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Determination of urea with special emphasis on biosensors: A review

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ABSTRACT

Urea is the major end product of nitrogen metabolism in humans, which is eliminated from the body mainly by the kidneys through urine but is also secreted in body fluids such as blood and saliva. Its level in urine ranges from 7 to 20 mg/dL, which drastically rises under patho-physiological conditions thus providing key information of renal function and diagnosis of various kidney and liver disorders. Increase in urea levels in blood, also referred to as azotemia or uremia. The chronic kidney disease (CKD) or end stage renal disease (ESRD) is generally caused due to the progressive loss of kidney function. Hence, there is an urgent need of determination of urea in biological fluids to diagnose these diseases at their early stage. Among the various methods available for detection of urea, most are complicated and require time-consuming sample pre-treatment, expensive instrumental set-up and trained persons to operate, specifically for chromatographic methods. The biosensing methods overcome these drawbacks, as these are simple, fast, specific and highly sensitive and can also be applied for detection of urea in vivo. This review presents the principles of various analytical methods for determination of urea with special emphasis on biosensors. The use of various nanostructures and electrochemical microfluidic paper based analytical device (EμPAD) are suggested for further development of urea biosensors.

1. Introduction

Urea is non-toxic, nitrogenous organic end product of protein metabolism, enabling 80–90% of nitrogen elimination from human body. Normally, urea level in blood serum/plasma is in the concentration range, 3.3–6.7 mM, while its level > 30 mM concentration signify the requirement of dialysis, in addition to other possible factors such as saline, water depletion and gastrointestinal tract destruction (Ezimah and Abijah, 2005). On the other hand, decreased blood urea level could be pointing to the impaired renal function, inadequate protein diet, or alcohol use. The elevations in blood urea levels > 30 mM may also indicate the malfunctioning of kidneys, being one of the reference metabolites for hemodialysis. According to national kidney foundation (NKF), chronic kidney disease (CKD) affects around 10% of world's population and ranked 18th among the various causes of deaths worldwide with an annual death rate of 16.3 per 100,000 or over 1 million in totals (Morton and Nussey, 1940). The kidney function tests play an important role in the diagnosis of renal disorders at early stages. Several tests such as urinalysis, urine protein, creatinine clearance, serum creatinine, blood urea nitrogen (BUN), glomerular filtration rate (GFR) involving either urine or blood samples are commonly grouped under kidney function tests (KFT). Most important of these tests are BUN and serum creatinine, which are frequently used in every diagnostic laboratory for monitoring of renal function (Karsli, 2015). These tests also form an essential part of radiological screening procedures such as magnetic resonance imaging (MRI) and computed tomography (CT) prior to the administration of radiological contrast agents such as iodinated contrast or gadolinium based contrast agents so as to prevent complications such as contrast medium induced nephropathy and nephrogenic systemic fibrosis (Zhou and Lu, 2013).

The urea level in biological fluids has been analyzed quantitatively by different methods such as chromatographic, chemiluminometric, colorimetric, spectrophotometric, fluorimetric beside electrochemical methods. However, the conventional methods such as chromatography and colorimetric analysis suffer from relatively long time consuming sample preparation, high cost of equipment, requirement of skilled persons to operate and long time for analysis (Soni and Jha, 2017). The colorimetric procedures include nesslerization, phenol-hypochlorite or Berthelot method and diacetyl monoxime method. BUN test is most commonly used test for the assessment of blood urea levels and the test is frequently combined along with a serum creatinine test for the differential diagnosis of pre-renal, renal and post-renal hyperuremia. BUN measures the amount of nitrogen present in a person's blood. The main drawback of this procedure is that it is time-consuming as well as,

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requires withdrawal of blood from vein, which is painful and inconvenient to the subject undergoing the procedure. Therefore use of alternate body fluids for urea estimation is of great importance (Watson, 1958). Among non-invasive body fluids such as urine, saliva, sweat and tears, saliva is most easily accessible to the user due to ease in collection and its availability. Several research groups have also established a good correlation between salivary and blood urea levels using conventional methods (Evans et al., 2017). The biosensing methods overcome the aforementioned drawbacks and enhance the response in terms of sensitivity and selectivity. Biosensors are analytical devices integrating with target analyte recognition element, physical or chemical signal transduction element, and unit for signal display. The demand of sensitive, selective, inexpensive, and rapid detection of biologically important analytes has attracted the development of biosensors (Pundir et al., 2017).

During the past one and a half decades, the use of nanomaterials such as carbon nanotubes (CNTs), enzyme nanoparticles (ENPs), metal nanoparticles, and semiconducting nanoparticles have gained wide attraction for development of improved biosensors for urea detection. This has happened, because of their high chemical stability, unique structure and high surface-to-volume ratio of nanomaterials (Capek, 2009). These characteristics are the reasons for these nanomaterials to become more attractive for constructing chemical sensors. In recent times, composite materials based on redox mediators, conducting polymers, metal nanoparticles, nanocomposites and nanoclusters have been utilized to combine characteristics of the individual components with a cooperative improved performance in biosensor fabrication (Pundir and Narwal, 2018). However, now a day's efforts are also being made to achieve fabrication of a low cost urea biosensor along with their improved sensitivity and stability (Jakhar and Pundir, 2018).

The present review highlights the wide ranging approaches of urea biosensors, including the use of various materials, such as conductive polymers, non-conductive polymers, redox dyes, redox polymers, oxides, clays, zeolites and sol gels for this purpose. The recent and future trends towards the use of nanomaterials (nanoparticles, nanotubes, nanolayers, nanofibers, nanohybrids and nanocomposites) for fabrication of more sensitive and robust urea biosensors have also been highlighted in this review.

2. Basic concept of a biosensor

According to a recently proposed IUPAC definition, “A biosensor is a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor), which is in direct spatial contact with a transducer element (Fig. 1) (Qian et al., 2017). The biosensors are widely used in diagnostic, medical, quality control, agriculture industry, veterinary, dairy sciences, bacterial and viral diagnostic, drug production, control of industrial waste water, mining, military and defense (Pundir et al., 2018a, 2018b). The electrochemical biosensors combine the advantage of the electrochemical techniques with high substrate specificity of the enzyme, quick response time and

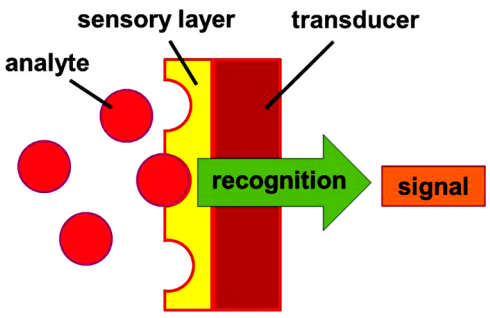

Fig. 1. Basic principle of biosensor (Pundir et al., 2018a, 2018b).

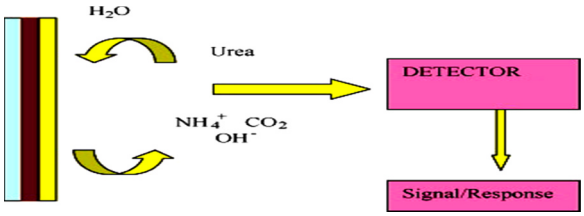

Fig. 2. Scheme of urea biosensor (Guilbault and Montalvo, 1969).

ease of procedure. Recently, analytic performance of electrochemical biosensors has been improved using nanomaterials (Pundir et al., 2017). Nanostructure of metal oxides is known to have unique ability to promote faster electron transfer kinetics between electrode and the active site of the desired enzyme. In recent years, with the development of nanotechnology, a lot of novel nanomaterials have been prepared and their novel properties are being gradually discovered and their applications have also advanced biosensors (Pundir et al., 2017). During the last decades, great attention has been paid to various biosensors for detection of urea level in different biological samples.

3. Urea biosensors

Urea biosensors are based on the urease enzyme, which catalyzes the hydrolysis of urea generating ammonium and bicarbonate ions. These species influences the pH of the surrounding environment, which is directly proportional to urea concentration. In particular, the ammonium ion (NH_4^+) in traces can be easily detected by employing a specific transducer i.e. NH_4^+ specific electrode (Ammonium ion specific electrode,AISE),which is in accordance of urea concentration Limbut, 2004. Fig. 2 shows the schematic presentation of urea biosensor.

Guilbault and Montalvo (1969) fabricated for the first time a urea biosensor, a potentiometric urease electrode for determination of urea through its urease catalyzed hydrolysis. The analytical signal of this early urea biosensor was the potential of a cation-selective glass electrode for detection of ammonium ions, generated by urease, which was proportional to urea concentration. The urea biosensor was stable for 3 weeks at 25 °C. The initially used glass electrode-based sensing element was interchanged with a more selective, neutral carrier-type ion-selective electrode (NH_4^+/CO_2) measuring cells. A number of urea electrodes, many of them using ion sensitive field effect transistor (ISFET) transducers, have been fabricated by now. Achievements have been made to improve the enzyme layer based biosensor, which has resulted in higher enzyme stability and lower limits of urea determination.

4. Classification of urea biosensors

A number of criteria like bio-receptors, transducers and different types of physical and chemical interaction can be used for classification of urea biosensors. On the basis of transducers, urea biosensors reported till date, can be classified into 6 types: – 1. Electrochemical (Amperometric and potentiometric) 2 Conductometric 3. Thermal 4. Optical 5. Manometric and 6. Piezoelectric

4.1. (A) Amperometric urea biosensors

Urease, which is often present in most biological systems, plays a very important role in this process by catalyzing the decomposition reaction of urea. This urease-catalyzed reaction produces an increase in pH, which is in proportion to urea concentration. The electrochemical measurements are carried out in a three-electrode cell system consisting a working electrode, a reference electrode (Ag/AgCl) and a counter electrode.

An amperometric urea biosensor was fabricated employing the nanostructured polypyrrole (PPy) and poly ortho- phenylenediamine

(POPDA). The effect of supporting electrolyte and number of deposition cycles on the OPDA and Py electropolymerization were studied. It was proved that POPDA and PPy were affected by pH changes and responded to the ammonium ions and product of urease catalyzed reaction. Scanning electron microscopic(SEM) images of the modified Pt/PPy electrode were taken. The cycle voltammograms (CV) and chrono amperometric curves of Pt/POPDA/urease and Pt/PPy/urease electrodes were studied. A good linear relationship was observed for Pt/POPDA/urease& Pt/PPy/urease electrode in a concentration range from 6.7 to 54 mM and 0.02–0.16 mM of urea, respectively. The carbon nanotubes (CNT) entrapment method was used for construction of biosensors and the two biosensors (Pt/PPy/POPDA/urease and Pt/POPDA/PPy/urease) reduced their sensitivity to urea. Pt/PPy/CNT/urease and Pt/PPy/ urease biosensors were 173 and 138 times more sensitive to urea than biosensor without PPy (Pt/POPDA/urease biosensor (Branzoi et al., 2012)).

4.2. (B) Potentiometric urea biosensors

Potentiometry is an analytical method that measures electrical potential differences between two electrodes. Increases or decreases in potential based on a chemical reaction, such as the hydrolysis of urea, could be recorded, which provides a quantitative determination of urea levels in real-time samples (Pundir and Narwal, 2018). One of the most attractive modes of detection of urea commonly used in many urea biosensors was based on the potentiometric detection of ammonium ion, ammonia gas, carbon dioxide or pH changed generated by the enzymatic hydrolysis of urea in accordance with urea concentration. Notably, the required devices for this mode of transduction are simple to construct and are readily available. As the biocatalytic reaction of urease results mainly in a pH change, most urea biosensors are based on potentiometric mode of pH detection. However, a major problem for pH-sensitive electrodes is that the sensor response is strongly dependent on the buffer capacity of the sample solution. Therefore the small pH change produced by the enzyme catalyzed reaction may be suppressed, when a high buffer concentration is employed and can lead to a narrow dynamic range and in some cases it results into loss of biosensor sensitivity (Nouira et al., 2012).

4.3. Conductometric urea biosensors

Transduction based on conductometric measurement is also useful for biosensing of urea, based on its ability to detect the change in solution resistance (reciprocal of conductance) during enzymatic reaction. These sensors are also simple to construct, suitable for miniaturization and mass production. These sensors do not require the use of a reference electrode and are unaffected by colour or turbidity, but suffer from lack of specificity. Substances that can contribute to the conductivity of a solution can influence the reliability of this sensor.

Lee et al. (2000a, 2000b) have used sol-gel immobilised urease on planar interdigitated electrode arrays for fabrication of urea biosensors. In this case, the charged products of the urease-catalyzed hydrolysis of urea increase the solution conductivity around the vicinity of the sensor surface and the measured conductivity is related to urea concentration.

4.4. Thermal urea biosensors

The detection of thermal change has not gained much interest for urea biosensors, but has been used in some cases. In this method, heat produced during a biochemical reaction (urease catalyzed hydrolysis) is measured and it is believed that the heat produced through the specific enthalpy, of the reaction is related to the amount of reacted substrate (urea). The benefit of this method, compared to other methods such as spectrophotometric and electrochemical methods, is the universal detection principle of specificity of biological reactions, as all biological reactions are exothermic. However, a major disadvantage of this method is non-specific heat effects from mixing, changes in pH, viscosity and ionic strength can also produce signals. The sensitivity of this method is directly related to the apparent molar enthalpy of a reaction and, as such, only more endo or exothermic reactions are often considered for detection by this approach. The urease catalyzed urea hydrolysis has an enthalpy of $-14.97\ \text{Kcal mol}^{-1}$, which is adequate for thermal bio sensing of urea (Bjarnason et al., 1998).

4.5. Optical urea biosensors

The use of optical chemical sensors has also attracted some interests as an alternative means of detection of urea. Several optical biosensors have been described for urea determination that involves immobilization of urease onto different matrices, such as PVC, N-+indicator. A distinct disadvantage of using optical biosensors for urea is that none of the catalytic products (ammonia or carbon dioxide) or the analyte show optical properties for adequate detection by optical sensors. Due to this reason, a second reaction or an indicator dye is often used to achieve adequate detection. An exceptional example of an optical urea biosensor which does not require such reaction or the addition of dye has been reported and it is based on the use of a conductive polymer (polypyrrole) and has been used successfully for urea determination in biological samples (Marcos et al., 1999).

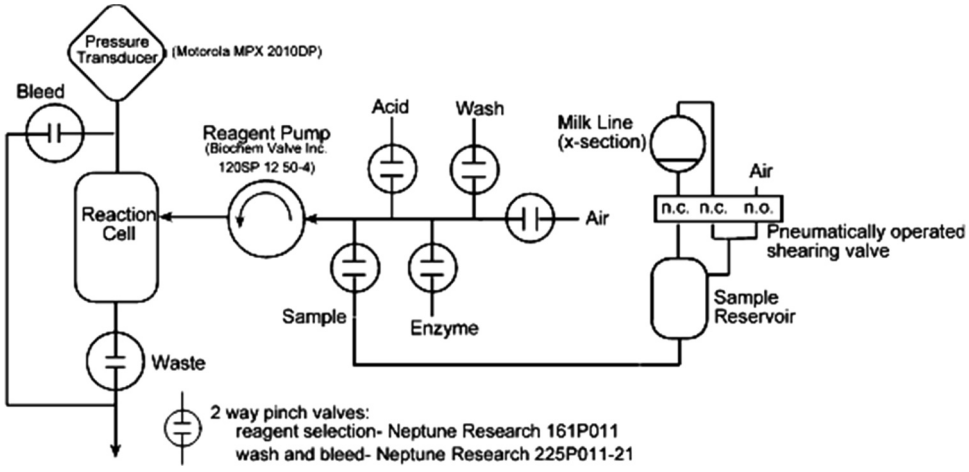

Fig. 3. Fluidic schematic of biosensor (Jenkins and Delwiche, 2003).

4.6. Manometric urea biosensors

The measurement of the total quantity of a gas (such as NH_3 or CO_2) produced during the urease catalyzed hydrolysis of urea, have also been made by simple manometric methods (Fig. 3). These methods are based on the ideal gas law, which states that the volume occupied by a gas is directly proportional to the number of moles of the gas at constant pressure and temperature. The gas volume is thus measured as a function of time or at completion of the reaction. Using this principle, Jenkins and Delwiche (2003) developed an on-line biosensor and used it for measurement of urea level in milk with a piezo-resistive pressure transducer during milking. The sensor is robust to milk and the complete measurement cycle, including a wash cycle between samples and correction for background dissolved gases lasted about 5 min. The main disadvantage of these manometric sensors compared to other modes of transduction is that the devices could only be used for the analysis of discrete samples in a sealed system. Another limitation arose from practical considerations of the dimension of the headspace gas volume relative to the sample volume. For effective mass transfer and reproducible sensitivity, this volume was required to be relatively large. This can result in a loss of sensitivity compared to the theoretical maximum. Jenkins and Delwiche (2003) proposed a modification to solve these problems, wherein the gaseous volume and pressure sensor would be contained within a porous membrane. Soluble gases could then move across the membrane to and from the sample, but the gas phase would be held inside the cavity due to the surface tension of the sample on the membrane. The cavity pressure could then be independent of the pressure in the sample, and the entire sensor could be immersed in the sample. Furthermore, the volume of the gaseous cavity could be made constant and much smaller so that the sensitivity of the sensor is reproducible and as large as possible (Jenkins and Delwiche, 2003).

4.7. Piezoelectric urea biosensors

Another transduction mode that has gained some interest for biosensing of urea is piezoelectric detection. In general, the detection of the change in mechanical characteristics such as mass loading effect and elastic characteristic due to immunity reaction on a propagation plane or electrode can be achieved with an acoustic device. However, the acoustic sensor, which normally detects change in mechanical properties cannot detect an enzymatic reaction. By using a piezoelectric crystal in the acoustic device, a piezoelectric field is formed according to the wave propagation. Since the piezoelectric field penetrates into the medium in contact with the device, the electrical properties (dielectric contact and conductivity) of the medium can be detected (Kondoh et al., 1996). This approach was used to develop urea piezoelectric biosensors, where the interaction between immobilized urease on these devices and urea in solution is detected based on change in conductivity monitored by acoustoelectric interaction. Wei and Shih (2001) used another approach, which involved the coating of piezoelectric surface with an adsorbent, which can adsorb the products of the enzymatic reaction (NH_3 or CO_2), which serve as a basis for measurement of urea concentration.

5. Urea biosensors based on polymer matrices fabrication

5.1. Urea biosensors based on conductive polymer

One of the areas, which have generated much interest in the fabrication of urea biosensors is in the use of conducting polymers. These polymers provide suitable matrices for immobilization of bioactive substances and can be used to enhance stability, speed, and sensitivity (Ahuja et al., 2008). A number of immobilization techniques including ethyl-dimethylaminopropylcarbodiimide (EDC) and N-hydroxy-succinimide (NHS) coupling chemistry, have been used to achieve improved stability of the desired biomolecules within or on conducting polymers. Several conducting polymer-based urea biosensors have been fabricated by entrapment of urease within electropolymerized polypyrrole(PPy) films,polyaniline (PANI) films and polythiophene films (Borole et al., 2006). Some of the significant urea biosensors reported in this area are discussed in this review. These matrices are used as supports for biomolecules, resulting in biosensors that have enhanced speed, sensitivity and versatility in diagnostics to measure desired analytes. These devices are finding ever-increasing use in clinical diagnostics (Kaku et al., 1994).

Various conducting polymers, like PANI, PPy and polythiophene, have been used for the fabrication of biosensors. Among them, PPy is one of the most extensively used conducting polymers in the fabrication of urea biosensors. The versatility of this polymer is determined by (i) its biocompatibility, (ii) capability to transduce energy arising from the interaction of analytes and analyte recognizing sites into electrical signals that are easily monitored, (iii) capability to protect electrodes from interfering material, and (iv) easy ways for electro-chemical deposition on the surface of any type of electrode. Among other conducting polymers, PANI is often used as the immobilizing substrate for biomolecules and as an efficient electrocatalyst. However, the necessity to detect bioanalytes in neutral pH ranges leads to electro-inactivity of the deposited film, discouraging the use of polyaniline and polythiophene as biosensing materials for the detection of urea (Dhawan et al., 2009).

5.2. Urea biosensors based on non-conducting polymer matrices

Polymers are finding wide usage in the field of electronic measuring devices, especially sensors owing to their ability to have their chemical and physical properties tailored over a wide range of characteristics. The suitably chosen polymeric matrices are found to be biocompatible, flexible, and cost-effective. Besides this, they can be obtained in the form of free-standing films for the fabrication of biosensors (Grieshaber et al., 2008). Extensive efforts have been made to fabricate urea biosensors using different polymer matrices, such as phosphine oxide polyether, polygalacturonate, polyurethane-acrylate, acrylonitrile, hydroxyapatite,alginate, bovine serum albumin, poly(vinyl ferrocenium), polyethylenimine, synthetic latex polymer, activated polyvinyl alcohol (PVA), polyvinyl chloride (PVC) and chitosan (CHIT). These materials provide mechanical strength and long-term stability as supports for the immobilization of the enzymes. There are a variety of methods by which enzymes can be immobilized, ranging from covalent chemical bonding to physical entrapment (Soldatkin et al., 2000).

A potentiometric enzymatic biosensor, with a detection range for urea between 8.9×10^{-5} to 1.1×10^{-3} M, consisting of urease immobilized on PVA activated with 2-flouro-1-methylpyridiniumtoluene-4-sulfonate (FMP) was developed by Sehitogullari (2002). This sensor has a limit of detection (LOD) of 1 mM and did not cover the physiological range of urea. Lakard et al. (2004) used polyethylenimine films coated onto glass sealed, metal microelectrodes for the potentiometric detection of urea. The biosensor include a short response time (15–30 s), sigmoidal response for urea concentrations in the working range from $1 \times 10^{-2.5}$ to $1 \times 10^{-1.5}$ M and a lifetime of about 4 weeks. It also included benefits such as low diffusional resistance, as well as strong binding forces between enzyme and matrix, thus reducing the loss of enzymes and increasing stability.

PVC-based ammonium ion-selective electrodes have been extensively used for the fabrication of potentiometric urea biosensors. These conventional potentiometric urea biosensors, pH electrodes and ammonium ions-selective electrodes detect changes in H^+ or NH_4^+, respectively, produced in an enzymatic reaction. However, these biosensors suffer with the associated problem of strong dependence on sample buffer capacity, which is known to be suppressed by the buffer used, leading to a narrow dynamic range and loss in sensor sensitivity. PVC membrane electrode-based biosensors, with suitable ionophores,

offer many advantages that include short response time, good reproducibility, simple design and longer lifetime, up to 12 months (Jaworska et al., 2015).

Functionalized PVCs have been investigated for use as electrode membrane materials to facilitate efficient enzyme immobilization. Suitable ionophores have also been utilized with PVC ion electrodes to enhance response characteristics, such as response time, reproducibility, simple design and longer lifetime. The response characteristics and potential use of laminated PVC membranes for urea biosensor fabrication was also studied. An ionophore-free PVC–NH_2 pH membrane electrode covalently immobilized with enzyme was applied by Wałcerz et al. (1996, 1998) for urea estimation by measuring hydronium ions after enzymatic hydrolysis. Later, Tinkilic et al. (2002) fabricated a miniaturized urea sensor by immobilizing urease directly onto an all solid-state, contact PVC–NH_2membrane,AISE with improved sensitivity, longer lifetime and low cost. The biosensors showed a linear range between 5×10^{-2} and 5×10^{-4} M urea in unbuffered solution. However, the sensitivity was highly dependent on the H^+, Na^+ and K^+ concentrations in the sample solution, as severe interference from Na^+ and K^+ ions was observed. Efforts were made to reduce the effect of interferants to achieve improved performance over carboxylated PVC-based biosensors. This was in terms of sensitivity, dynamic stability over 2 months and a response time of 1–2 min. The serum urea concentration was also measured by these urease-biosensing electrodes that were based on PVC-membrane ammonium-selective electrodes containing palmitic acid and nonactin.

An optical biosensor was developed based on an ion-selective optode membrane, which contained nonactin as the ion-selective ionophore and ETH 5294 chromoionophore in a thin (1 mm) plasticized poly(vinylchloride) film. This urea biosensor was used for flow injection urea measurements in the 0.01–2 mM concentration range. These sensors suffer from the influence of interferants such as ammonium, potassium and sodium ions. In this context, several strategies were used to overcome the effect of interferences e.g. differential measurements including separation by gas diffusion and electronic tongues have been used. Ion-selective-electrode arrays and multivariate calibration models have proved to be effective for increasing selectivity, enabling the simultaneous determination of several species in different types of samples, ranging from biological fluids to natural waters. Many non-conductive organic polymers were also used with various immobilization methods to construct a range of urea biosensors (Kovács et al., 2003a, 2003b). In one of these studies, Magalhaes and Machado, 1998 fabricated potentiometric urea biosensors by immobilizing urease into chitosan membranes coupled to all-solid-state nonactin ammonium ion selective electrodes. They compared the response characteristics of the biosensors prepared with four different immobilization methods for the incorporation of urease into the chitosan membranes by adsorption; adsorption followed by reticulation with dilute aqueous glutaraldehyde (GA) solution; activation with GA followed by contact with enzyme solution; and activation with GA, contact with the enzyme solutionand reduction of the Schiff base with sodium borohydride (Magalhaes et al., 1998). They found that the potentiometric urea biosensor prepared by above method gave the best response characteristics and was able to detect urea within a linear concentration range of 0.1–10 mM, with a sensitivity of 56 mV decade-1, response time of about 1 min and life time of 2 months. Lakard et al. (2004) used a glass-sealed platinum electrode coated with a thin polyethylenimine film, which was sensitive to pH to construct a potentiometric pH sensor. They then used this electrode as a basis for constructing a potentiometric urea biosensor and compared the use of a number of immobilization methods for the incorporation of urease onto the electrode. It revealed that immobilization of urease by crosslinking with GA has many advantages, compared with those achieved with other reagents (Massafera and Torresi, 2009). The GA immobilised urease biosensor exhibited a short response time of 15–30 s, sigmoidal responses for urea within a linear concentration range of 3.1–31.6 mM and a life time of 4 weeks. Karakuş et al. (2005) also investigated four different immobilization methods for immobilizing urease onto a PVC ammonium membrane, which contained palmitic acid and involved nonactin as an ammonium-ionophore. The resulting potentiometric urea biosensor gave relatively better analytical characteristics and performance with a relatively high sensitivity of 40–54 mV/decade, a dynamic stability over 2 months with relatively small decrease in sensitivity and a response time of 1–2 min. The potentiometric biosensor was successfully used for rapid determination of urea concentrations in serum samples. There was a good correlation between the results obtained with this biosensor and a standard spectrophotometric method for the determination of urea in human serum samples. Koncki et al. (1999a, 1999b) constructed potentiometric urea biosensors by combining urease with ammonium-selective membranes. Carboxylated PVC (PVC-COOH) was used as a support polymer in ion-selective membrane and nonactin was applied as an ammonium selective ionophore. The carboxyl groups located on the membrane surface enabled covalent attachment of urease directly on the membrane, without the need for additional protective membrane. The biosensor was able to determine urea within a linear concentration range of 0.3–70 mM at a response time of < 20 s and has a dynamic stability of over 2 months without a decrease in sensitivity (Wałcerz et al., 1996). This biosensor was subsequently employed in a flow injection analysis (FIA) system and achieved a linear concentration range of 0.5–8 mM with a response time of ≤ 2 mins. In the FIA mode, the biosensor was used for fast and accurate human serum analysis. The results obtained were in good agreement with the reference values for the samples. Other advantages included the simplicity of the system and low costs of measurements (Wałcerz et al., 1998). The biosensor was further used in a simple flow injection analysis system for monitoring of hemodialysis progress by potentiometric determination of dialysate urea nitrogen (DUN). Over 25 DUN determinations were achieved in an hour, within a linear concentration range of 1.6–5 mM DUN. The urea biosensor/FIA system was also successfully used to evaluate clinical parameters of urea kinetic modeling: parameters KT/V, urea reduction ratio (URR), percentage removal of urea (PRU) and total urea removal (TUR) for intradialytic intervals and urea generation rate (G) and protein catabolic rate (PCRn) for interdialytic intervals. These biomedical parameters are used for describing the adequacy of hemodialysis therapy. In another study, n-tridodecylamine was composited as the hydrogen-ion selective ionophore with PVC-COOH as the support and urease was covalently attached to the membrane surface via carboxyl groups on PVC network. The resulting potentiometric biosensor was again successfully applied to the determination of urea in urine, cosmetic creams and pharmacutical samples. Koncki et al. (1995) also used a lipophilic pH indicator, 9-(4-diethylamine-2-octadecyloxystyryl)-acridine, with plasticized PVC-COOH for the preparation of a pH optode membrane. Urease was then linked covalently to the surface of the pH optode membrane to form a urea optical biosensor. The resulting biosensor was used for rapid determination of urea within a linear concentration range of 0.3–100 mM. Kovacs et al., 2003 also developed a planar waveguide type optical urea sensor based on the detection of the NH_4^+ liberated during the catalytic reaction with an ion-selective optode membrane which contained nonactin as ion selective ionophore and ETH 5294 chromo-ionophore in a thin plasticized PVC film. The sensor contained an ammonium sensitive, 1 mm thick optode layer and a second covering layer made of polyurethane. Urease was immobilised by cross-linking with glutaraldehyde on the surface of the secondary layer. The biosensor achieved a linear concentration range between 0.08 and 2 mM, as well as a fast response time, which enabled the analysis of about 30 samples/hour. The electrospinning of a urease solution with polyvinylpyrrolidone (PVP) was used to develop a urea biosensor by Sawicka et al. (2005). The resulting material was tested for the detection of urea. In the process of electro-spinning,mixtures of polymer and enzyme solutions were injected from a small nozzle under the influence of an electric field as high as 30 kV. The buildup of electrostatic charges on the surface of a liquid droplet induces the

Table 1
A comparison of analytical properties of various urea biosensors.

Type of biosensor	Type of transducer/support for immobilization	Electrode used	Method of immobilization	Optimum pH	Linear range (mM/l)	Detection Limit (mM)	Response Time (s)	Sensitivity (mV/decade)	Interference	Storage stability at 4 °C (days)	Reference
Potentiometric	Polypyrrole and polyion complex	Pt	Covalent binding	NR	3–300	0.030	20	110	NR	NR	(Osaka et al., 1996)
Potentiometric	Polyurethane acylate polymeric membrane	NR	Entrapment	NR	0.04–36	0.04	30–300	NR	NR	30	(Puig-Lleixà et al., 1999)
Potentiometric	Poly(carbamoylsufonate) hydrogel	NR	Entrapment	NR	0.1543–45	0.0043	60–120	54.8 ± 0.3	K^+,Na^+	90–120	(Eggenstein et al., 1999)
Potentiometric	Poly (3-cyclohexyl thiophene)	Pt	Physical adsorption	NR	NR	0.04	50	55	NR	90	(Pandey et al., 2000)
Potentiometric	Gelatin beads	NR	Entrapment & cross linking	NR	1.785–50	1.7857	120	NR	NR	240	(Srivastava et al., 2001)
Potentiometric	Poly(N-vinyl carbazole/Stearic acid) Langmuir-Blodgett film	ITO	Entrapment	NR	0.5–68	0.5	120	NR	NR	35	(Singhal et al., 2002)
Potentiometric	Caboxylic poly (vinyl chloride)	NR	Electrostatic adsorption	NR	0.1–1000	0.028	30	56.1	NR	25	(Wu et al., 2002)
Potentiometric	Poly(vinyl alcohols)with styrylpyridinium groups	ITO	Covalent bonding and crosslinking	6	0.1107–42.85	0.1071	60–120	60.9	NR	99	(Chen et al., 2003)
Potentiometric	Poly(vinyl alcohols)with styrylpyridinium groups	NR	Covalent bonding and crosslinking	NR	1.0714–107.14	0.8571	60–120	NR	NR	30	(Wang et al., 2003)
Potentiometric	Polyethylenimine	Pt	Physical adsorption and reticulation		1–10	1	15–30	50.24	NR	28	(Lakard et al., 2004)
Potentiometric	Latex polymers (Insulator semiconductor)	NR	Electrostatic interaction	NR	0.1–10	0.1	NR	NR	NR	30	(Barhoumi et al., 2005)
Potentiometric	Biocomposite nonwoven nanofiber mat/ polyvinylpyrrolidone (PVP)	NR	Encapsulation	NR	05–2.5	5	1200	NR	NR	7	(Sawicka et al., 2005)
Potentiometric	Poly(vinyl ferrocenium)matrix	Pt	Ionic binding	7	0.001–0.25	0.001	60	2.4	NR	24	(Kuralay et al., 2005)
Potentiometric	Polymeric coating	NR	Microencapsulation	NR	0.2–20	0.2	NR	NR	NR	21	(Starodub and Rebriev, 2007)
Potentiometric	PVC-COOH polymer	NR	Covalent binding	NR	1–100	0.1	60–120	30	NR	20	(Ternovskii et al., 2007)
		NR	Covalent binding	NR	0.99–100	0.1	60–120	50	NH_3,K^+,Na^+	NR	
Potentiometric	Whatman paper	NR	Physical adsorption	NR	1.66–16.6	1.66	120	NR	NR	Disposable	(Gutiérrez et al., 2007)
Potentiometric	Polyvinyl alcohol	NR	NR	NR	NR	1	120–240	NR	NR	30	(Gutiérrez et al., 2008)
Potentiometric	Polyvinyl chloride-palmitic acid	NR	NR	NR	NR	NR	60–120	54.3 ± 0.4	NR	60	(Verma and Singh, 2003)
Potentiometric	PVC-NH_2	NR	NR	NR	NR	0.03	10	48 ± 5	NR	30	(Ciurli et al., 1996)
Potentiometric	Chitosan	NR	NR	NR	NR	NR	30–120	56	NR	60	(Godjevargova and Dimov, 1997)
Potentiometric	Polypyrrole	NR	NR	NR	NR	NR		31.8	NR		(Marzadori et al., 1998)
Potentiometric	Poly(N-3-aminopropylpyrrole-co-pyrrole)film	NR	NR	NR	NR	NR	25–30	27.5	NR	60	(Gutiérrez et al., 2007)
Potentiometric	Tetraphenyl doped polyaniline	NR	NR	NR	NR	0.02	NR	86	NR	60	(Komaba et al., 1997)
Potentiometric	Poly(N-vinylcarbazole)and stearic acid	NR	NR	NR	NR	5	NR	10	NR	35	(Rajesh et al., 2005a, 2005b, 2005c)
Potentiometric	Υ-AL_2O_3	NR	NR	NR	NR	0.01	NR	NR	NR	NR	(Komaba et al., 1996)
Potentiometric	Teflon membrane	NR	NR	NR	0.05–10	NR	120–180	NR	NR	60	(Seki et al., 1998)

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Type of biosensor	Type of transducer/support for immobilization	Electrode used	Method of immobilization	Optimum pH	Linear range (mM/l)	Detection Limit (mM)	Response Time (s)	Sensitivity (mV/decade)	Interference	Storage stability at 4 °C (days)	Reference
Potentiometric	Poly(vinyl alcohol)/ 2-fluoro-1-methylpyridinium salt	physically adsorbing	NR	NR	0.10–100	NR	90–240	NR	NR	28	(Eggenstein et al., 1999)
Potentiometric	IROF grown on silicon-based thinfilm	Pt.	electrodeposition	NR	0.2–5	78	60	72.97	NR	NR	(Mascini and Guilbault, 1977)
Potentiometric	PPy	NR	NR	NR	10–100	NR	NR	63	NR	NR	(Narinesingh et al., 1991a, 1991b)
Potentiometric	PPy/$NaHCO_3$	Pt	Electropolymerization	NR	0.1–300	NR	180	31.8	NR	NR	(Prats-Alfonso et al., 2013)
Potentiometric	Functionalized PPy	GC electrode	Covalent immobilization	NR	0.14–0.01	0.14	NR	93	NR	NR	(Jin et al., 2003)
Potentiometric	PPy/Polyion complex	NR	Electropolymerization	NR	0.3–30	3.0	30	110	NR	NR	(Komaba et al., 1997)
Potentiometric	PPy microspheres/PPy-PVS	ITO	Covalent bonding	7.2	5–60	0.16	60	12	NR	40	(Osaka et al., 1996)
Potentiometric	PolyN-(3-aminopyropyl pyrrole-co-pyrrole)	ITO	Covalent bonding	NR	0.006–0.4	NR	25–50	28	NR	60	(Osaka, 1999)
Potentiometric	PPy	ITO	Covalent bonding	NR	0.006–0.4	NR	NR	28	NR	NR	(Gambhir et al., 2002)
Potentiometric	Tin-oxide (SnO2)-coated hybrid nanocomposite	physical absorption	–	NR	0–42	0.5	NR	NR	NR	NR	(Rajesh et al., 2005a)
Potentiometric	1-PANI/PVA	NR	NR	NR	10–100	NR	NR	41	NR	NR	(Rajesh et al.,
	2-PANI/Sol-gel	NR	NR	NR	NR	0.02	NR	45	NR		2005b)
Potentiometric	Poly(3-cyclohexyl thiophen)				0.001–0.004	0.04	55	55		90	(Saeedfar et al., 2013)
Potentiometric	Poly(carbamoylsulfonate)/ Polyethylenimine				0.072–21	0.02	120	52	4	4	(Pandey, 2001)
Potentiometric	PVC/Palmitic acid	AISE	NR	NR	0.01–10	NR	120	40–54	NR	60	(Pandey et al., 2000)
Potentiometric	PVC-COOH/Nonactin	NR	NR	NR	0.5–8	NR	180	NR	NR	60	(Eggenstein et al., 1999)
Potentiometric	PVC-COOH/Nonactin	Covalent binding	NR	NR	0.1–70	0.01	15	50	K^+	60	(Karakuş et al., 2005)
Potentiometric	Chitosan	NR	NR	NR	0.1–10	NR	120	56	NR	60	(Wałcerz et al., 1998)
Potentiometric	Polyethylenimine	NR	NR	NR	3.1–31.6	NR	30	7–25	NR	NR	(Wałcerz et al., 1996)
Potentiometric	PVC-COOH/Nonactin	NR	NR	NR	1.6–5	NR	15	NR	NR	NR	(Magalhaes and Machado, 1998)
Potentiometric	PVC-COOH/Nonactin	AISE	NR	NR	2–16	NR	144	39	NR	K^+	(Lakard et al., 2004)
Potentiometric	PVC-COOH/n-tridodecylamine	NR	NR	NR	1–13	NR	240	40	NR	30	(Koncki et al. (2000)(a)
Potentiometric	Polyvinylpyrrolidone	NR	NR	NR	0.5–2.5	NR	1200	NR	NR	NR	(Koncki et al. (2000)(b)
Potentiometric	Poly(N-vinylcarbazol)/Stearic acid	NR	NR	NR	0.5–93	0.5	120	10	NR	35	(Koncki et al., 1999a, 1999b)
Potentiometric	PVC-BSA	NR	NR	NR	1–100	NR	60	30	NR	NR	(Sawicka et al., 2005)
Potentiometric	Gelatin	NR	NR	NR	2.5–23	NR	120	67	NR	80	(Singhal et al., 2002)
Potentiometric	PVC-COOH/Nonactin	NR	NR	NR	1–15	NR	120	21	NR	30	(Zamponi et al., 1996)

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Type of biosensor	Type of transducer/support for immobilization	Electrode used	Method of immobilization	Optimum pH	Linear range (mM/l)	Detection Limit (mM)	Response Time (s)	Sensitivity (mV/decade)	Interference	Storage stability at 4 °C (days)	Reference
Potentiometric	Poly-o-phenylenediamine	GC electrode	NR	NR	0.01–1	NR	NR	15	NR	35	(Srivastava et al., 2001)
Potentiometric	PVC/AISE	NR	NR	NR	0.02–1	NR	60–120	40	NR	60	(Koncki, 2000)
Potentiometric	y- AL_2O_3	NR	NR	NR	0.03–14	0.01	240	54	NR	90	(Chirizzi and Malitesta, 2011)
Potentiometric	RuO_2/graphite	NR	NR	NR	1–31	NR	120	53	NR	NR	(Dindar et al., 2011)
Potentiometric	RuO_2/graphite	NR	NR	NR	6–17.5	NR	NR	30	NR	NR	(Liu et al., 1997)
Potentiometric	SiO_2/AL_2O_3	NR	NR	NR	5–25	15	NR	52	NR	NR	(Tymecki et al., 2005)
Potentiometric	Poly(venylferrocenium)	Pt	NR	NR	0.05–100	0.005	NR	13	NR	NR	(Tymecki et al., 2005)
Potentiometric	Fe_3O_4NPs	NR	NR	NR	0.01–0.3	0.00001	60	NR	NR	NR	(Seki et al., 1998)
Potentiometric	MWCNT/silica matrix on ITO glass plate	NR	NR	NR	0.002–1.07	NR	18	23	NR	60	(Kuralay et al., 2006)
Potentiometric	Poly(glycidylmethacrylat)-grafted Iron oxideNPs	Au	NR	NR	0.25–5	0.05	7.8	20	NR	60	(Sahraoui et al., 2011)
Potentiometric	Immobilon-AV membrane	NR	NR	NR	0.5–30	NR	60	59	NR	30	(Ahuja et al., 2011)
Potentiometric	AISE,nylon screen	covalent immobilization	NR	6.0	0.035–0.714	NR	315	NR	NR	30	(Çevik et al., 2013)
Potentiometric	ZnO nanowires	NR	–	7.4	0.1–100	0.1	4	52.8	NR	NR	(Cunningham, 2004)
Potentiometric	Indium tin oxide, polyethylene terephthalate(ITO/PET-EGFET)	NR	NR	NR	NR	NR	180–540	51 ± 4	NR	NR	(Maria et al., 2011)
Potentiometric	Chitosan-iron oxide magnetic nanoparticles	NR	NR	NR	0.1–80	NR	12	42	NR	NR	(Syed et al., 2011)
Potentiometric	Fullerene-immobilized urease (C60-urease) poly(*n*-butyl acrylate) (P*n*BA) membrane	NR	NR	NR	2.31–0.0828	NR	120	59.67 ± 0.91	NR	140	(I Shun Wang et al., 2012)
Potentiometric	**Highly porous sol-gel**	NR	NR	NR	NR	**0.001**	2–3	NR	NR	NR	(Ali et al., 2011)
Potentiometric	Nylon net, ammonium ion-selective electrode	NR	NR	NR	0.0357–0.714	NR	900	NR	NR	70	(Saeedfar et al., 2013)
Potentiometric	Poly(glycidylmethacrylate) (PGMA)-grafted iron oxide nanoparticles	Covalentl binding	Au electrode	7.5	0.25–5.0	NR	8	20	NR	60	(Bhardwaj1 et al., 2013)
Potentiometric	**Carbon nanotubes,** poly(vinyl chloride)	NR	NR	7.19	1000–0.01	0.1	NR	59.4 ± 0.9	NR	NR	(Rafaela et al. (2013)
Potentiometric	Polyion complex (mixture of poly-L-lysine hydrobromide and poly(sodium 4-styrenesulfonate), PIC)	Carbon nanotube (CNT),	NR	NR	0.01–3	NR	60–90	59.1	NR	15–17	(Çevik et al., 2013)
Potentiometric	Screen-Printed Carbon Electrode	NR	NR	NR	0.05–40	0.012	50	NR	NR	180	(Ewa Jaworska et al., 2015)
Potentiometric	Thiophene Copolymer	NR	(ITO) glass electrodes.	NR	0.99–4.97	NR	600	NR	NR	NR	(JieChen et al., 2015)
Amperometric	Nylon net	NH4+electrodes.	NR	NR	0.2	0.01	NR	NR	NR	20 days	(Bertocchi et al., 1996)
Amperometric	Natural dye hematein	Graphite and Pt. composite electrode	Physical adsorption	NR	0.01–0.25	0.003	120–180	1.95	NR	90	(Pizzariello, 2001)
Amperometric	Electropolymerized toluidine blue film	GC electrode	Physical adsorption,	4.0	0.02–0.8	0.02	20–30	980	NR	24 h	(Vostiar et al., 2002)

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Type of biosensor	Type of transducer/support for immobilization	Electrode used	Method of immobilization	Optimum pH	Linear range (mM/l)	Detection Limit (mM)	Response Time (s)	Sensitivity (mV/decade)	Interference	Storage stability at 4 °C (days)	Reference
Amperometric	polyaniline-Nafion, Cyclic voltammetry	Au electrode	Covalent binding and crosslinking,	NR	0.05–0.5	0.005	NR	316	NR	NR	(Luo and Do, 2004)
Amperometric	poly(vinyl ferrocenuim) matrix	Pt electrode	Anion-exchange	NR	0.001–0.25	0.001	60	NR	NR	29	(Kuralay et al., 2006)
Amperometric	Poly(vinylferrocium)	NR	NR	NR	NR	0.001	60	NR	NR	29	(Mascini M and Townshend, 1995)
Amperometric	Polyethylenimine	NR	NR	NR	NR	NR	15–30	NR	NR	28	(Adhikari, Majumdar, 2004)
	Polypyrrole	NR	NR	NR	05–0.25	0.107	60	1.02	NR	28	(Adeloju et al., 1996)
Amperometric	Carbon paste electrode	NR	NR	NR	NR	0.005	NR	NR	NR	15	(Jin et al., 2003)
Amperometric	polyester functionalized gold nanoparticles	covalently immobilization	ITO	NR	0.01–35	0.01	3	7.48	NNRR-	NR	(Tiwari et al., 2009)
Amperometric	Natural dye hematein		Pt electrode	NR	0.01–0.25	0.003	120	1.95	NR	90	(Pizzariello, 2001)
Amperometric	enzyme-based probes nylon net	covalently immobilization	Pt electrode	NR	0.01–0.3	0.01	240	NR	NR	NR	(Bertocchi et al., 1996)
Amperometr+ic	polyaniline	cross-linking	Nafion Electrodes	NR	0.001–1	0.0005	40	0.76	NR	14	(Cho, Huang, 1998)
Amperometric	PPy	NR	NR	NR	0.002–0.075	0.001	240	NR	NR	14	(Adeloju et al., 1997)
Amperometric	PPy	Pt electrode	entrapmen		1.7–56	0.0006	180	0.001	NR	3	(Trojanowicz et al., 1996)
Amperometric	Poly(3-aminopyropyl-pyrrole-*co*-pyrrole)	ITO glass	Covalent immobilization	NR	0.16–5.05	0.02	42	470	NR	60	(Rajesh et al., 2005a, 2005b, 2005c)
Amperometric	PPy	NR	NR	NR	0.01–100	NR	60	7.6	NR	NR	(Jin et al., 2003)
Amperometric	PANI/Poly (carbamoylsulphonat)	Pt-C	NR	NR	0.1	0.005	NR	40 ± 20	NR	30	(Strehlitz et al., 2000)
Amperometric	Hematein/Graphite powder	NR	NR	NR	0.01–0.25	0.003	120	0.195	NR	92	(Pizzariello, 2001)
Amperometric	pH-sensitive redox probe	Pt.,Au and solid composite electrodes	NR	NR	0.002–1.2	0.002–0.01	31–301	0.015–0.0001	NR	30	(Stredanský et al., 2000)
Amperometric	Nickel hexacyanoferrate	Covalent immobilization	Nickel electrode	NR	0.01–1	NR	60	314	NR	45	(Milardovi et al., 1999)
Amperometric	Poly(glycidyl methacrylate-covinylferrocene)	Covalent immobilization	NR	NR	0.1–4	0.06	3	0.0003	NR	44	(Çevik et al., 2011)
Amperometric	PANI nanofiber/ Pt nanoflower	NR	Glassy carbon	NR	Up to 20	0.01	180	0.116	Cl^-	NR	(Jia et al., 2011)
Amperometric	ZnOnanorods	electrostatic interactions	ITO	NR	1–20	0.13	4	0.4	NR	NR	(Palomera et al., 2011)
Amperometric	Rhodium NPs / chitosan	NR	NR	NR	1.6–8.2	0.5	NR	3.19	NR	10	(Gabrovska et al., 2011)
Amperometric	PANI/CNT/ nanocomposite film	Covalent immobilization	Pt electrode	NR	4–10	NR	13	4	NR	24	(Branzoi et al., 2012)
Amperometric	nanoporous silicon (NPSi)	Covalent immobilization	Au electrode	NR	0.3–4.5	NR	NR	2.05	NR	NR	(Yun et al., 2012)
Amperometric	NADH	NR	Carbon paste electrode	NR	0.02–0.2	0.005	NR	500	NR	20	(Yang et al., 2004)
Conductometric	porous glass	NR	NR	NR	5–50	NR	360	NR	NR	30–150	(Thavarungkul et al., 1991)
Conductometric	controlled pore glass (CPG), silica gel and Poraver	NR	NR	NR	1–150	0.5	240–300	NR	NR	210	(Limbut, 2004)
Conductometric	Sol-gel	NR	Au electrode	7.5	0.2–50	0.2	300–720	NR	NR	21	(Lee et al., 2000a, 2000b)
Conductometric	PPy	NR	Pt electrode	NR	0.05–5.8	NR	NR	140		25	(Ghourchian et al., 2004)

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Conductometric	PPy/bilayer lipid membranes (sBLM)	NR	Pt electrode	NR	Up to 120	0.5	NR	NR	NR	NR	(Hianik et al., 1998)
Conductometric	Poly(pyrrolepyridinium)/ Laponite clay gel	NR	NR	NR	Up to 1.5	0.01	NR	0.085	NR	7	(Senillou et al., 1999)
Conductometric	PANI /Poly(n-butyl methacrilate)(Pan-PBMA)	NR	Physical adsorption	NR	0.33–25	0.33	600	0.7	NR	NR	(Castillo-Ortega et al., 2002)
Conductometric	Cartridge/magnetoinductive conductive cells	NR	NR	NR	3.5–35	NR	NR	NR	NR	90	(Ciana and Caputo, 1996)
Conductometric	Controlled porous glass	NR	NR	NR	5–50	NR	300	NR	NR	350	(Shu and Wu, 2000)
Conductometric	Controlled Pore glass(CPG), Silica gel,Poraver	NR	NR	NR	Up to 150	0.5	240–300	51–53	NR	310	(Limbut, 2004)
Conductometric	Silicalite/Zeolite Beta	NR	NR	NR	Up to 6	NR	78	11.6	NR	7	(Kirdeciler et al., 2011)
Conductometric	Sol-gel	Au electrode	NR	7.5	0.2–50	0.2	240	8.2	NR	21	(Lee et al., 2000a)
Conductometric	Sol-gel	NR	NR	7.5	0.03–2.5	0.0300	480	204	NR	25	(Lee et al., 2000b)
Conductometric	Sol-gel	interdigitated electrode	NR	NR	0.01–5	NR	90	NR	NR	NR	(Sheppard et al., 1996)
Conductometric	NiO NPs/indium tin oxide (ITO) coated glass	NR	NR	NR	0.83–16.6	NR	6	21.3	NR	14	(Tyagi et al., 2013)
Conductometric	titania–zirconia(TiO2-ZrO2)	ITO	NR	NR	0.8–16.6	0.07	9.6	2.74	NR	NR	(Srivastava et al., 2013)
Conductometric	Gold NPs / poly(allylamine)	NR	Layer-by-Layer	NR	Up to 0.003	0.002	180	10–107	NR	NR	(Nouira et al., 2012)
Optical	PPy/Polyacrylamide	NR	NR	NR	60–1000	NR	120	0.013	NR	NR	(Marcos et al., 1999)
Optical	Triacetylcellulose membrane	NR	Entrapment	NR	1–500	1	60–300	NR	NR	60	(Krysteva and Hallak, 2003)
Optical	polyurethane film	NR	Entrapment	NR	0.7–8	20,000	16–20	NR	NR	NR	(Kovacs et al., 2003)
Optical	Sol–gel	NR	Encapsulation	NR	0.0025–5	0.0025	600	NR	NR	Unstable	(Tsai and Doong, 2005)
Optical	PVC-polyurethane	AISE	NR	NR	0.1–10	NR	240	0.03	NR	3	(Chen and Chiu, 2000)
Optical	PVP nanocomposite nanofibers Quantum dots	NR	Electrodeposition	NR	NR	0.01	NR	NR	NR	NR	(Crumbliss et al., 1992)
Optical	PVC-COOH	NR	NR	NR	0.3–100	NR	180	0.003	NR	21	(Koncki et al., 1995)
Optical	PB/4-(pyrrolyl)- benzoic acid	NR	NR	NR	2–16	NR	NR	NR	NR	60	(Koncki et al., 2001)
Optical	PB/4-(pyrrolyl)- benzoic acid	NR	covalent immobilization	NR	3–100	NR	NR	0.002	NR	60	(Koncki et al., 2001)
Optical	Prussian blue and N-substituted polypyrroles	NR	covalent immobilization	NR	0.2–100	100	NR	0.119	NR	60	(Koncki et al., 1999a, 1999b)
Optical	Sol-gel (TEOS)	NR	Encapsulation	NR	3.5–9	NR	300	0.07	NR	NR	(Olatowska-Jarza and Podbielska, 2002)
Optical	PAH/PSS nanofilms	NR	Encapsulation	NR	0.1–60	NR	480	0.09	NR	NR	(Swati et al., 2010)
Colorimetric	DEAE cellulose paper	NR	Ionic binding	NR	3.57–142.85	0.01	240–300	NR	NR	14	(Reddy et al., 2004)
ISFET	BSA/Glycerol	NR	NR	NR	Up to 2	NR	180	18	NR	NR	(Boubriak et al., 1995)

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ISFET	Poly(N-Vinyl pyrrolidone)	NR	NR	NR	0.05–20	NR	600	− 38	NR	40	(Rebriiev and Starodub, 2004)
ISFET	PVA Styrylpyridinium (SbQ)	controlled pore glass (CPG), silica gel and Poraver.	NR	NR	0.8–13	NR	120	132	NR	99	(Limbut, 2004)
ISFET	Clinoptilolite/Silopre ne	NR	NR	NR	0.03–0.5	0.0300	300	15	NR	NR	(Vadgama et al., 1992)
ENFET	1- [Zn-Al] LDH 2- Laponite	NR	N R	NR	0.04–1.37 0.005–0.5	0.0035 0.002	< 12 < 240	37	NR	NR	(Melo et al., 2002)
ISFET	[Zn-Al] LDH	NR	NR	NR	3–100	NR	NR	19	NR	NR	(Vial et al., 2006)
ISFET	Si_3N_4	NR	NR	NR	0.3–10	NR	72	66	NR	35	(Pijanowska, Torbicz, 1997)
ISFET	Al_2O_3-ISFET	NR	NR	NR	0–200	NR	NR	55	NR	NR	(Premanode and Toumazou, 2007)
Manometric	Free enzyme	NR	NR	NR	2–11	2	300	NR	NR	30	(Jenkins and Delwiche, 2002)
Manometric	Free enzyme	NR	NR	NR	0–100	NR	180	NR	NR	–	(Renny et al., 2005)
Piezoelectric	nanoporous alumina (Piezoelectric)	Adsorption	NR	NR	0.002–0.003	0.0002	30	NR	NR	30	(Yang et al., 2007)
Piezoelectric	PVC/C_{60}	adsorption	NR	NR	0.1–100	0.001	NR	15	NR	NR	(Wei and Shih, 2001)
Piezoelectric	Nylon nets	NR	NR	NR	0.01–3	NR	180	0.33	NR	28	(Xu et al., 1996)
Piezoelectric	Sol-gel (APTES)	NR	NR	NR	0.07–5	NR	180	NR	NR	NR	(Kondoh et al., 1996)
Piezoelectric	Nano porous alumina	NR	NR	NR	0.0005 μM/L	0.0002	30	NR	NR	36	(Yang et al., 2007)
Impedometric	Polyester nonwoven	(p(NIPAAm-NAS-HEMA)	Cross-linking	NR	NR	NR	NR	21	NR	NR	(Chen and Chiu, 2000)
Impedometry	CNT-PPy	NR	NR	NR	0.0006–1	0.6	30	NR	NR	30	(Bourigua et al., 2011)
Chemiluminesence		NR	NR	NR	0.004–0.4	2	300	7 I	NR	5	(Qin et al., 2000)
Thermal	Alumina plates	NR	NR	NR	0.5–15	0.5	NR	2.5	NR	NR	(Bjarnason et al., 1998)
Thermal	Quartz chip	NR	NR	NR	1–25	NR	NR	1.2	NR	NR	(Xie and Danielsson, 1996)
Thermal	Agarose beads	NR	NR	NR	0.5–20	0.5	15	2.6	NR	NR	(Xie et al., 1995)

Iridium oxide films (IROF), NR = Not reported.

formation of a jet. The jet was subsequently stretched to form a continuous fiber. Before it reaches the concurrently charged collecting screen, the solvent evaporated or solidified. The fibers were collected on a conductor surface and formed non-woven mats that were characterized by high surface areas and relatively small pore sizes. This improved the adsorption properties and enhanced the sensitivity of the biosensor. Urease-PVP nanofibers prepared by this approach achieved a linear concentration range of 0.5–2.5 mM, and a response time between 10 and 30 s. In another interesting study, urease was immobilized in mixed monolayers of poly (N-vinyl carbazole) and stearic acid formed at an air/water interface. The monolayers were transferred onto indium-tin-oxide (ITO) coated glass plates using Langmuir/Blodgett film deposition technique. This technique for monolayer deposition is known to facilitate the desired orientation of a biomolecule. Only a few investigations have been reported by this approach. The resulting biosensor gave a linear concentration range of 0.5–93 mM and the shelf-life was up to 5 weeks at 4 °C.

Rebriiev and Starodub (2004) developed a simple and rapid method for the immobilization of urease on a ISFET gate surface based on the use of liquid photo-polymerisable composite (LPhPC) as a target material. N-vinyl pyrrolidone was used as a principal component of the LPhPC and was applied for immobilization of urease. The potentiometric biosensor gave a linear concentration range of 0.05–20 mM, a sensitivity of 38 mV/decade and was stable for about 40 days. Eggenstein et al. (1999) developed a potentiometric urea-sensitive biosensor based on a NH^{4+}-sensitive disposable electrode in a double matrix membrane. The ion-sensitive polymer matrix membrane was formed in the presence of an additional electrochemical inert filter paper matrix to improve the reproducibility of the sensor production. Poly(carbamoylsulfonate)hydrogel, produced from hydrophilic polyurethane pre-polymer blocked with bisulfite, served as the immobilization material in urea biosensor. The biosensor was successfully used for the direct determination of urea in blood samples. There was a good agreement between the results obtained with the biosensor and a spectrophotometric method. The biosensor achieved a LOD of 20 μM and a linear concentration range of 72 μM-21 mM. Narinesingh et al. (1991a, 1991b) also used SiO2 thin films on an Indium Tin Oxide(ITO)-FET to fabricate pH sensors. Photocross linkable PVA bearing styrylpyridinium groups was then used to immobilize urease on the electrode. The resulting biosensor was stable for longer than 99 days and a linear concentration range of 0.8–13 mM was achieved for urea determination. Boubriak et al. (1995) constructed a urea biosensor by immobilization of urease into to a BSA/glycerol membrane on the surface of an ISFET. However, the resulting potentiometric biosensor was not very sensitive achieving a sensitivity of 8 mV/mM with a linear concentration range from 0 to 2 mM. Nevertheless, the biosensor was successfully applied to the determination of urea in rat and rabbit blood serum samples. Using a very different approach, Xu et al., (1995, 1996) constructed a urea biosensor with a series piezoelectric crystal (SPC) device. This involved covalent immobilization of urease layer on nylonnets or by fixing jack bean tissue slices on the probe surfaces of the SPC ammonia sensor (Yuanjin et al., 1996). The urease sensor exhibited favourable frequency response to 0.01–3 mM urea with a response.

5.3. Enzyme nanoparticles based urea biosensor

The nanoparticles (NPs) aggregates of commercial urease from jack beans (Canavalia ensiformis) were prepared by desolvation and glutaraldehyde crosslinking and functionalized by cysteamine dihydrochloride. The biosensor exhibited optimum response within 10 s at pH 5.5 and 40 °C. The biosensor was employed for measurement of potentiometric determination of urea in sera of apparently healthy and persons suffering from kidney disorders. The biosensor displayed a low detection limit of 1 μM/L with a wide working range of 2–80 μM/L (0.002–0.08 mM) and sensitivity of 23 mV/decade. The analytical recovery of added urea in serum was 106.33%. The within and between-batch coefficient of variations (CVs) of present biosensor were 0.18% and 0.32% respectively. There was a good correlation (r = 0.99) between sera urea values obtained by reference method (Enzymic colorimetric kit method) and the present biosensor. The ENPs bound NC membrane was used maximally 8–9 times per day over a period of 180 days, when stored in 0.01 M sodium acetate buffer pH 5.5 at 4 °C (Jakhar and Pundir, 2018).

6. Summary & conclusion

In this review, we have provided a brief comprehensive literature/report of successful fabrication of urea biosensors, in which careful consideration of optimum factors including the choice of enzyme/bioactive substances, method of immobilization and transduction mode was undertaken.

This review presented some of the evident works in the development of urea biosensors with an emphasis on the types of immobilization techniques and the different types of transducers used. The comparative analytic features of the various urea biosensors have been summarized in Table 1 for an easier overview.

The study highlights the importance of urea biosensors encompassing its fields of application from analysis of clinical samples, to food safety, to heavy metal analysis. Urease in urea biosensor, catalyzes the hydrolysis of urea producing NH_4^+ and HCO_3^-causing a pH increase in the aqueous reaction medium. Specific transducers can detect NH_4^+, NH_3 gas and CO_2, or pH changes. Immobilization of urease onto a suitable matrix is crucial in developing an electrochemical urea biosensor. Many electrode materials, such as polymers, sol–gels, conducting polymers, Langmuir–Blodgett films, nanomaterials, and self-assembled monolayers have been used to obtain enzyme biosensors. Recently, composite materials based on conducting polymers, redox mediators, metal nanoparticles, nanocomposites, and nanoclusters have been used to combine the properties of the individual components with a synergistic performance in biosensor fabrication. However, biosensors containing electrochemical transducers are the most used and in these cases urea is detected via either potentiometric or amperometric methods. This first one has attracted a lot of attention due to both its effectiveness and simplicity of preparation. Potentiometric biosensors appear promising in determining urea concentration due to their effectiveness and simplicity (Emami and Haghjoo, 2014). Without doubt, the various urea biosensors that have been reported to date have had many positive impacts in the analysis of urea in various samples such as urine, blood, milk, wine, coastal waters, fertilizers, soil, cosmetic and pharmaceutical samples.

7. Future perspectives

Urea sensors have the potential to be a billion-dollar market and the technology needs improvement in biological stability, signal transduction, precision and cost effectiveness. The development of cheap and disposable array biosensors for the simultaneous detection of clinically important metabolites and rapid screening of renal related diseases is still needed (Pundir and Narwal, 2018).

The use of biological molecules for NPs formation and nanowires has shown great promises in the future of bio sensing and designing of bioelectronics systems. We believe that the use of bio catalytic nanostructure growth, using dip-pen nanolithography as a patterning method and biomolecules as templates for nanostructure synthesis, has great promise in future nanotechnologies. Moreover, we can miniaturize the urea biosensors and design them as a wearable sensor to benefit the health sector more effectively.

Conflict of interest

There is no conflict of interest among the authors.

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