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Urea biosensors: A comprehensive review

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Abstract

Present study is specially designed for the recent advances in biosensors to detect and quantify urea concentration. Urea (carbamide) is an organic compound made up of the carbonyl ($C{=}O$) functional group with two $-NH_2$ groups having chemical formula $CO(NH_2)_2$. In nature, urea is found everywhere as the result of various processes, and in the human body, urea is an end product of nitrogen metabolism. An excessive concentration of urea in the human body is responsible for different critical diseases such as indigestion, acidity, ulcers, cancer, malfunctioning of kidneys, renal failure, urinary tract obstruction, dehydration, shock, burns, gastrointestinal bleeding, and so on. Moreover, below the normal level may cause hepatic failure, nephritic syndrome, cachexia, and so on. As well as in various fields such as fishery, dairy, food preservation, agriculture, and so on, urea is normally found and its detection is necessary. In urea biosensors, enzyme urease (Urs) is used as a bioreceptor element and retains its long last activity is the critical issue in front of the researcher. During recent decades, different nanoparticles (zinc oxide, nickel oxide, iron oxide, titanium dioxide, tin(IV) oxide, etc.), conducting polymer (polyaniline, polypyrrole, etc.), conducting polymer–nanoparticles composites, carbon materials (carbon nanotubes, graphene oxide, reduced graphene oxide graphene), and so on are used in urea biosensors. The main emphasis of the present study is to provide cumulative and comprehensive information about the sensing parameters of urea

Abbreviations: Ag/NiOOH, silver nickel(II) hydroxide; AgNPs, silver nanoparticles; CNTs, carbon nanotubes; CNW, cellulose nanowhiskers; CPH, conducting polymer hydrogels; CPH/Urs, conducting polymer hydrogels/urease; C_S/Co_3O_4, chitosan/cobalt oxide; $CuInS_2$, chalcopyrite copper indium sulfide; EIS, electrolyte-insulator-semiconductor; Fc-PAMAM, ferrocene-poly(amidoamine); Fe_3O_4, iron oxide; FTO, fluorine doped tin oxide; GCE, glassy carbon electrode; GLDH, glutamate dehydrogenase; GO, graphene oxide; GSH, glutathione; HANCD, high-content nanocellulosedialdehyde; LbL, layer-by-layer; LOD, lowest detection limit; NC, nanocellulose; $NiCo_2O_4$, nickel cobalt oxide; Ni–MOF, nickel–metal organic framework; NiO, nickel oxide; Nr-NiO, nio nanorods; OCPs, organic conducting polymers; PAMAM, polyamidoamine; PANI, polyaniline; PANI/MWCNTs, polyaniline-multiwalled carbon nanotubes; p-DMAB, p-dimethylaminobenzaldehyde; PGMA, poly (glycidylmethacrylate); PnBA, nonplasticized poly (N-butyl acrylate); PPy, polypyrrole; PUB, photoelectrochemical urea biosensor; PVA/SbQ, poly(vinyl alcohol), N-methyl-4(4'-formylstyryl)pyridinium methosulfate acetal; PVdF-HFP, polyvinylidene fluoride-co-hexafluoropropylene; RF sputtering, radio frequency sputtering; RGO, reduced graphene oxide; SF, silk fibroin; SiO_2NPs, silica-gel nanospheres; SNARF-1-dextran, carboxy seminaphthorhodamine-1-dextran; SnO_2, tin(IV) oxide; SPCE, screen-printed carbon electrode; ssDNA, single-stranded DNA; TCFH, *N*,*N*,*N*′,*N*′-tetramethylchloroformamidinium hexafluorophosphate; TiO_2, titanium dioxide; Urs, urease; Urs/ZnONR/ITO, urease/zinc oxide nanorods/indium tin oxide; ZnO, zinc oxide; ZnO NRs, zinc oxide nanorods; ZnO-en/PANI-g-CHIT, zinc oxide encapsulated polyaniline-grafted chitosan; ZrO_2, zirconia.

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biosensors based on the materials used for enzyme immobilization. Besides this special task, this review provides a fruitful discussion on the basics of biosensors briefly for new and upcoming researchers. Thus, the present study may act as a gift for a large audience that come from different fields and are working in biosensors research.

KEYWORDS
biosensor classification, biosensors, carbon nanomaterials, conducting polymers, immobilization matrix, nanomaterials, urea biosensor

1 | INTRODUCTION

Major and high-risk diseases such as diabetes, cardiovascular diseases, heart disease, cancer, kidney and liver disorders, chronic respiratory diseases, and tuberculosis, and so on are originated in the human body due to an imbalance of blood substances. As a concern to maintain human health, it is necessary to monitor ingredients of blood such as urea, uric acid, glucose, cholesterol, and any other body serum contents.[1,2] Thus, with time being, it is necessary to develop such a device that can measure these components rapidly and accurately. In the early days, to measure the biomolecules, numerous conventional techniques were found to be used viz. chromatography, chemiluminometric, colorimetric, spectrophotometric, and so on. However, these techniques suffered from imprecision, sample pretreatment, on-site monitoring, long time consumption, expensive equipment, skilled operator, long time analysis, and so on.[3,4] In 1962, a new device was invented by Cleark and Lyons termed as glucose biosensor (oxygen elctrode) which overcomes the all above mentioned drawbacks.

A biosensor has excellent features viz. quick response, high sensitivity, realistic selectivity, handheld, cost effective, miniatures, and so on.[5–8] Due to these superior features, the applicability of biosensors increased in many fields such as environmental, clinical, agricultural, food, dairy, fishery, homeland security, military, and so on. To fulfill the requirement of different types of biosensors for diverse fields, undefined efforts are taken from the various research groups. Entirely, no doubt, the progress of biosensor technology comforts human life to protect the health and local ecosystems, still it leaves the challenges for the upcoming researcher to improve the sensor parameters.

A biosensor is an analytical device that explore the qualitative and quantitative information of the targeted analyte in the form of a recognizable signal using a suitable transducer that is modified with bioreceptors.[9] Biosensor commonly comprises main components viz. transducer, biorecognition or bioreceptor species, immobilization matrix, and results display unit. Different transducers were employed to fabricate the biosensors such as optical, electrochemical, thermometric, magnetic, piezoelectric, and so on. In bioreceptor species included enzymes, nucleic acids (DNA and RNA), microorganisms, whole cells, antibodies, cell receptors or biologically derived materials. The variety of immobilization matrices were exploited in biosensors such as polymers, sol–gels, conducting polymers, Langmuir–Blodgett films, nanomaterials, and self-assembled monolayers).[10–13] The general design of the biosensor system is visualized in Figure 1 and the following sections provide brief information on various components of the biosensor.

Urea is a toxic organic compound that exists everywhere in the nature. Its abnormal level in the human body creates many diseases. Therefore, the quantification of urea in various working fields such as the milk industry, fisheries, food industries, medicine, and so on is also necessary. The broad area of the research community has been working to develop a better device for urea detection. For upcoming researchers as well as curious readers, it is must to know the earlier development in urea biosensors. The main aim of this study is to provide clear and concise information about the efforts taken to detect analyte urea using biosensors. However, the special emphasis on the recent advances in urea biosensors is based on the enzyme immobilization materials over the last decades. Hence, the authors have presented here the comprehensive review on the development of urea biosensors.

2 | BIOSENSOR COMPONENTS

2.1 | Bioreceptor

Bioreceptor or biological recognition species is the heart of the biosensor system and its over long-time activity decides the sensing performance of the biosensor. Bioreceptor species connects over a matrix-modified physiochemical transducer by implementing different immobilization techniques. Possibly, various species such as enzymes, nucleic acids (DNA and RNA), aptamers, cells, and so on have been used as bioreceptor elements in the

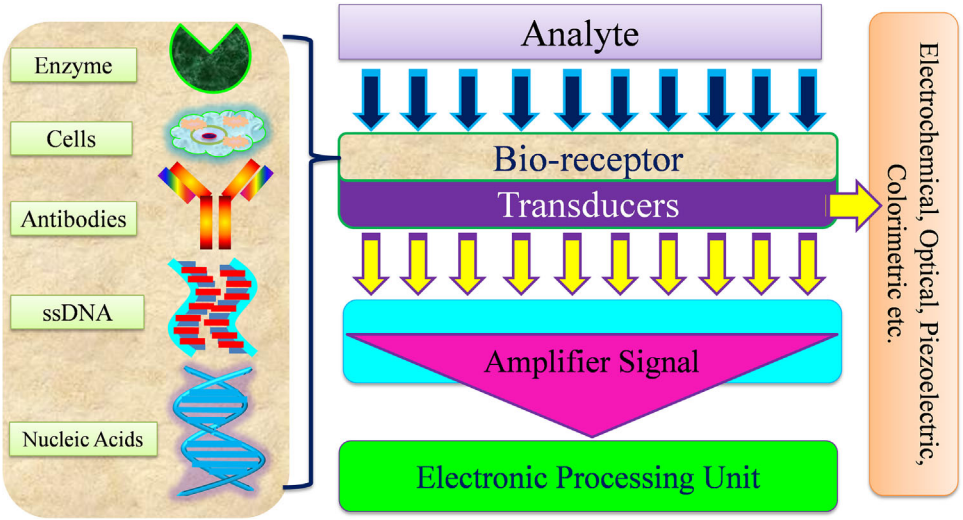

FIGURE 1 General design of biosensor

biosensor. The selection of these species is based on the affinity toward specific analytes. In the last few decades, the progress of enzyme-based biosensors increased exponentially. under tha catalytic porecess between enzyme and analyte origiantes the measurable end products (protons, electrons, light, heat and so on). The enzyme kinetics can be specifically explained by using the Michaelis–Menten equation and "lock and key" and "induced fit" hypotheses. The performance of enzyme-based biosensors highly depends on substrate concentration, temperature, pH, and presence of a competitive and noncompetitive inhibitor.[14,15] The use of antigen/antibody (Y-shaped immunoglobin) in biosensors is coined as immunosensors. The highly specific ability of the antibody to bind with the corresponding antigen provides the selective sensing response in immunosensors. Different transducers can be used in immunosensors owing to some drawbacks and superiority. In nucleic acids-based biosensors, the output response is produced by the hybridization of complementary ssDNA to form dsDNA by exposing the target analyte on the sensing probe. A special feature of this biosensor is the detection of a single molecule species in a complex mixture. The living cells (microorganisms such as bacteria and fungi) are used in cell-based biosensors. The cell-based biosensors detect the change in intracellular and extracellular microenvironment conditions and measured the change in physiological parameters through the interaction between stimuli and cell. The drawback of the cell-based biosensor is that it works in natural environmental conditions where the cell can stay alive for a long period. Thus, to work with a cell-based biosensor, it is necessary to control the physical and chemical parameters of the environment. Biomimetic biosensors or artificial sensors are those in which aptamers are used as the bioreceptor components. An aptamers-based biosensor has a few advantages over antibody-based biosensors in terms of high binding efficiency, no use of animals, less complex, and miniature. However, due to the properties of nucleic acids such as structural pleomorphic and chemical simplicity that reduces the assay efficiency and increases the production cost of aptamers-based biosensors.[16] The classification of the biosensor is shown in Figure 2 based on transducers and bioreceptor species.

2.2 | Transducer

The main function of a transducer is to convert the biochemical interaction between the bioreceptor and target analyte in the form of a measurable signal. A transducer is a very crucial part of biosensors and the basis of transducers biosensors can be classified as electrochemical, optical, piezoelectric (mass detection methods), and thermal.[17] An electrochemical biosensor measures the electric parameters produced during the biochemical interaction using an electrode. Further, electrochemical biosensors are categorized as conductometric, potentiometric, and amperometric based on the form of signal production.[18,19] Piezoelectric biosensors are fabricated by coating piezoelectric material on the transducer. In a piezoelectric biosensor, a transducer vibrates at a natural frequency and a certain value of current is generated by the controlled frequency as a response to an external electrical signal. Piezoelectric biosensors measure the frequency shifts that occurred by the interaction between analyte and sensing materials. Based on the frequency and small current change generated, the mass of the detected species can be measured.[20] The calorimetric transducers detect the temperature changes that occur during the biochemical reactions. The temperature change is related to the amount

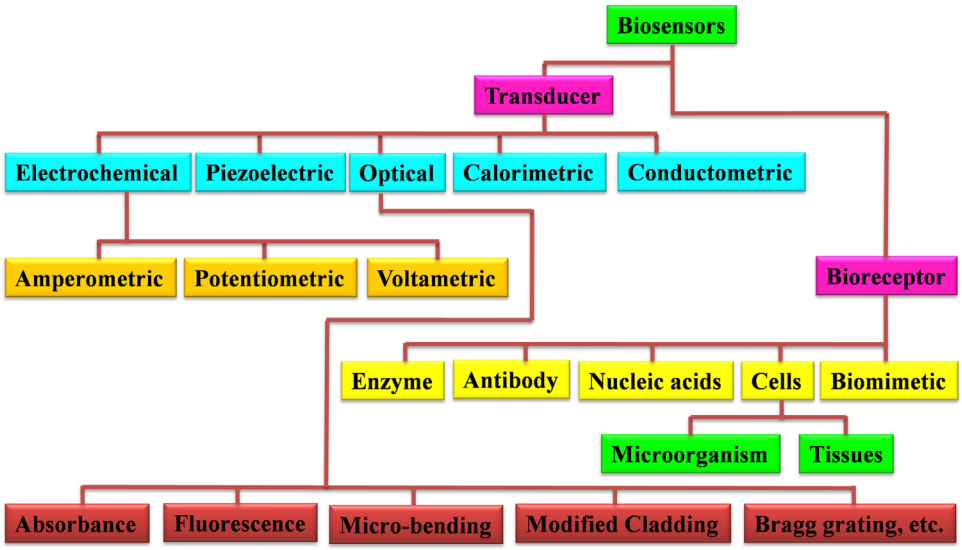

FIGURE 2 Classification of biosensor

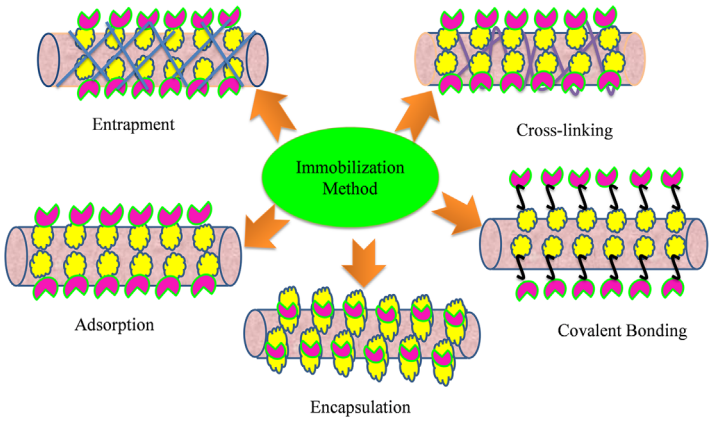

FIGURE 3 Immobilization techniques

of reactants consumed or end product formed. The calorimetric biosensors have the features such as improved sensitivity, high stability, and miniaturization.[21–23] In optical fiber biosensors, optical fiber itself works as a transducer. The optical fiber provides the sensing response in the form of a change in light intensity. Optical fiber biosensors can give the choice to use different types of spectroscopic techniques for the measurement of output signal such as fluorescence, absorbance, and chemiluminescence.[24]

2.3 | Methods of immobilizations

The sensing parameters of biosensors such as sensitivity, selectivity, good operational and storage stability, short response time, and high reproducibility are decided by the activeness of bioreceptor species on the transducer. These characteristic features can be achieved by adopting the judicious and appropriate immobilization technique. The proper immobilization techniques maintained the biological activity, physical structure, unstoppable function to react with the target species of bioreceptor, and provides a tight binding to the bioreceptor over the transducer surface and avoids desorption during the measurement. The selection of immobilization technique in the design of biosensors decides by the factors such as simplicity, cost, processing time, reproducibility, and so on. The nature of enzyme, transducer, and associated detection mode in biosensors decides the selection of immobilization technique.[25,26] Different immobilization strategies were available to immobilize the bioreceptor species viz. physical and chemical adsorption, covalent bonding, entrapment, cross-linking, affinity, and so on. Figure 3 pictures the immobilization techniques and table 1 elaborates[] he advantages and disadvantages of these techniques. Adsorption is the simplest immobilization method in which the transducer is kept in enzyme solution for a defined period and subsequently washing off any non-absorbed biomolecules. However, this technique suffers from biomolecules desorption from the support due to the

TABLE 1 Advantages and disadvantages of immobilization methods

Immobilization technique	Binding nature	Advantages	Disadvantages
Adsorption	Weak bonds	Simple, easy, and gentle treatment of biocatalyst, limited loss of enzyme activity	Desorption, nonspecific adsorption, responsible for external environment
Covalent binding	Functional groups of the enzyme and the supporting matrix	No diffusion barrier, stable short response time, low affection of adverse conditions	Nonregenerable matrix, use of toxic binding chemicals, loss of bioreceptor activity
Entrapment and encapsulation	Mixing of bioreceptor within an immobilization matrix	Inactive chemical reaction between the monomer and the bioreceptor, more than one bioreceptor can be immobilized within the same matrix	Diffusion barrier and enzyme leakage, only for small analyte detection, necessity of high concentrations of monomer and enzyme
Cross-linking	Bonding between cross-linker and bioreceptor	Simple, strong bonding between matrix and bioreceptor, highly adopted to adverse condition	Loss of enzyme activity and use of harsh treatment with toxic chemicals
Affinity	Bonding between a functional group on a support and affinity tag on a protein sequence	Controlled and oriented immobilization	Need of the presence of specific groups on enzyme (e.g., His, biotin)

weak binding and reversible nature of the binding.[27–29] In an entrapment method, bioreceptor is entrapped by three-dimensional matrices such as electro polymers, photopolymers, or silica gels. This technique is faced with a long response time and low stability due to no interaction between immobilization matrix and enzyme.[30,31] Cross-linking technique of immobilization is extensively found in biosensor fabrication. In this technique, two or more molecules are bound by a covalent bond via cross-linking agents such as glutarldehyde and carbodiimide. However, due to the use of toxic cross-linking agents in cross-linking technique decreases the activity of enzymes.[32–35] In affinity immobilization technique, specific groups of enzymes that may naturally present or genetically engineered at a specific location in the enzyme (e.g., biotin, carbohydrate, histidine) bind between a functional group of the support (e.g., avidin, lectin, metal chelates).[25,36]

3 | UREA BIOSENSORS

3.1 | Importance and sensing mechanism of urea biosensor

An organic compound urea (carbamide) is made up of the chemical composition of the carbamyl (C-O) functional group with two $-NH_2$ groups with chemical formula $CO(NH_2)_2$. Urea is generated in human body from protein breakdown as an end product of nitrogen metabolism. As the protein degrades in human body, toxic chemical specie ammonia (NH_3) is produced. The normal level of urea in body serum is from 15 to 40 mg/dl (2.5–7.5 mM/L) and above this permissible level causes many dangerous diseases. Excessive concentration of urea is responsible for the diseases such as indigestion, acidity, ulcers, cancer, malfunctioning of kidneys, renal failure, urinary tract obstruction, dehydration, shock, burns, gastrointestinal bleeding, and so on. Moreover, below the normal level may cause hepatic failure, nephritic syndrome, and cachexia.[37–39] In milk, the concentrations of urea in the typical range of 18–40 mg/dl exist as well as urea is used as an adulterant for milk.[40] In the milk industry, urea testing over a period is very necessary because it results in lower feed costs, greater milk-protein yield, increased reproductive performance, and minimal nitrogen excretion into the environment. In marine nitrogen, cycle urea plays a strategic role. In the pharmaceutical industry, urea is used as a component of many ointments; its level in these products must be strictly controlled. Urea levels in rivers or groundwater give evidence of contamination with sewage. The high content of urea is one of the reasons for algae blooming.[41] Annual world production of urea exceeds 100 million metric tons, the majority of which is used as fertilizer.

TABLE 2 Analytical properties of urea biosensors using different matrices

Transducer	Immobilization matrix	Immobilization technique	Linear range	Sensitivity	Response time (s)	Detection limit	Stability (days)	Ref.
Ratiometric	Carboxy seminaphthorhodamine-1-dextran	Covalent binding	0.01–10 mM in pure form and 3.1–7.8 M in serum				30	[90]
Optical	Lipid membranes	Entrapment	–		–	1 nM		[42]
Electrochemical	Zirconia	Entrapment	40 mg/dl	0.071 μA/mM/cm^2		0.5 mM	120	[41]
Potentiometric	Poly(O-phenylenediamine)	Entrapment	10/μM/mM			10 μM		[37]
Potentiometric	Zno nanowires	Physical adsorption	0.1–100 mM		4	0.1 mM	21	[91]
Electrochemical	Polycarbonate (PC) foils irradiated swift heavy ions	–	1×10^{-7}/M M		–	10 mM	–	[38]
Amperometric	Chitosan incorporating rhodium particles with modified AN copolymer	Cross-linking	1.6–8.2 mM	3.1927 A/mM/cm^2		0.5 mM	10	[92]
Electrochemical	Multilayered graphene	Covalent binding	10–100 mg/dl,	5.43 μA/mg/dl/cm^2	10	3.9 mg/dl,	42	[93]
Conductometric	Natural zeolite clinoptilolite	Cross-linking	0–64 mM,	20.36 μS/mM	45–70	1 μM	150	[39]
Conductometric	Zeolite beta with Si/Al ratio of 40 and 50	Cross-linking	1 mM		8		60	[94]
Potentiometric	Charged polysaccharides (carboxymethylpullulan and chitosan deposited on pani	Covalent binding	10^{-5}–10^{-3} M	55–125 mV/decade	120		21	[95]
Optical	Acrylic microspheres	Covalent bonding	0.01–1000 mM		600	0.01 mM	45	[96]
Amperometric	Rhodinized PAN membranes	Covalent binding	0.1–2.67 mM	1.85 A/mM/cm^2	15	50 μM	27	[97]
Optical	Silver, silicon	–	0–160 mM		–	–	–	[98]
Electrochemical	Zinc oxide (Zno) and multiwalled carbon nanotubes (Mwcnts)	Physical adsorption	0.8–16.6 mM	43.02 A/mM/cm^2	4	0.23 mM	28	[73]
Electrochemical	Nio	Physical adsorption	0.83–16.65 mM	21.33 mA/mM/cm^2	5	–	140	[51]
Potentiometric	Poly(glycidylmethacrylate) (PGMA)-grafted iron oxide nanoparticles	Covalent binding	0.25–5.0 mM		8		60	[63]
Piezoelectric	Single-enzyme nanoparticles	Encapsulation	0.08 μM to 1 mM		12	0.05 μM	70	[75]

(Continues)

TABLE 2 (Continued)

Transducer	Immobilization matrix	Immobilization technique	Linear range	Sensitivity	Response time (s)	Detection limit	Stability (days)	Ref.
Potentiometric	Fullerene	Physical adsorption	2.31×10^{-3} to 8.28×10^{-5} M	59.67 ± 0.91 mV/decade	120	–	140	71
Amperometric, Impedimetric	Polypyrrole	Nonenzyme	80–1440 μM	1.11 μA/μM/cm^2	1	40 μM	–	84
Electrochemical	Zinc oxide nanorods	Physical adsorption	0.001–24.0 mM	41.64 μA/mM/cm^2	–	10 μM	140	55
Reflectometric	Silica-gel nanoparticles	Cross-linking	50–500 mM		54	10 mM	55	52
Potentiometric	Zeolites	Physical adsorption	0–1 Mm		–	55 μM	–	2
Electrochemical	Nio nanorods	Physical adsorption	0.83–16.65 Mm	48.0 μA/mM	–	–	140	54
Coloumbometric	Polypyrrole	Physical entrapment	5.79 μC/μM					48
Electrochemical	Zirconia-poly(propylene imine) dendrimer	Cross-linking	0.01–2.99 mM	3.89 μA/mM/cm$^{2.}$	4	–	–	64
Capacitive	Polyamidoamine (Pamam) dendrimer and carbon nanotubes (Cnts)	Physical adsorption	0.1–100 mM	18 mV/decade	–	–	–	72
Optical	Dichlorofluorescein octadecyl ester (Dcfoe)	Cross-linking	10^{-2}–0.1 M		30		6	99
Impedimetric	Zno-PVA	Covalent binding	5-125 mg/dl		3	3.0 mg/dl	60	65
Amperometric	PANI-Mwcnts	Physical adsorption And electrochemical entrapment	10^{-2}–10^{-5} M	12×10^{-5} A/mM/cm^2	50	0.04 mM	15	67
Fluorescence	Dopamine-functionalized $cuins_2$ Qds	–	0.2–6 mmol/l		–	0.1 mmol/L	–	56
Potentiometric	Polyaniline/chitosan-carboxymethylpullulan	Physical adsorption	0.01–100 mM	32.8 mV/decade	–	–	> 21	49
Potentiometric	PVA/Sbq photopolymer	Entrapment	0.5–15 mM		60–120	–	150	50
Potentiometric	Chitosan/cobalt oxide (CS/Co3O4	Physical adsorption	19×10^{-4}–89×10^{-2} M	45 mV/decade	12	–	–	57
Colorometric	Polydopamine	Entrapment	1×10^{-7} to 1×10^{-3} M		3600	100 nM	–	58

(Continues)

TABLE 2 (Continued)

Transducer	Immobilization matrix	Immobilization technique	Linear range	Sensitivity	Response time (s)	Detection limit	Stability (days)	Ref.
Optical	Nileblue (NB) chromoionophore-doped kappa-carrageenan (KC)	Physical adsorption	0.001–100 mM		60	0.001 mM	14	100
Impedimetric	Zno–PVA	Electrostatic	8.0–110.0 mg/dl		10	5.0 mg/dl	21	59
Electrochemical	Sulfonated graphene/polyaniline	Cross-linking	0.12–12.3 mM	0.85 μA/cm^2/mM	5	0.050 mM	15	68
Optoelectronic (Photometric)	Prussian blue (Pb)	Carbodiimide method	1–8 mM	6.8 $\pm$ 1.1 mV/mM	–	0.6 mM	–	101
Electrochemical	Polyaniline grafted conducting hydrogel	Covalent binding	1.5–1000 μM	878 μA/mM/cm^2		60 nM	60	47
Colorimetric	Opal photonic crystal polymer (Iopp) films	Entrapment	1–9 mM		–	–	–	76
Electrochemical	Nickel-metal organic framework and multiwalled carbon nanotubes	Nonenzyme	–	685 μA/mM/cm^2	10	3 μM	30	89
Amperometric	Ferrocene-poly(amidoamine) and multiwalled carbon nanotubes	Physical adsorption	0.2–1.8 mM	1.085 μA/cm^2/mM)	3	0.05 mM	–	74
Electrochemical	Silk fibroin scaffold	Cross-linking	0.3–2.7 mM	112.3 A/mM/cm	–	0.163 mM	–	79
Potentiometric	Poly(3-hexylthiophene-co-3-thiopheneacetic acid) (P(3HT-Co-3TAA))	Covalent binding	0–6.94 mM		360	–	–	102
Electrochemical	Pvdf-HFP/Ni-Co nanofiber	Nonenzyme	20 μM to 2 mM		5	12 μM	30	85
Electrochemical	Nickel cobalt oxide (Nico2o4) nanoneedles	Nonenzyme	0.01–5 mM		–	1.0 μM	–	86
Electrochemical	Nickel–metal–organic framework (Ni-MOF) nanobelts	Nonenzyme	0.01–7.0 mM	118.77 μA/mM/cm^2	–	2.23 μM	30	87
Electrochemical	Ag/NiOOH nanorods	Nonenzyme	0.2–26.0 mM	233.7 μA/mM/cm^2	3	5.0 μM	–	88
Electrochemical	Graphene nanoplatelet/graphitized nanodiamond	Cross-linking		806.3 μA (mg/ml)/cm^2	20	5 μg/ml	–	61
Electrochemical	Fe3O4/MWCNT/PANI-Nafion	Cross-linking	1.0–25.0 mM			67 μM	60	69
Electrochemical	ZnO-en/PANI-g-CHIT	Cross-linking	20–500 ppm	187.5 μV/ppm/cm^2	180	29.84 ppm	–	66
Electrochemical	Ni nanotube	Nonenzyme	1–20,000 μM	902.8 μA/mM/cm^2	–	3 μM,	–	103
Electrochemical	AgNP	Nonenzyme	1–8 mM	9.212 μA/mM	–	0.14 mM	–	62
Optical Fiber	zeolitic imidazolate framework (ZIF-8/Urs)	Encapsulation	1–10 mM	0.8 mM/RIU		0.1 mM	–	104

Excessive nitrogen fertilizer applications can lead to pest problems by increasing the birth rate, longevity, and overall fitness of certain pests. Urea can be responsible for the reduction of the soil pH. High urea content in the milk occurs from the unbalanced feeding of cows, which influences milk production and fertility.[42] Normally in milk, urea constitutes about 55% of the total nonprotein nitrogen. However, milk is a daily need of human beings as a high protein food. Due to the increasing demand, adulteration of milk by urea has been very common to enhance its heat stability and increase the amount of milk. Thus, it is necessary to determine the urea concentration in milk to check the purity of milk.[43,44] Thus, the quantitative and qualitative detection of urea is of great interest and an utmost need in the fields of pharmaceutical, food industry, environmental protection, fertilizers, dairy, and fishery industries.

The maximum existed urea biosensors were used the enzyme urease (Urs) (EC 3.5.1.5) as the bioreceptor. Enzyme Urs is a highly efficient catalyst for the hydrolysis of urea with a rate of approximately 10^{14} times the rate of the noncatalyzed reaction. Enzyme Urs catalyzes the hydrolysis of urea to ammonium and bicarbonate as an end product in the form of the following reaction.

$$NH_2CONH_2 + 3H_2O \overset{\text{urease}}{\rightarrow} 2NH_4 + OH^- + HCO_3^-$$

As the reaction takes place on the transducer surface, the microenvironment of the biosensor system changes in the form of pH, ammonium ions, and so on, which gives the change in the output signal. The output signal may change based on the used transducer for specific biosensor fabrication. The different transducers obtained the sensing response by measuring different parameters such as an amperometric (change in current), potentiometric (change in voltage), optical fiber (change in refractive index), colorimetric (change in color), and so on.

4 | DEVELOPMENT OF UREA BIOSENSOR BASED ON IMMOBILIZATION MATRIX

The use of appropriate immobilization technique in biosensor provides the enzyme stability, improved activity, specific selectivity, resistance to inhibitors, and, in certain cases, it purifies the enzymes. Surface for immobilization the bioreceptor should have the features such as high ability to interact with enzymes (without significantly changing its activity), chemical and mechanical stability/resistance, hydrophobic or hydrophilic surfaces, defined porous morphology, the possibility to medium-long term storage, and low costs.[45,46] From the last decades, various synthesized materials were used for the immobilization of enzyme Urs to fabricate the urea biosensor.

4.1 | Urea biosensors based on organic conducting polymers

Organic conducting polymers (OCPs) become the most promising and efficient matrix material for enzyme immobilization. The applicability of OCPs is found in different fields due to their ability to convert reversible interchange between redox states. The class of OCPs is very sensitive to the chemical potential of the microenvironment, thus it acts as both immobilization matrices as well as the physicochemical transducer to convert the chemical signal into an electrical signal.[2] Some of the recent urea biosensors using the OCPs are summarized as follows.

Conducting polymer hydrogels (CPH) are prepared by electro polymerized aniline in the nonconducting hydrogels of polyacrylamide and polyvinyl alcohol (PVA). An electrochemically synthesized CPH was further employed for immobilization of the enzyme Urs. The developed CPH/Urs working electrode measured the urea concentration by electrochemical techniques. In this method, the change in partial capacitive behavior of the bioelectrode in the frequency range of 10^2–10^3 Hz was analyzed by a Bode plot. The detection range of a developed sensor is 1.5–1000 μM with a low detection limit (LOD) of 60 nM and sensitivity of 878 μA/mM/cm^2 using differential pulse voltammetry measurement. The scan rate and pH of the electrolyte were optimized at the time of measurement. The developed sensor can be applied for the detection of urea in milk, puffed rice, soil, and human blood owing to the features such as the low detection limit, high sensitivity, low K_m value, easy preparation method, low cost, sample preparation, reproducibility, and high stability and selectivity.[47]

In another study, enzyme Urs containing polypyrrole (PPy) films, PPy–Urs–chloride (PPy–Urs–Cl), and PPy–Urs–sulfonated cyclodextrin (PPy–Urs–SCD) were fabricated for quantitative analysis of urea. The coulomb metric results show the PPy–Urs–SCD film has a superior sensitivity of 5.79 μC/μM compared with 0.76 μC/μM for the PPy–Urs–Cl polymer film.[48]

An electrodeposited polyaniline film has been prepared and employed to develop of potentiometric urea biosensor. Two kinds of assemblies were done: the first one consisted enzyme Urs adsorption onto a polyaniline film followed by the adsorption of a chitosan-carboxymethylpullulan multilayer film, whereas the second one consisted of the adsorption of an enzyme Urs–chitosan multilayer film. The electrochemical response of resulting sensors was fast and sensitive for both types of assemblies, but the stability

was much better for the films obtained from alternative adsorption of Urs and chitosan onto a layer of enzyme Urs adsorbed over electrodeposited polyaniline.[49]

PVA/SbQ photopolymer entrapped enzyme Urs potentiometric biosensor was for urea determination based on two identical pH-sensitive field-effect transistors. The parameters of the biosensor were studied for urea concentration in the linear range 0.5–15 mM and operational detection range 0.5–40 mM. The biosensor shows the ∼1–2 min response time with 5-month stability.

Further, the biosensor is used to analyze the samples with different concentrations of urea for hemodialysis control.[50]

4.2 | Urea biosensors based on nanomaterials

Recently, nanotechnology explored the big opportunity to utilize the various types of nanomaterials for different applications. The interest in nanomaterials increased in biosensor device due to the features of nanomaterials such as an optimum surface-to-volume ratio, a high catalytic efficiency, and an ability to absorb biomolecules as well as high electro catalytic properties, oxygen ion conductivity, biocompatibility, nontoxicity, high chemical stability, and high electron transfer. Moreover, it becomes the most promising and efficient candidate for bioreceptor immobilization as a matrix due to the flexibility in obtaining the desired properties.[51] Some of the recently developed urea biosensors by using nanomaterials are described briefly as follows.

An optical urea biosensor has been developed by silica-gel nanospheres (SiO_2NPs), which are synthesized by sol–gel chemistry using a modified Stober method. The SiO_2NPs surfaces were modified with amine (-NH_2) functional groups and the pH-sensitive chromoionophore ETH 5294 for enzyme Urs immobilization in the presence of glutaric acid cross-linker over a transducer. Enzymatic hydrolysis of urea in the presence of immobilized enzyme Urs quantifies the urea concentration by reflectometric technique. The linear response range of urea was found to be 50–500 mM ($R_2 = 0.96$) with a 10 mM detection limit. The changes in pH value were correlated with the urea concentrations. The developed sensor shows high selectivity and reproducibility toward urea with ∼9 min response time. The developed sensor was used for the analysis of urea in urine samples. Also, it showed a good correlation ($R_2 = 0.996$ and regression slope of 1.0307) with a p-dimethylaminobenzaldehyde (p-DMAB) reagent used nonenzymatic sensor.[52]

Multianlaytes (glucose, urea, and glutamate) in serum samples were simultaneously determined by a novel optical array-based titanium dioxide (TiO_2) biosensor. In sensor development, carboxy seminaphthorhodamine-1-dextran (SNARF-1-dextran) as a single-molecule fluorescent probe was used. Furthermore, it was coimmobilized with enzyme Urs, glucose dehydrogenase, and glutamate dehydrogenase (GLDH) for simultaneous determination of urea, glucose, and glutamate. The sensor detects the concentration range 2.4–5.5 μM in water and 3.1–7.8 M in serum samples.[12]

Biocompatible zirconia (ZrO_2) film was electrochemically deposited over a gold-coated glass electrode with covalent attachment of enzyme Urs and GLDH for urea detection. The bioelectrode shows linear response up to 40 mg/dl for urea with 0.071 μA/(mM/cm^2) sensitivity. The sensor reveals 4 months stable response for urea when stored at 4°C. The activity of enzyme over ZrO_2 biocompatible matrix determined by a low value of Michaelis–Menten constant (K_m) estimated using Hans plot as 0.5 mM.[41]

Zinc oxide nanorods (ZnO NRs) were grown onto an ITO surface with an RF-sputtered ZnO seed layer. Enzyme Urs physically binds with a modified electrode, which was further used for the estimation of urea. Amperometric result shows the sensitivity of 0.9 μA/mM, with 1–11 mM linearity. LOD of the developed sensor for urea was found as 0.12 mM and a K_m value of 5.01 mM. When the same sensor (Urs/ZnONR/ITO) studied via electrochemical measurement reveals the quasi-reversible behavior. Two weeks' stability validates by the bioelectrode using clinical samples.[53]

Enzyme Urs immobilized over indium tin oxide (ITO) film, which was modified with NiO nanoparticles to study the urea concentration determination. Electrochemical and spectroscopic techniques are utilized here to analyze the sensor performance. The sensor shows high sensitivity (21.3 μA/(mM cm^2)) and a good linearity (0.83–16.65 Mm) toward urea with 5-s response time. The own redox couples (Ni^{+2}/Ni^{+3}) of NiO-NPs enhanced the enzyme Urs immobilization, which was understood by the low value of the Michaelis–Menten constant ($K_m = 0.34$ mM).[51]

In another study,[54] vertically aligned cone-shaped NiO nanorods (Nr-NiO) was synthesized under glancing angle deposition configuration in radio frequency (RF) sputtering. Further, as synthesized nanorods deposited over ITO-coated glass (Nr-NiO/ITO/Glass) was used for enzyme Urs immobilization. The developed bioelectrode (Ur/Nr-NiO/ITO/ glass) shows excellent response toward urea with high sensitivity of about 48.0 μA/(mM) and a good linearity over a wide range (0.83–16.65 Mm). The sensor shows long shelf life of about 20 weeks and a low value of the Michaelis–Menten constant ($K_m = 0.47$ mM). Hence, the authors concluded that the Nr-NiO is an efficient matrix for the realization of a reagent less urea biosensor

owing to the high electrocatalytic activity along with the redox behavior.

Ahmad et al.[55] prepared the ZnO NRs via a low-temperature solution route and grown vertically aligned directly on Ag sputtered glass substrate. The bioelectrode (glass/Ag/ZnO NRs/Urs) shows selective response toward urea in the wide linear range 0.001–24.0 mM with the sensitivity of 41.64 μA/mM cm^2. Sensor detects 10 μM LOD with Michaelis–Menten constant $K_{mapp} = 0.328$ mM.

A fluorescence probe, using dopamine-functionalized chalcopyrite copper indium sulfide ($CuInS_2$) quantum dots (QDs) was developed for selective detection of urea. The developed probe was used to detect the urea concentration ranging from 0.2 to 6 mmol/L.[56]

Physically adsorbed enzyme Urs over chitosan/cobalt oxide (CS/Co_3O_4) nanocomposite was used to fabricate a potentiometric biosensor on glass filter paper. Fabricated biosensor shows good sensitivity between a range of 1×10^4 and 8×10^2 M, linear slope curve of 45 mV/decade, and response time as ~12 s.[57]

The polydopamine nanoparticles have been used to fabricate urea biosensors with dynamic sensing features. The detection range of urea found to be 1×10^7 to 1×10^3 M with LOD of 100 nM in the linear range 5×10^6 to 1×10^4 M under optimal reaction conditions.[58]

A semiconductor-based urea biosensor was reported using the F-doped SnO_2 conducting glass fluorine-doped tin oxide (FTO). The film was deposited with ZnO nanoparticles and enzyme Urs electrostatically immobilize over the film. Developed FTO/ZnO/Urs biosensor shows a high sensitivity for urea detection within the range of 8–110 mg/dl with a 5 mg/dl detection limit.[59]

Nien et al.[60] used the RF system and screen-printing technology to fabricate urea biosensors. The sensor was fabricated based on urease-maghemite nanoparticles (γ-Fe_2O_3 NPs)/graphene oxide (GO)/nickel oxide (NiO) sensing membrane on silver conductive wire printed polyethylene terephthalate substrate. The sensing characteristics of developed were sensor studied by V–T measurement system. The sensor shows the sensitivity = 52.36 mV/decade with 0.996 μM linearity and 7.91 μM LOD. The sensor reveals a stable response toward urea for 29 days.[60]

A new mediator-free, sophisticated, and direct current–voltage (I–V) based sensor was developed for the detection of urea. The sensor was developed by nanocomposites made up of enzyme Urs immobilized graphene nanoplatelets and graphitized nanodiamonds. The performance of the sensor analyzed by the sensitivity 806.3 μA (mg/ml) $^{-1}$/cm^2) and the limit of detection of 5 μg/ml with 20 s response time. The authors claimed that the proposed strategy is helpful for the enhancement of sensor performance with high enzyme loading capacity.[61]

A commercially available glucose test strip was used for urea detection by modifying it with silver nanoparticles (AgNPs) via electrochemistry. The evaluation of the prepared strip shows the sensing response for urea concentration in the linear range is 1–8 mM with 0.14 mM LOD.[62]

4.3 | Urea biosensors based on polymer–nanoparticles composite

Au electrode was modified and developed with poly (glycidylmethacrylate) (PGMA)-grafted iron oxide nanoparticles via covalently attached Urs. The developed potentiometric bioelectrode responds to urea concentration in the linear range of 0.25–5.0 mM with a response time of ~8 s. The sensor shows stable response over 2 months. The study analyzed the optimum pH value of 7.5 at which the sensor shows the maximum potential difference. Increasing the temperatures between 20and 45°C increases the rate of the reaction, which results in the response increases. After this temperature range, sensor decreases its sensitivity due to the denaturation of the enzyme by thermal effect.[63]

ZrO_2 nanoparticles were dispersed in PPI solution and electro-co-deposited by cyclic voltammetry onto a screen-printed carbon electrode (SPCE) surface. The prepared SPCE electrode was further immobilized by enzyme Urs and employed for urea detection using electrochemical technique. The biosensor parameters were measured by an amperometric method such as the sensitivity of 3.89 μA/mM/cm^2 in a linear range from 0.01 to 2.99 mM with a response time of 4 s.[64]

The F-doped SnO_2 conducting glass (FTO) electrochemically modified platform with PVA tuned ZnO nanoparticles. The modified platform was further immobilized by enzyme Urs via covalent linking. The developed platform studied urea detection using an impedimetric technique. Results of the fabricated disposable biosensor exhibit high sensitivity toward urea in the range of 5.0–125.0 and 3.0 mg/dl LOD.[65]

ZnO-en/PANI-g-CHIT biocompatible ternary hybrid matrix was synthesized by *in situ* polymerization technique and used to modify ITO film for enzyme immobilization. The modified film shows improved electrical conductivity, chemical stability, and self-activation. The sensor response toward urea was studied by the potentiometric technique. The sensor exhibits a sensitivity of 187.5 μV/ppm/cm^2 in the range of 20–500 ppm. The response and recovery time for detection shows 3 min and 30 s respectively with 29.84 ppm LOD.[66]

4.4 | Urea biosensors based on the polymer–carbon material composite

Due to the excellent properties of the carbon materials viz. significant mechanical strength, high surface area, high electrical conductivity, and good stability promote the promising platform for enzyme immobilization. Thus, polymer–carbon material nanocomposites were found to be used extremely in biosensors for enzyme immobilization. The carbon materials such as CNTs, graphene and graphene oxide, and so on used in urea biosensors are described below.

An electrochemical urea biosensor was developed by using the polyaniline-multiwalled carbon nanotubes (PANI/MWCNTs). An electro-polymerization method was adapted for the deposition of PANI/MWCNTs over an electrochemical urea biosensor. An amperometric measurement of biosensors reveals the sensitivity $(I_{max}/K_m) = 12 \times 10^{-5}$ A/mM/cm^2 in a linear range from 10^{-2} to 10^{-5} M. The detection limit of the sensor was found as 0.04 mM with 2.02 mM Michaelis–Menten constant.[67]

Electrochemical urea biosensor was developed by enzyme Urs immobilized over sulfonated graphene/polyaniline nanocomposite film. The film shows impressive performance for urea detection in the broad linear range of 0.12–12.3 mM with a sensitivity of 0.85 μA/cm^2/mM^{-1}. The sensor responds toward 0.05 mM urea concentration as a LOD and 5 s response time with 15 days when stored at 4°C.[68]

Glassy carbon electrode (GCE) was electrochemically modified with Fe_3O_4/MWCNT/PANI-Nafion nanocomposite and used for urea detection in milk samples. The cross-linking technique is used to immobilize enzymes with help of glutarldehyde over the modified film. A good linear range of 1.0–25.0 mM with a detection limit of 67 μM with storage stability of 60 days was measured by electrochemical techniques and chronoamperometry.[69]

4.5 | Urea biosensors based on the nanoparticles–carbon material composite

ZnO-multiwalled carbon nanotube (MWCNT) nanocomposite was prepared by sol–gel technique and used to fabricate potentiometric urea sensor. The developed sensor reveals the sensing response for the concentration range of 0–200 ppm by varying temperatures of 0–300°C.[70]

A novel screen-printed electrode containing a nonplasticized poly (n-butyl acrylate) (PnBA) membrane entrapped with a hydrogen ionophore was modified with fullerene-immobilized enzyme Urs (C60–Urs) bioconjugate. Fabricated potentiometric urea biosensor shows the sensitivity of 59.67 ± 0.91 mV/decade in the range from 2.31×10^{-3} to 8.28×10^{-5} M at pH (7.0). A highly selective urea sensor shows stable response up to 140 days. The good adhesion and low leaching of enzyme Urs by fullerene–Urs bioconjugate and an acrylic membrane show higher stability.[71]

Report[72] discussed the benefits of a polyamidoamine (PAMAM), dendrimer, and carbon nanotubes (CNTs) layer-by-layer (LbL) nanofilm in electrolyte-insulator-semiconductor (EIS) field-effect sensor for urea detection. Three different sensor arrangement are studied viz. a bare EIS directly immobilized Urs [EIS–Urs], LbL film over the EIS [EIS–(PAMAM/CNT)–Urs], and Urs sandwiched between the LbL film and another CNT layer [EIS–(PAMAM/CNT)–Urs–CNT]. The dynamic constant-capacitance measurement was carried out to detect urea concentrations range from 0.1 to 100 mM. The EIS–Urs and EIS–(PAMAM/CNT)–Urs films show similar sensitivity (~18 mV/decade) with nonregular signal behavior as the increased urea concentration. Superior output signal obtained by EIS–(PAMAM/CNT)–Urs–CNT sensor with sensitivity about 33 mV/decade.

A hybrid ZnO/MWCNTs nanocomposite was deposited over ITO-coated glass slides (ITO/Glass) by the chemical route deposition technique. Further, enzyme Urs (Urs) immobilized over ZnO–MWCNT/ITO electrode by physical immobilization method. Sensing response of the developed sensor was analyzed by electrochemical techniques. The (Urs/ZnO–MWCNT/ITO) bioelectrode reveals a long shelf-life of more than 4 months with high sensitivity of about 43.02 mA/mM/cm^2. The low Michaelis– Menten parameter (K_m) value 0.85 mM sensor exhibits the high affinity toward urea of the immobilized enzyme Urs on the surface of the hybrid nanocomposite matrix.[73]

Ferrocene-poly(amidoamine) (Fc-PAMAM) dendrimers incorporated with MWCNTs-modified electrochemical urea biosensor was fabricated. The developed sensor was successfully applied to detect the urea concentration for blood samples of healthy humans. Under optimum temperature and pH condition sensor shows sensitivity toward urea 1.085 μA/cm^2/mM in a wide linear range of 0.2–1.8 mM. The detection limit of 0.05 mM and fast response of 3 s shows the excellent performance of the developed sensor.[74]

4.6 | Urea biosensors based on miscellaneous matrices and sensing methods

A novel piezoelectric biosensor was developed using the single-enzyme nanoparticles immobilized onto nanoporous alumina membranes prepared by electrical anodization. The presented biosensor detects the urea concentration of 0.08/M^{-1} mM with a short response

time of 12 s with a lower limit of detection of 0.05 M. The developed sensor also confirms its feasibility in the clinical analysis.[75]

A novel label-free urea sensor has been successfully fabricated by adopting a special enzyme catalytic reaction and the unique optical property of the pH-sensitive IOPP film. The sensor shows the visual response toward the urea in the form of change in different colors for different concentration ranges of urea in the different environmental systems. The sensor shows high stability, reusability, and selectivity toward urea when it was compared with inference species. The authors believed that the detection of urea with this methodology will provide a useful way for clinical and environmental systems.[76]

Enzyme Urs covalently attached over glutathione (GSH) modified gold microelectrode via glutarldehyde as a coupling agent. The H-NMR analysis highlights the interaction between urea and the GSH layer by forming hydrogen bonds before enzyme Urs grafting. The hydrophobic/hydrophilic features of the modified gold surface were determined by contact angle measurements. An impedimetric study of developed sensors shows the sensitivity of 8.73–10^{-8}/Ω/mM for low concentrations whereas 7.03–10^{-9}/Ω/mM for high concentration ranges.[77]

Organic field-effect transistor sensor detects the urea concentration by ammonia measurement. The sensor was developed by covalent attachments of enzyme Urs over 100 nm parylene-C layer, which serves as a semipermeable top gate dielectric. Enzyme Urs catalyzes the hydrolysis of urea into ammonia and carbon dioxide, as increases ammonia in concentrations decreased in transistor currents. The sensor covers the sensitivity as several mM in the range of physiological concentrations.[78]

Urs/silk fibroin (SF)/aminated GCE electrode was successfully fabricated and employed for electrochemical sensing of urea concentration. Electrode–Urs/SF/aminated GCE was developed with SF by placing enzyme Urs near the surface of the aminated GCE. High sensitivity of 112.3 μA/mM/cm^2 with a linear correlation between current and urea concentrations (0.3–2.7 mM) was observed. The sensor calculated the lower detection limit as 0.163 mM LOD and limit of quantification (LOQ) 0.394 mM.[79]

Tamaddon and Arab[80] mentioned the HANCD@Urs contained an environment-friendly green protocol for urea detection. The HANCD was prepared by nanocellulose-dialdehyde (HANCD), which was obtained from cotton-derived nanocellulose (NC) via tandem acid-hydrolysis and periodate-oxidation reactions. Further, enzyme Urs covalently attached to HANCD gives the HANCD@Urs. The sensor shows 90% preservation of the enzyme activity after the six cycles of reuse in enzymatic reactions.

Khattab et al.[81] developed cellulose nanowhiskers/Urs matrix (CNWTCFH) as a colorimetric nanocomposite film over N,N,N′,N′-tetra methylchloroformamidinium hexafluorophosphate spectroscopic probe for visual detection of urea. The colorimetric sensor responds toward urea by changing colors from yellow to pink in the 50–1100 ppm range. The pH range of 6.55–7.15 was used in the study of sensor response. The developed sensor can be utilized as a naked-eye sensing label or test strip for urea detection.[81]

A novel fiber-shaped photo electrochemical urea biosensor (PUB) was developed based on the highly flexible graphene (G) fiber. PUB responds to urea concentration in a wide linear range from 0.01 to 1500 μM with 1 nM low detection limit. The flexible microelectrode biosensors are a promising tool to use for monitoring personal healthcare.[82]

Polymer network entrapped p-DMAB sol–gel is employed for urea determination in feedstuffs using the colorimetric method. The urea analysis was monitored by a smartphone when the formation of Schiff base under acidic conditions. The sensor shows the response in a linear range of 2.5–100 and 100–1000 mg/L ($R_2 > 0.99$) toward urea concentration under optimized conditions. The sensor detects the LOD = 0.1 mg/L and LOQ = 0.5 mg/L with −1.15–2.76% accuracy and shows stability at least 90 days.[83]

4.7 | Nonenzymatic urea biosensors

A polypyrrole-coated platinum electrode was developed for urea detection without using any enzyme. The amperometric and impedimetric methods were employed to the estimation of urea in mildly acidic conditions. The developed biosensor reveals the sensitivity of 1.11 μA/μM/cm^2 in the linear range of 80–1440 μM along with 40 μM detection limit. Also, the sensor shows specific selectivity toward urea among the various interference species.[84]

The efficient electrochemical probes were developed by freestanding and binder-free electrospun polyvinylidene fluoride-co-hexafluoropropylene (PVdF-HFP) nanofiber membrane. Nickel (Ni)/Ni–cobalt (Ni–Co) nanoparticles were homogeneously distributed over the smooth surface of PVdF-HFP nanofibers and used for nonenzymatic urea detection. The sensor detects the urea in wide linear 20 μM/mM with high sensitivity 2.4 μA/mM/cm^2. The sensor reveals the rapid response of 5 s and a lower detection limit of 12 μM toward urea detection.[85]

A GCE modified with low-temperature aqueous chemical method synthesized nickel cobalt oxide ($NiCo_2O_4$) nanoneedles, urea electrochemical was successfully developed. The developed nonenzymatic sensing platform

shows the linear response (R_2 = 0.99) over the concentration range 0.01–5 mM and with a detection limit of 1.0 mM. The typical poor conductivity of bare NiO and Co_3O_4 nanoparticles can be overcome by the nickel–cobalt oxide ($NiCo_2O_4$) nanoneedles. The highly selective owing to the simple and cost-effective fabrication of the proposed nonenzymatic urea sensor can be used as a promising analytical tool for urea estimation.[86]

A nonenzymatic urea sensor was successfully fabricated by using ultrathin nickel–metal–organic framework (Ni–MOF) nanobelts. Sensor based on Ni–MOF ultrathin nanobelts exhibits a high sensitivity of 118.77 μA/mM/cm^2 in a wide linear range of 0.01–7.0 mM. The low detection limit shows 2.23 mM (LOD) with (S/N = 3). This highly sensitive, selective, and stable biosensor can be applied for the detection of urea in human body fluids.[87]

Ag/NiOOH nanorods-modified electrode was used to detect the urea with no enzymatic reaction. The developed electrode overcomes the drawback of catalytic response only to alkaline or OH- environment work by working in a neutral pH. Electrochemical measurement of nonenzymatic urea biosensor shows the high sensitivity of 233.7 μA/mM/cm^2 in a wide linear range of 0.2–26.0 mM. The sensor exhibits a fast response for urea concentration ~3.0 s and detection limit of 5.0 μM (S/N = 3) at applied potential in neutral phosphate-buffered saline solution.[88]

Nonenzymatic electrochemical urea biosensor was fabricated via porous composite catalyst based on a Ni–MOF and MWCNTs. The novel hybrid inorganic–organic Ni–MOF/MWCNT (ITO glass) electrode detects urea concentration with a sensitivity of 685 μA/mM/cm^2 in 10 s response time. The sensor shows a detection limit of 3 μM with 30 days of storage stability.[89]

5 | CONCLUSION AND FUTURE PERSPECTIVES

Advanced techniques and a variety of synthesized materials used in biosensors explored their applicability in diverse fields. Recent improvements in biosensors are found due to the efforts of academic and industrial researchers. The efforts taken by various research groups have tried to summarize in the present study. The study especially accomplishes the summaries of urea biosensors with their salient features as reported from the last decade. As a part of the biosensor, different components perform excellently according to their ability. The performance and suitability of urea biosensors decide by the active appearance of bioreceptor species. The protection of bioreceptor species carried out used an immobilization matrix to fabricate the urea biosensor. Thus, the present review report briefly described the various attempts that were made up in urea biosensor using different types of immobilization matrices. Conducting polymers, conducting polymer composite with nanomaterials, pure nanomaterials with different shapes and sizes, carbon materials especially carbon nanotubes, graphene, graphene oxide, and polymer carbon nanotube composite were fruitfully discussed in different sections. The special achievement of the last decades was the nonenzymatic detection of urea. However, the improvement in the nonenzymatic detection of urea usage is a great task for the upcoming researcher.

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