urease enzyme was immobilised on the electrode with the help of glutaraldehyde. Polyaniline-sulphonated-graphene based sensor can be used reliably as a biosensor of urea with a relatively good sensitivity ($0.850 \mu\text{A}\cdot\text{cm}^{-2}\cdot\text{mm}^{-1}$) with a quick response and improved stability [107]. An impedimetric polymeric zinc-oxide based disposable urea biosensor has been synthesised. An efficient zinc-oxide based transducer was developed on tin-oxide conducting glass. In the presence of ferricyanide, electron transport pathways were performed on the tin-oxide-zinc oxide-urease electrode. The proposed biosensor's impedimetric analysis indicated a fast response time of less than 10 s with a wide line arrangement of 8.0–110.0 mgdL⁻¹ and the detection limit for urea as 5.0 mgdL⁻¹ [108]. A dendrimer shaped PPy silver nanocomposite has been used to develop a urea biosensor. The nanocomposite dendrimer was characterised by XRD, EDAX, SEM, TEM and TGA. In addition NH⁴⁺ ion-selective electrode was also synthesised on a PVC

matrix membrane. NH_4^+ electrodes were synthesised to immobilise the urease enzyme [109]. In similar way an innovative amperometric urea biosensor has been developed. MWCNTs were synthesised with electric arc deposition method. A bionanocomposite film was developed by incorporated the Fc-PAMAM dendrimer with MWCNTs. And the biosensing of electrode was characterised by the voltammograms to study about the reusability and storage according to the temperature and pH. The bionanocomposite based urea biosensor had wide linear range of 0.2–1.8 mM, very fast response of 3.0 s, good detection limit of 0.05 mM, and excellent sensitivity $0.1085 \mu\text{A}/\text{cm}^2/\text{m}$ [110]. There are many studies on incorporation of CNTs in the manufacturing of biosensor and some of them are reviewed here. Based on the studies, another urea biosensor was developed by incorporating the CNT with poly-o-toluidine (PoT) similar. Due the conversion of the lower electro catalytic activity into high electro catalytic of PoT, there is quick transfer of electrons. This biosensor also preserved the catalytic function of the urease enzyme. This disposable biosensor had a reasonable detection period for urea in the range 0.1 to 11 mM with a detection limit of 0.03 mM [111]. A fast response time, stable and reportable urea biosensor has been developed by sonochemical method. Graphene oxide was synthesised using graphite as a precursor by hummer method. These graphene oxides were modified by zinc phthalocyanine by simple sonication and drop casting technique. Zinc phthalocyanine graphene oxide (ZnPh/GO) based nanocomposite urea biosensor had excellent electro catalytic property. Urease enzyme was immobilised by covalent bonding on Zinc phthalocyanine graphene oxide electrode for urea detection. The biosensor permitted a wide range of linear urea concentrations from 0.4 to 22 μM with a mere 0.034 μM detection limit [112]. A potentiometric urea biosensor based on $\text{Fe}_3\text{O}_4\text{-CH}$ nanobiocomposite was fabricated by immobilising the urease enzyme. The urea biosensor had a broad logarithmic urea concentration range of 0.1–80 mM with a 42 mV/decade sensitivity and a latency of about 12 s [113]. The urease enzyme has been extracted and further purified from *Bacillus sphearicus* MTCC 5100 for developed a urea biosensor. The pristine MWCNTs were grafted with carboxylation. The co-precipitation method was employed for the synthesis of iron oxide NPs. Glassy carbon electrode with a surface area of approximately 0.03 cm^2 is coated sequentially with alumina powder of different particle sizes (μm) to acquire the mirror as a clean surface. MWCNTs were grafted with carboxylic group. Then electro polymerisation method was adopted for adsorption of iron oxide nanoparticle on the surface of functionalised electrode. Nafion solution was dropped onto the surface of the cleaned glassy carbon electrode and kept dry for air. Use glutaraldehyde as just another cross linker, the extracted bacterial urease enzyme was immobilised onto the electropolymerised electrode. For detection, the urea in milk samples concentration range of 1.0–25.0 mM was achieved with a limit of detection of about 67 μM . The nanobiocomposite biosensor developed exhibited fast response and great stability. It was used to measure the urea in the milk samples [114]. A simple way has been developed to prepare a nanocomposite made up of MWCNT and poly(urethane-sulphide) grafted with urea, having strong mechanical strength,

durability and good self-healing capacity. The MWCNT and poly(urethane-sulphide) is used as a functional cross-linking agent to enhance mechanical properties and self-healing capacity of nanocomposite. It also absorbs near-infrared irradiation and converts it to local auto-healing thermal energy. The prepared nanocomposite can enhance more than 90% of the original strength efficiently. The tensile strength of the said nanocomposite is 3.58 MPa by modification of the cross-linking degree and the strain increases by 258%. The nanocomposite also has excellent water proofing, and can retain robust lap-shear property of 1.01 MPa after 6 h of water immersion. [115]. A real evidence-of-concept micro-droplet sensor, which can detect unknown amounts of urea in blood serum, has been established. Anhydrous solution of gold-cadmium sulphide composite NP coated with urease enzyme was synthesised. The analyte mixture comprising urea moieties was eventually combined with the suspension described above and distributed on the suitable substrate. The acoustics caused mechanical noise enabling the enzymatic lock-and-key reaction among analyte urea and enzyme urease through amplified mixing. The latest conceptual prototype could become a low-cost and viable solution for point-of-care detection [116]. A potentiometric nanocomposite urea biosensor has been developed. Highly ordered homogenous iron oxide NPs were synthesised by hydrothermal technique. Then hybrid nanocomposite was formed by mixing the chitosan solution with iron oxide NPs under magnetic stirring. The morphology and crystal nature of chitosan based iron oxide nanocomposite was characterised by scanning electrode microscopy, XRD technique. The urease enzyme was entrapped on iron oxide nanoparticle with the help of PPy by nanofabrication technique. This nanocomposite urea biosensor has a potential application in clinical diagnosis [117]. Another direct current voltage based unsophisticated graphene polymorphs sensor was fabricated. Here, the researcher synthesised an f-GNPlts by generating the carboxylic group on graphene nanoplate's surfaces. Then self-organisation process was employed to synthesise the nanocomposite (f-GNPlts/GNDs) of functionalized graphene nanodiamond and functionalised nanograpene. The amine group of urease enzyme and carboxylic group of f-GNPlts/GNDs nanocomposite were covalently bonded and resulted in formation of urease grafted graphene polymorphs biocomposite. The activity of urease enzymes on to the surface of the nanobiocomposite was confirmed by indothymol test. The ion produced due to the hydrolysis of urea which takes place at the surface of nanobiocomposite generates a current at 0 V. The current produced at zero voltage displayed a considerable relationship with the amount of urea in the sample [118]. In the authors experiment on application of biosensor here, microbial urease has been synthesised by purification, then characterisation was completed and after that it is immobilised on metal electrode. Purification of the enzyme was done progressively, and it was purified by salt precipitation and chromatography by ion exchange at first and then by gel filtration. Having specific activity 32.5 U/mg, the purification fold was reached 6.67. For the synthesis of biosensors, the isolated urease was immobilised on metal electrode with the aid of glutaraldehyde. The purified urease was also immobilised on metal electrode modified for

PANi for the development of biosensors. For operating reliability over 60 days, the immobilisation performance was calculated to be 97%. The storage durability was obtained for up to 60 days when placed in PBS buffer at 4°C [119].

A bio-inspired approach to nanoarchitectonics was developed for the preparation of optical probes by Lorente et al. This chemosensor depends on a nanodevice that combine i) subunit acting as enzymatic receptor, ii) signalling subunit of labelled-reporter (attached on silica surface) and iii) a communication method between the two sites for the generation of messengers chemically by the enzymatic subunit that induces the dissociation of the molecules from the surface of silica. A nanosensor of urea which depends on the release rate of oligonucleotide labelled with Alexa Fluor 647 from gold mesoporous NPs of silica was synthesised as proof of concept. Gold mesoporous NPs of silica are functionalised with amino groups on the face of the silica which is attached with oligonucleotide through electrostatic forces, while the urease enzyme is grafted with the gold face. This nanodevice is capable of releasing fluorescent oligonucleotide to ammonia through enzyme-mediated urea hydrolysis and further amino groups deprotonation on the face of silica. This nanodevice has been used to measure the urea in blood samples and to identify the adulterated milk [120]. A lifetime technique of fluorescence has been developed for the distinguishing wet arterial and venous blood from dry arterial and venous blood. The study has great potential to determine the true source of the detected blood, and even if it is arterial or venous blood, the developed technique could effectively support analysis of bloodstain patterns. The urea, platelets and fibrin were perfect indicators for the detection of arteriovenous blood which is saturated with oxygen. The imaging method that depends on the oxygen level of blood is not able to differentiate arteriovenous blood saturated with oxygen. In order to avoid this constraint, it is suggested here to apply the lifetime of eosin fluorescence to whole wet blood, which would differentiate between wet arterial and venous blood. The amount of urea in wet whole blood can be used as an indicator for further verification of wet arterial and venous blood depending on the eosin lifetime in the urease-urea response. The clots made in arteries mostly consist of platelets in dried blood while clots made in venous produce higher amount of fibrin. In addition, for distinguishing dried arterial and venous blood clots, different lifetime parameters of eosin like short and mean lifetime can be applied. In addition, lifetime fluorescence imaging microscopy could be a possible tool for examining blood-stain patterns in criminal cases [121]. Examination of flow injection was used to evaluate the urea and creatinine from urine of human. For the selectivity of the urea study, a minicolumn of urease combined with an on-line gas propagation module was used. It assesses the urea and creatinine without the sample being specially prepared. This approach demonstrated strong linearity accuracy and range of detection [122].

4.10 | Non-enzymatic-based urea biosensor

Nevertheless, enzymatic biosensors have drawbacks, because enzymes used are not only costly, but also have small lifetimes.

Non-enzymatic biosensors utilising nanosize bare and functionalised metals and metal oxides in the place of the enzymes were developed because of the high cost of biosensors. Apart from cost savings, nanosize bare and functionalised metals and metal oxides are strong candidates to be used in non-enzymatic biosensors because they are highly sensitive and stable. The electrochemical non-enzymatic urea sensor was developed using reduced graphene oxide probe doped with tin oxide quantum dot. XRD, XPS and TEM revealed well-ordered nano-porous structure of graphene oxide composite. The porous composition of the composite matrix provides good conductivity and therefore facilitates fast diffusion of the ionic particles from the solvent to the electrode, resulting in a fast response of 5 s and a volumetric sensitivity of 1.380 $\mu\text{A}/\text{fM}$ [123].

For the analysis of urea, a non-enzymatic graphene-PANi composite electrochemical sensor was designed. Polymer graphene composite was synthesised with surface grafting of graphene with PANi using cyclic voltammetry technique. Graphene grafting with PANi has been verified by Raman spectroscopy. Cyclic voltammetry analysed the electrochemical activity of urea at the composite surface while urea sensing was conducted via basic current-potential approach. With increased sensitivity, the designed sensor demonstrated lower detection limit, excellent reproducibility, specificity and durability. The durability of the as-made sensor has been effectively tested by using it to measure concentrations of urea in water supply and milk specimens. This composite-based urea sensor provides a simple, low-cost, non-enzymatic urea detection solution that finds various applications in clinical diagnostics, milk products, pesticide plants and pollution monitoring [124]. A non-enzymatic MWCNT composite based urea biosensor has been developed. Solvothermal approach has developed a composite of the nickel-trimesic acid and metal oxide. Also, an electrode was prepared by dispersing the mixture of MWCNT and hybrid of Ni and MOF on indium tin oxide glass substrate. Potentiostat/galvanostat was used to measure the electrochemical activity of the electrode. This non-enzymatic hybrid MWCNT based biosensor demonstrated excellent sensitivity about 685 $\mu\text{A}/\text{mM}/\text{cm}^2$. Urea biosensor exhibited long stability, due to the no losses of detection activity after the 1 month storage at ambient temperature [125]. An electrochemical non-enzymatic urea bio-sensor has been designed utilising silver doped SWCNT glass electrode. Non-enzymatic nanocomposite urea biosensor has been developed to remove the disadvantages such as high cost and poor stability of enzymatic biosensor. This electrochemical biosensor was developed by a single step thermal reduction method in which silver NPs were dropped on SWCNT. The nanosensor reported a good concentration limit of 4.70 nM, and excellent sensitivity of 141.0 $\mu\text{A}/\text{mM}^{-1}\text{cm}^{-2}$ towards urea detection in the limit of 66.0–20.6 mM. The nano sensor's durability was effectively tested by using the sensor to measure urea in the water from the tap and dairy milk specimen [126]. Moreover, for the detection of urea a non-enzymatic nanocomposite urea biosensor was developed. ZnO nanorods and nanoflakes were synthesised by the solution growth process and coated with silver by sputter deposition technique. Electrochemical activity

of nanobiocomposite was based on electro oxidation of urea which was analysed by CVs and CAs. The composite electrode may therefore be a prospective candidate for exposure of urea with no assistance of urease enzyme [127]. Urease enzyme-less-based urea biosensor has been investigated to determine the urea in real sample. An amperometric urea biosensor was developed by using glassy carbon electrode. Nickel oxide NPs were synthesised by using the extract of pod of *pisumsativum* by ultrasound assisted method. Nickel oxide NPs were immobilised on glassy carbon electrode. This green biosensor offered a good linear range 100–1200 μM urea with R^2 value of 0.991 and LOD of 8 μM [128]. The authors used the solvothermally developed CdV₂O₄-V₆O₁₃ micro flowers as an electro catalyst to illustrate non-enzymatic electro-chemical evaluation of urea. During the solvothermal process, the flower-like CdV₂O₄-V₆O₁₃ is constructed by assembling nano-dimensional petals, arranged across all directions that offer good substrate access to the substance for electro-chemical urea study. From the electrochemical studies, it was observed that the CdV₂O₄-V₆O₁₃ micro flower exhibits high electroactive contact area and displays easy detection of urea without any enzyme aid. It was also observed that the mass-transfer response undergoes a diffusion-mediated cycle in the presence of 20 μM urea, which has been found from the cyclic voltammograms obtained at different scan speeds [129]. Also, an electrochemical nanobelt based non-enzymatic urea biosensor has been fabricated for the evaluation of urea in biological fluid as well as in environment. Further, an innovative electrode decorated with the non-enzymatic urea sensor, which is an ultrathin Ni-MOF nanobelt, was evaluated. For the detection of urea, nano nickel metal acting as a proficient catalyst for the oxidation of urea is used. The prepared urea sensor shows superior sensing efficiency and also low detection limit, large detection range, reasonable ability to prevent interference, reproducibility of stability and high selectivity [130].

5 | CONCLUSION

The authors have provided a brief study on efficient urea biosensor synthesis and application. During the review, careful consideration was taken on all of the factors involved in fabricating a biosensor such as type of material/nano-material, matrix/substrate, enzymatic/non-enzymatic, immobilisation/binding process and transduction methods. The authors also presented some of the related works in the technology advancement in synthesis of urea biosensors with an emphasis on the modes of immobilisation techniques and the different types of transducer employed. For a simplified description, the comparative output characteristics of the specific urea biosensors were listed in Table 1.

The report emphasises the importance of the application of urea biosensors in nearly all fields such as clinical diagnosis, food and soil identification, heavy metal detection and environmental monitoring. The multiple urea biosensors identified

so far definitely have had many positive impacts in the analysis of urea in various recorded samples such as urine, sweat, serum, edible flour, food, beef, milk, coastal and mineral waters, insecticides, pesticides, synthetic fertiliser, medicinal and beauty products. The electrochemical has earned significant credit among several transduction mechanisms, due to its simplicity, low instrumentation expense, miniaturisation capacity and automation.

Moreover, the benefit of screen-printed electrodes emerged well into the 1990s, due to their durability, reproducibility, mass production and inexpensive added significantly to this prosperity.

Nonetheless, in most instances, improvements with a number of nanomaterials and artificial sensing components have been used with good results in order to boost their analytical functionality. For the synthesis of highly sensitive urea biosensor, nano-range materials like SWCNTs, MWCNTs, magnetic beads, bare and functionalised graphene, metal and metal oxide NPs (gold, zinc oxide, nickel oxide, titanium, iron, silver etc.), and, mediator nano-range particles like Prussian blue and cobalt phthalocyanine, were used. The health sector will certainly drive the overall development of biosensors with its requirements for point-of-care testing for advanced treatment, but environmental and food safety monitoring are the areas where people are becoming more aware of the potential risks involved. The latest nanotechnology developments can be better integrated with production of these existing biosensors, thus growing their diagnostic efficiency and practical applications.

Growing industrialisation and degradation of the atmosphere have adversely affected both life and ecology. The polluting nature directly deteriorates human health and, in particular, its vital organ such as the liver and kidney. Ideal biosensors still need to be established for effective identification of metabolite-causing disease. So, that renal related disease can be examined rapidly. The urea biosensors have the possibilities of having a potential market worth billions. There is therefore a need for technology that can effectively eradicate urea biosensor disadvantages and enhance its efficiency, accuracy, reliability, lifespan, scope of detection and cost-effectiveness.

The utilisation of nanotechnology and its modified structure showed great assurances well into the future for smart biodetection and the design of efficient and rapid bio-electronic technologies. We assume that the use of nano-dendrimer, quantum dot, nanowires, green and phyto-constituent based nanometallic particles and their oxide have already shown great catalytic activity which will provide the best foundation for the development of efficient, inexpensive and smart urea biosensor. In addition the urea biosensors can be miniaturised and developed to be eco-friendly wearable bio-sensors, which will be more efficient in serving the health care sector.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ORCID

Sarajeet Singh  <https://orcid.org/0000-0002-8394-9013>

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