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Review

A review of recent advances in non-enzymatic electrochemical creatinine biosensing

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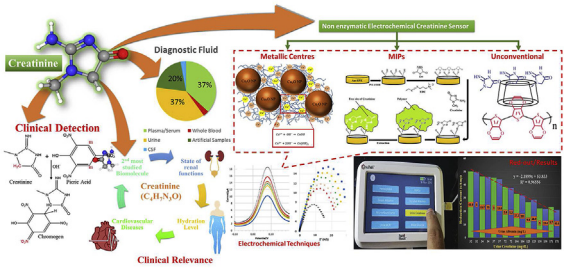
HIGHLIGHTS

- The review encompasses non-enzymatic electrochemical creatinine detection depending upon the modified electrode.
- The use of nanoparticles for creatinine detection and the underlying principle of creatinine as a ligand is elaborated.
- The review also evaluates few unconventional detection approaches such as non-enzymatic potentiometric creatinine detection etc.

GRAPHICAL ABSTRACT



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ABSTRACT

Creatinine biosensing is a rapidly developing field owing to the clinical relevance of creatinine as a vital biomarker for several diseases associated with renal, thyroidal, and muscular dysfunctions. Over the years, we have observed numerous creatinine biosensing strategies, including the most widely studied enzymatic creatinine biosensors. Though the enzymatic approach provides excellent selectivity and reliability, it has certain drawbacks, which include high fabrication cost and poor storage stability (that is inherent to every enzyme-based biosensors). This has led to the development of non-enzymatic creatinine biosensors, of which electrochemical sensors are the most promising for point-of-care applications. However, only a limited number of studies have been conducted and there is a lack of reviews addressing the recent advances in this research area. Herein, we present for the first time, a review with a prime focus on the various strategies implemented in non-enzymatic electrochemical creatinine biosensing. We aim to offer a comprehensive context on the achievements and limitations of currently available non-enzymatic electrochemical creatinine biosensors and address the underlying factors pertaining to the interplay of modification/fabrication techniques with the sensitivity, selectivity, interferences, and long-term storage stability of the biosensor. We hope that this work shall prove to be seminal in the conception and advancement of future non-enzymatic electrochemical creatinine biosensors.

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Contents

1. Introduction

Creatinine is one of the most clinically analysed biomolecule coming second only to glucose as it is widely accepted as an important biomarker for various renal and cardiovascular diseases [1]. For instance, creatinine concentration is analysed in a typical blood test as an indication of the patient's renal functions. Creatinine levels are also associated with the estimated glomerular filtration rate (eGFR), based on which, organizations such as Kidney Foundation in New York classify five different stages of Chronic Kidney Disease (CKD). Advancement in CKD and the consequent cardiovascular diseases is estimated to directly attribute towards 1.2 million deaths, 19 million disability-adjusted life-years, and 18 million years of life lost. Additionally, creatinine is vital in the early detection of muscular diseases [2], monitoring post-surgery renal functions and, is also a direct indication of the body's hydration level [3,4]. Owing to such clinical relevance, creatinine has been established as an important diagnostic biomarker.

Traditionally and up until today, creatinine has been clinically analysed using the colorimetric Jaffé method at centralized laboratories. However, in recent years, reports of creatinine biosensors have surfaced which are cost-effective, reliable, efficient, and amenable to point-of-care (POC) settings. Unfortunately, there is limited literature that concisely collects and summarizes the vast techniques used for the detection of creatinine. Killard and Smith [5] summarized enzyme based potentiometric and amperometric biosensors available till 2000 and discussed the advantages and drawbacks of these enzymatic creatinine sensors. A review by Shephard [6] highlighted numerous creatinine POC devices using blood, serum and plasma reported till 2011. Mohabbati-Kalejahi et al. [7] published a study in 2012 which stressed the improved limit of detection (~0.28 nM) provided by implementing chromatographic techniques, with insights on fast response times for potentiometric electrodes and the enhanced sensitivity of molecular imprinted polymer-based detection methods. Randviir and Banks concisely gathered and discussed the available enzymatic and non-enzymatic creatinine detection methodologies reported up till 2013 [8] Pundir et al. [9] highlighted the state of enzymatic and non-enzymatic electrochemical creatinine detection and mentioned, in brief, the use of nanomaterials for sensing applications. Cánovas et al. [10] eloquently discussed the modern techniques for POC detection of creatinine and the various challenges it faces. This study also addressed the multitude of biological fluids exploited for creatinine detection. Evidently, it appears that all available reviews on creatinine detection focuses exclusively on enzymatic electrochemical creatinine detection, with only trivial mentions about the recently developing field of non-enzymatic electrochemical creatinine biosensors.

Herein, we present for the first time a detailed review with the prime focus being non-enzymatic electrochemical creatinine biosensing. The review is divided into eight major parts. The first section comprises of a brief introduction to the clinical versatility of creatinine and the available reviews on creatinine detection. The second section introduces the reader to the background knowledge required to comprehend the working and the development of creatinine biosensors, including the chemical structure of creatinine and the various biofluids in which creatinine can be detected. The third section discusses creatinine detection methods

(colorimetric and electrochemical), followed by a section addressed to non-enzymatic electrochemical creatinine detection via metallic centres, which is sub-divided into two sections that comprise of copper-based metallic centres and other metallic centres. The next section highlights the non-enzymatic electrochemical creatinine detection based on the use of MIPs (Molecularly imprinted polymers), discussing in detail the use of MIP grafted electrodes and nanoparticles modified/enhanced MIP based sensors. This section also introduces the different electrochemical detection techniques exploited in MIP based creatinine biosensing, such as EIS (Electrochemical Impedance Spectroscopy), DPV (Differential Pulse Voltammetry), SPCSV (Scanning Pulse Cathodic Stripping Voltammetry). This is followed by a section about unconventional creatinine biosensors including a non-enzymatic potentiometric detection scheme and a biosensor with enhanced electron transfer activity based on surface modification via pre-anodization. The subsequent section summarizes the commonly used pre-treatment processes required to remove interference which plays a crucial role in the enhancement of the selectivity and specificity of the creatinine biosensor. The final section gives an insight into the current status of non-enzymatic electrochemical creatinine biosensors and their future outlook.

2. Background knowledge for creatinine detection

2.1. Discovery of creatine and creatinine

Creatine (2- [Carbamimidoyl (methyl) amino] acetic acid) is a nitrogenous organic acid present naturally in all vertebrates. It is typically synthesised in the liver, pancreas and kidneys. Creatine functions as the primary supplier of energy in the form of ATP during muscular activity where it is metabolized to creatinine. Michel Eugene Chevreul, a French scientist first discovered creatine in 1832 when he extracted a new organic compound from meat and named it creatine after the Greek word for flesh “Kreas”. Later in 1847, Justus von Liebig, established that creatine was a consistent component of animal flesh. In the mid-1880s, creatinine (2-amino-1-methyl-imidasolidinone) was discovered in the urine, and later researchers speculated that creatinine was derived from creatine and was related to the total muscle mass.

2.2. Creatine/creatinine metabolism and its clinical relevance

Creatinine ($C_4H_7N_3O$) is a nitrogenous natural compound that can be considered as a cyclic derivative of creatine or as a condensation product of guanidine and glycolic acid. Creatinine is found in muscle tissues and blood and is typically discharged in urine as metabolic waste. Urinary discharge of creatinine is moderately consistent from day to day and reflects the amount of muscle tissue in the body. The mechanism and pathway of the interconversion of creatine to creatinine and vice versa is still not very well comprehended. However, it has been reported that the metabolism of creatine to creatinine in vertebrates is, for the most part, a spontaneous, nonenzymatic, monomolecular process (in both directions), as illustrated in Fig. 1 [11]. The vitro equilibrium is both pH dependent and temperature dependent and starting with pure creatine solutions, 1.0–1.3% of the creatine per day is converted into creatinine at pH 7.0–7.2 and 38 °C [11]. Creatine is favoured at high pH and low temperature, whereas Creatinine is favoured at elevated temperatures and in acidic solutions [12]. Moreover, creatinine is known to be a poor substrate for the creatine transporter [13,14], thus is devoid of uptake mechanism of any kind [15] and furthermore, due to its non-ionic nature, creatinine becomes membrane permeable, which results in creatinine being constantly diffusing out of the tissues into the blood, which then is excreted by the kidneys into the urine.

Though the mechanism is not completely understood, it is well established that based on the muscle mass the net amount of creatinine in the human body is fairly constant [9,16] and consequently its level in excreted urine as well as in blood is also quantifiably constant. Typically, in a healthy individual, the creatinine concentration in blood/serum ranges from 60 to 110 μM for men and 45–90 μM for women. However, creatinine levels may reach well over 500 μM, which is a clear indication of extreme renal dysfunction that requires immediate intervention via dialysis or kidney transplants [5]. Due to the asymptomatic nature of CKD (Chronic Kidney Disease), early diagnosis is of utmost importance and hence routine clinical analysis of creatinine level is crucial [17]. It is noted that creatinine levels must be analysed based on the total muscle mass, as different muscle contents can translate to different ranges of healthy and harmful creatinine levels. For instance, the value of creatinine level in a healthy young male with high muscle mass may well correspond to a harmful creatinine level in a female.

Moreover, it has also been observed that consistently high creatinine levels relate to higher risk of mortality [18]. Creatinine serves as a valuable biomarker to diagnose and prevent mortality due to renal disorders and therefore is the second most clinically studied biological compound after glucose [1]. A high creatinine level is also indicative of side effects of drugs like acetylcholine inhibitors, cyclosporine and clinical treatments such as chemotherapy, which can cause kidney damage and result in elevated creatinine levels [19–21]. Numerous studies have reported the presence of quantifiable amounts of creatinine in various biological fluids, including the most commonly used blood/serum and urine [22,23], which can be utilized to analyse the risk of renal dysfunction and various other diseases including muscular diseases like Duchenne muscular dystrophy [2]. In the next section, a range of biological fluids are discussed that can be clinically exploited to access creatinine levels.

2.3. Biological fluids applicable for creatinine detection

Creatinine has been detected and quantified in various biological fluids and its levels are studied to indicate renal functions and other related cardiovascular diseases [8]. Fig. 2 shows currently available techniques and the biological fluids that are employed for creatinine detection [26]. The presence of creatinine in such a multitude of biological fluids is due to its membrane permeable nature and the participation in numerous metabolic pathways [16], furthermore depending on the biological fluid the healthy and harmful creatinine levels also varies (Table 1). Some of the most extensively used biofluids exploited for creatinine detection are briefly discussed.

2.3.1. Blood/serum

Blood or serum is one of the most widely used diagnostic fluids for clinical analysis of creatinine. In fact, creatinine detection is generally included in a typical blood test as a measure to assess renal functions. Serum based creatinine detection is traditionally based on the Jaffé reaction, which is a colorimetric method based on the chromogen formation due to the reaction of alkaline picric acid and creatinine [29]. Colorimetric creatinine detection using whole blood is avoided due to the inherent red colour of blood and consequently serum as the detection fluid is utilized [28]. Serum has been proven to be a robust detection fluid which can be used to quantify creatinine via numerous methods, involving colorimetric as well as electrochemical detection techniques [23,30]. Due to its solubility in blood plasma and the membrane permeability property, creatinine can transport out of muscle cells and dissolve into the blood stream with ease, which is accepted as the main reason

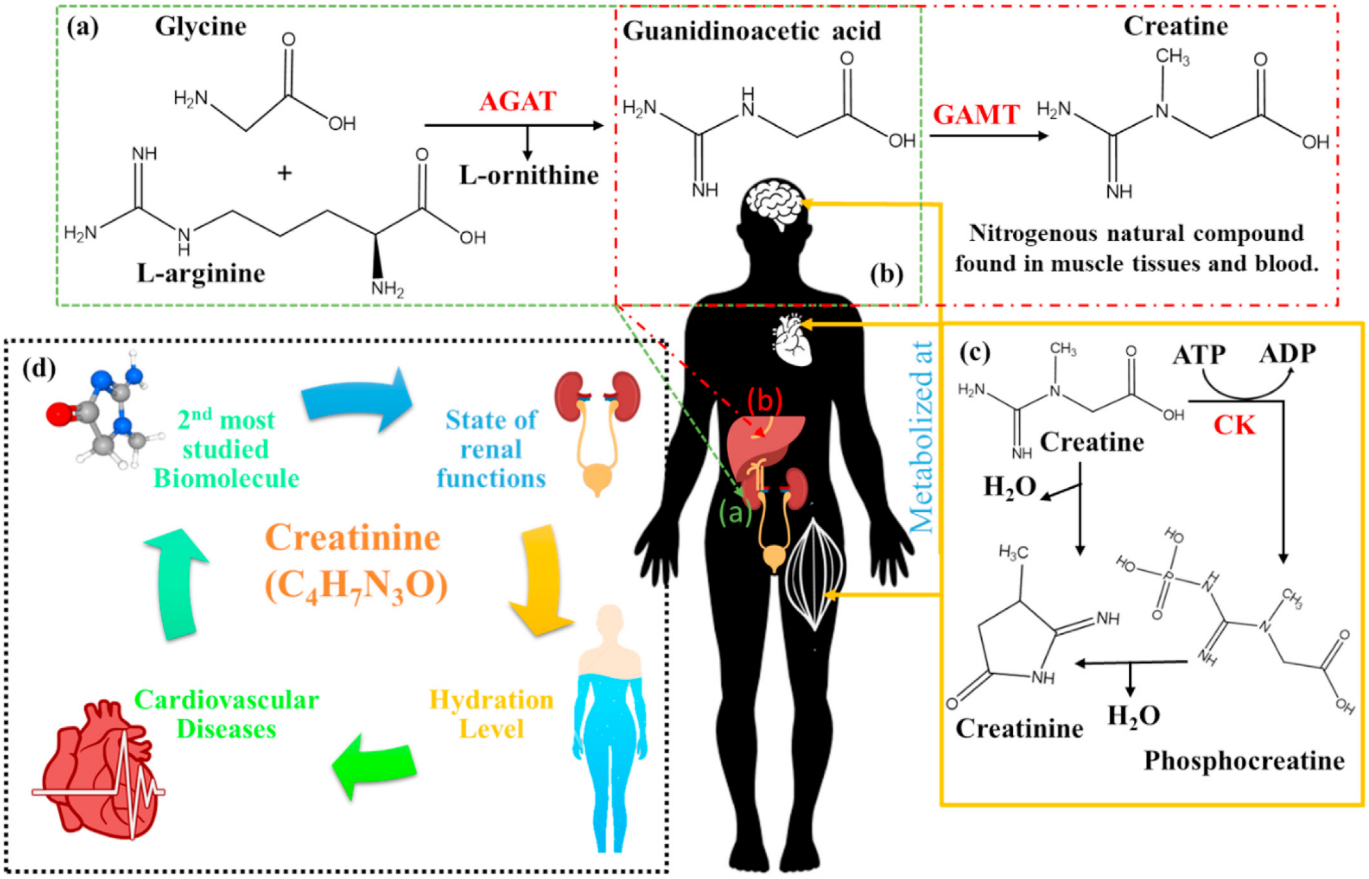

Fig. 1. (a) Initiation of creatine biosynthesis at the kidneys, via the reaction Glycine and L-arginine in the presence of AGAT(L-arginine glycine amidino transferase), producing GAA (Guanidinoacetic acid), (b) GAMT(S-adenosyl-L-methionine N-guanidinoacetate methyltransferase) catalysed methylation of GAA in the liver to give creatine, (c) Creatine is typically metabolized in muscles and brain to generate creatinine as a waste product, whereby muscular Creatine or Phosphorylcreatine (which is generated from creatine in the presence of CK i.e., creatine Kinase and ATP) is converted to creatinine, (d) Clinical versatility of creatinine, highlighting its association to renal functions and detection of cardiovascular dysfunctions.

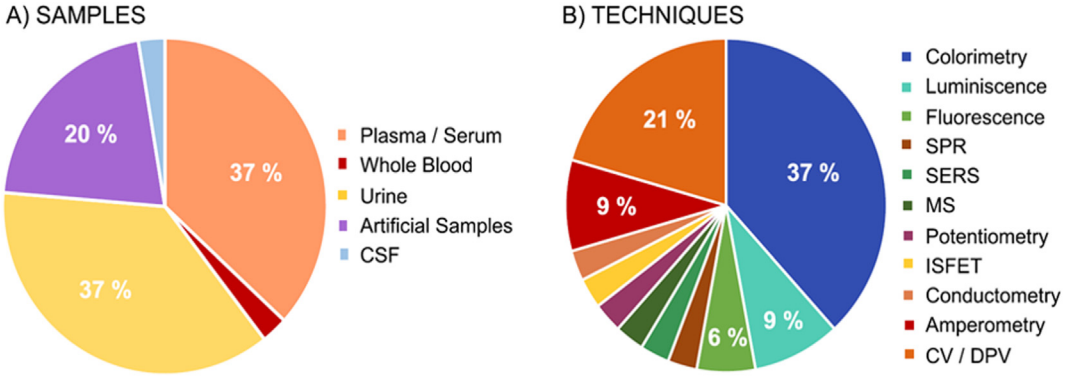

Fig. 2. Summary of (A) Diagnostic biofluid and (B) Techniques/methods used for analytical creatinine detection. [CSF: Cerebrospinal fluid, SPR: Surface plasmon resonance, SERS: Surface enhanced Raman Spectroscopy, MS: Mass Spectrometry, ISFET: Ion-sensitive field-effect transistor, CV: Cyclic Voltammetry, DPV: Differential Pulse Voltammetry]. Reprinted with permission from Ref. [10].

for the presence of creatinine in blood. Conventionally, for a healthy individual creatinine levels in blood ranges from 45 to 90 μM for females and 60–110 μM for males [25]. However, the level rises as a direct result of renal dysfunction. Creatinine level >150 μM is considered a clear indication of kidney damage and in extreme cases may reach well over 500 μM inferring high degree of kidney damage requiring immediate intervention via dialysis treatment and/or kidney transplantation. Additionally, blood/serum-based analysis can also generate various other clinically relevant information, such as the BUN-Cr ratio, which is the ratio of two serum laboratory values, the blood urea nitrogen (BUN) (mg/dL) and serum creatinine (Cr) (mg/dL). An abnormally high BUN-Cr value stipulates renal dysfunction as a result of kidney stones or tumours while low BUN-Cr value is indicative of low protein intake and critical liver damage [10].

Table 1
Healthy and harmful creatinine levels in various biological fluids.

Ref	Biological Fluid	pH range[1]	Healthy creatinine range[2]	Harmful Creatinine range
[24]	Urine	4 to 8	4.4–18 mM	>20 and <3 mM
[25]	Blood Serum	7.4 ± 5	$^{(M)}$45–90 μM $^{(F)}$60–110 μM	<40 μM and >150 μM*
[25,26]	Sweat	4 to 6.8	9.4–18 μM	60–200 μM
[27,28]	Saliva	6.2 to 7.4	4.4–17.7 μM	17.7–591 μM

Abbreviations.
[1] The range of pH can vary with dilution or other pre-treatment process, urine pH may show drastic change during infections [25].
[2] (M) = Male, (F) = Female.
* May reach over 500 μM in extreme cases that need immediate dialysis.

2.3.2. Urine

Creatinine detection in urine, which involves analysis of urine samples collected over a 24-h period, is also very common. Urine based creatinine analysis generally follow two methods:

(a) Using the Cockcroft-Gault formula (Eqn (1)) which predicts the creatinine clearance from serum creatinine. In this method, the age of the patient is considered to be fixed as 73 for male and 85 for females (which is substituted for "A" in equation (1)).

$$\text{Creatinine clearance} = \frac{(140 - \text{age})\text{x weight}}{2\text{A}[\text{Creatinine in Serum}]} \quad (1)$$

(b) Using a comparative analysis which considers both the creatinine levels in blood as well as the average creatinine level in urine samples collected over a 24-h time period. This method is generally more accurate as it reflects creatinine levels from two different sample types instead of only one.

Additionally, creatinine levels in urine are known to change as a function of hydration and other factors [10] and hence an elaborate analysis of samples collected over a period of 24-h is employed. Due to its non-evasive nature, urine is favoured against blood, however blood based samples provide much more rapid and clinically relevant results [31]. Urine samples can provide insights on kidney conditions based not only on creatinine detection but also via other approaches such as detection of albuminuria (presence of small amounts of albumin in urine), especially in patients at increased risk for CKD such as those with hypertension, diabetes, cardiovascular disease and a family history of CKD [32,33]. Diagnosis of albuminuria also requires periodic collection of urine over 24-h, which is cumbersome in routine clinical practice. One common problem associated with the quantitative measurement of urine biomarkers such as albumin, is that an appropriate correction factor is needed for variations in urine concentration. This problem can be solved by normalizing the measured concentration of a urine analyte with respect to urine creatinine concentration [34]. This leads to an alternative method of spot urine measurement of either albumin concentration or an albumin to creatinine ratio (UACR), which are relatively easy to obtain in a point-of-care setting. In general, albumin to creatinine ratio has been shown to have slightly better diagnostic accuracy than simply urinary albumin. UACR is defined as the ratio of milligrams of Albumin to grams of Creatinine per decilitre of urine, which is a representative of the amount of Albumin excreted per day in milligrams as shown in equation (2). Unlike a dipstick test for albumin, UACR is unaffected by variation in urine concentration. UACR levels above 10 mg/g indicating higher mortality risk due to kidney dysfunction and other cardiovascular diseases [35].Thus, urine based detection of creatinine using UACR can prove to be versatile tool in diagnostics of kidney dysfunctions and cardiovascular diseases as well as early detection of CKD [16,36].

$$x = \frac{\text{Urine Albumin (mg/dL)}}{\text{Urine Creatinine (g/dL)}} = \text{UACR in mg / g} \approx \text{Albumin excretion in mg / day} \quad (2)$$

2.3.3. Saliva

Saliva as a diagnostic fluid offers a very convenient non evasive collection method that has several advantages like low cost, applicability to all ages and the usability to conduct tests on a large population with ease [37]. Moreover, saliva is reported to exhibit analogous diagnostic results relative to the ones provided by blood-based analysis for various proteins and biomarkers [25,37–40], thereby proving a possible alternative for blood-based detection. Creatinine is present in saliva predominantly due to ultrafiltration and the level in saliva is about 10–15% relative to what is typically found in blood. Studies have reported a 90–95% accuracy based on creatinine levels detection in serum and saliva [17,28]. Healthy creatinine levels in saliva typically vary from 4.4 to 17.7 μM, while levels in the range of 17.7–591 μM indicate decreased renal functions. Though promising, saliva as a diagnostic fluid faces empirical assessment [41] due to the fact that salivary concentrations of analytes generally fluctuate and/or vary depending not only on the gender but also diet and genetics [42,43]. Additionally, of all the studies that reported saliva-based creatinine detection, very few were validated through a standard method, thereby questioning the reliability of saliva as a biofluid for creatinine detection.

2.3.4. Sweat

Sweat contains a wide range of biomarkers [44] with potential clinical interest. In cases of kidney damage, sweat play a major role in elimination of biological wastes. Moreover, using sweat as a detection medium presents a possible approach utilizing wearable sensors with sample collection in a non-invasive manner [45]. However, sweat based detection has numerous inherent disadvantages including the requirement of higher temperatures to induce an increased sweat production. Furthermore, there can be error in estimation due to unavoidable evaporation, thereby affecting sample volume while the net creatinine concentration remains unchanged, thus resulting in an overestimated result.

2.3.5. Tears

Tears have also been investigated for potential application as a biofluid for creatinine detection. A previous study conducted an examination of creatinine levels in blood and tears before and after haemodialysis treatment, and showed a 48% and 36% decrease in creatinine level in blood and in tears respectively [46]. Though tears contain various biological compounds like electrolytes, proteins, glucose etc. [47], creatinine is present only in trace amounts owing to its large size and solubility [48]. Furthermore, since details on the concentration range in healthy individuals as well as in patients

with renal dysfunction are currently unavailable, the use of tears as a potential diagnostic biofluid for creatinine detection is not a viable option and still requires further investigations.

3. Creatinine detection methods

Being a waste product of creatine metabolism, creatinine levels in the body is a direct reflection of the state of the kidneys and can relate to detection of various other muscular (cardiovascular) and thyroidal dysfunctions [49]. Owing to such clinical versatility, numerous creatinine detection methods have evolved over the years. The very first creatinine detection scheme was based on colorimetry; hence, to improve coherence and the background knowledge of the reader, we briefly introduce Colorimetric Creatinine detection, state its limitations and then move on to Electrochemical creatinine detection and how the latter is amendable to POC settings.

3.1. Colorimetric creatinine detection

Traditionally, almost all colorimetric detection of creatinine has been based on the Jaffé method which was the very first colorimetric creatinine detection method reported in 1886 [29]. Max Jaffé used potassium dichromate as an indicator to calorimetrically elucidate the amount of creatinine against a known standard. This method was later improved upon by Folin and Morris [50] wherein 1 mL of sodium hydrate solution of creatinine and picric acid was used as a standard and compared to 1 mL of urine sample which had been prepared in the same manner. Consequently, the colour of these two samples gave an evaluation of the creatinine concentration. This reaction of alkaline picric acid with creatinine that generates a chromogen with the characteristic red colour was named the Jaffé Reaction. This reaction follows a nucleophilic mechanism wherein the active ethylene group of creatinine acts as a nucleophilic donor and attacks the meta position of alkaline sodium picrate to give the characteristics red-yellow chromogen. In simpler words, the methylene anion of creatinine attacks the meta position of picric acid (Fig. 3), resulting in the formation of a red-yellow nitro anion, which is then spectrophotometrically analysed to quantify the concentration of creatinine.

However, colorimetric creatinine detection using Jaffé method has been proven to be non-specific in nature and the sensitivity of the method itself was found to be affected by variables including pH and temperature [51]. For e.g., 1 °C produces an error of ±0.7 μM in the estimated creatinine concentration, which can lead to erroneous decision-making in cases of a potential decrease in muscle mass [16,52]. However, due to the rapid analysis (~15 min per sample) and the method being compliant to automation, Jaffé method remains the gold standard of clinical creatinine detection. Though the Jaffé method is widely accepted as the gold standard for creatinine detection, one must also be aware of studies which have reported notable differences in results (3–10%) when compared to results from other analytical techniques, thereby questioning the accuracy of Jaffé method [51]. Moreover, the toxic and explosive nature of picric acid calls for skilled operators to countermeasure human error. Early studies have made extensive efforts to overcome the disadvantages of using picric acid. One such study was reported by Maw in 1948 which proposed the use of butanol, sec-butanol and collidine based chromatographic separation of creatinine from other interfering agents (that are Jaffé positive) followed by Jaffé method to quantify only the separated creatinine [53]. And studies as recent as 2018 by Alula et al. [54] reported colorimetric detection schemes using citrate covered silver nanoparticles (Ag NPs) that promote alkaline creatinine aggregation at an elevated pH of 12.

It must be noted that the Jaffé method cannot be directly used for creatinine detection in whole blood samples due to the simple fact that blood is red in colour. Moreover, the interference due to various components of the blood make an accurate analysis virtually impossible. Consequently, serum is typically used [55,56]. Urine samples on the other hand do not face problems due to coloration. is However, to avoid interference a 5-20-fold dilution, which has an inherent risk of generating error, is generally performed prior to analysis which is not required for serum-based samples. Though the Jaffé method is the most commonly used colorimetric assay of creatinine, several other assays were also studied. For instance, derivatives of 3,5-dinitrobenzioc acid have been reported. Sims et al. [57] reacted methyl-3,5-dinitrobenzoate with a mixture of dimethyl sulfoxide, methanol, and tetramethyl ammonium hydroxide, that resulted in a purple-rose colour. A pretreatment involving protein precipitation was utilized that significantly increases the specificity over the picric acid procedures. Another assay using the Skaguchi colour reaction [58] was reported based on the red coloured chromogen generated during the reaction of creatinine with o-nitro benzaldehyde in the presence of alkali, which forms oxalyl-methyl guanidine that is subsequently converted to methyl guanidine by heating after neutralization. This reaction reportedly eliminated the possibility of non-specific chromogens encountered in the Jaffé method; thereby reducing chances of false positives.

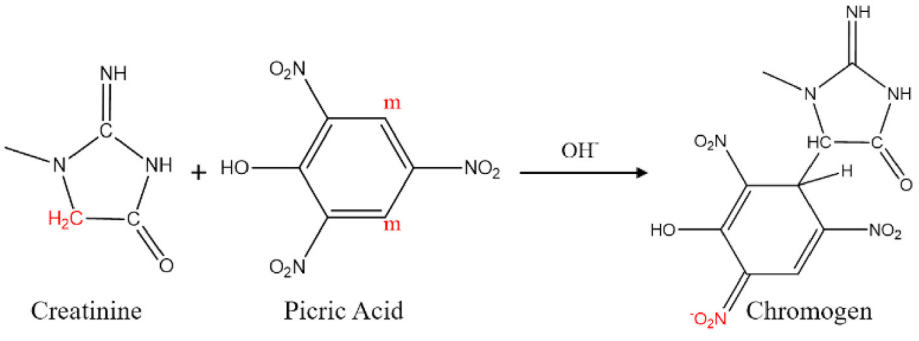

Fig. 3. Schematic representation of nucleophilic attack of the active methylene group of creatinine to the meta-position of picric acid in alkaline medium to give the red-yellow chromogen. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.2. Electrochemical creatinine detection

An extensive study of available literatures on creatinine detection suggests a growing popularity of electrochemical creatinine sensors. Based on the type of electrochemical techniques used, creatinine sensors can be broadly classified into amperometric and potentiometric sensors. Such classification has certain limitations considering both amperometric and potentiometric sensors have traditionally utilized enzymes. Herein, we have made a classification based rather on the nature of sensing mechanism and the sensing components and not on the electrochemical technique implemented. We have categorized electrochemical creatinine sensors into two categories, namely enzymatic and non-enzymatic. The limitations of enzymatic creatinine sensors led to the development of non-enzymatic creatinine sensors. Hence, to better illustrate the advantages of non-enzymatic creatinine sensors, one must first be familiarized to the basic working and design of enzymatic creatinine sensors followed by the reasons behind its limitations and how the advancements of non-enzymatic creatinine sensors strives to overcome said limitations (extensively addressed in the sections ahead).

3.2.1. Enzymatic creatinine sensors

The development of early electrochemical creatinine biosensors was kick started in 1976 by Meyerhoff and Rechnitz, when they developed a potentiometric creatinine sensor based on an ammonium ion-selective electrode. An enzyme catalysed reaction of creatinine iminohydrolase and creatinine results in N-methylhydantoin and ammonia [59]. This sensor however had a very limited lifetime of only 4 days and clinically irrelevant detection range of 0.52−9.6 mM. Such potentiometric sensors offer geat simplicity not only due to the use of a single enzyme but also due to the use of fairly well established gas sensing electrodes, nevertheless they had several flaws including the inability to detect the lower reference range for creatinine, suffered interference from endogenous NH_4^+ and exhibited poor long-term stability [5]. Continuous efforts were made to remedy all such shortcomings. For instance, Mascini et al. [60] significantly increased the operational range as well as the storage stability of such potentiometric gas sensors, to reach analytical ranges required to report normal, as well as pathological specimens. However it still fell short to reach commercial requirements.

On the other hand, amperometric biosensors based on the three-enzyme method have gained considerable attention. This method was first reported in 1983 by Tsuchida and Yoda [62], which exhibited robust stability of 500 assays requiring only 20 μL sample each time, a wide linear range of 1−100 mg/L and a response time of 20 s. They reported an enzyme electrode system for creatinine detection in human serum. This multi-enzyme sesnor consisted of three enzymes namely, creatinine amidohydrolase (CA), creatine amidinohydrolase (Cl), and sarcosine oxidase (SO), which performs a three stage conversion of creatinine to creatine, creatine to sarcosine and a final step that coverts sarcosine to glycine as shown in Fig. 4.6, thus giving detectable H_2O_2. Similar studies that used oxygen electrodes instead of peroxide detecting electrodes were also reported [63,64]. Traditionally, enzymatics sensors use Pt-anode and Ag-cathode with the enzymes immobalised between an inner assymetric membrane and an outer porous polymeric membrane (generally poly-carbonate) as shown in Fig. 4.2. The choice of Pt-electrode is based on constant reports of platinum exhibiting the best catalytic surface for the electrochemical oxidation of H_2O_2. Recent studies have reported that electordes fabricated by depositing Pt on Au have been observed to give the highest current response [61,65]. The method of enzyme immobalization has been observed to influence the sensor's analytical range and storage stability. Enzyme based sensors have an inherrent disadvantage of poor stability. To enhance its stability, the immobalization step has been thoroughly investigated. This commonly includes (i) gel-entrapment [66] that results in the enzyme trapped in an aqueous medium (ii) polymer entrapment [67] wherein the enzyme is entrapped in the electro-active (e.g. polyaniline), or non-electroactive (e.g. Nafion®) polymer matrix due to its growth, (iii) direct non-immobilized deposition [68] which involves a non-covalent interaction of the enzyme with the electrode surface and the method that provides most stablity, and (iv) crosslinking [65] (refer Fig. 4) which as the name suggest invloves the crosslinking of the enzyme(s) onto the electrode in the pressence of crosslinking agents or a polymeric film (e.g. Gelatin film). Evidently, even with all the efforts, enzymatic creatinine sensors never reached their full commercial promise, owing to the poor storage stability and restricted sensing conditions that are not ideal, especially for point of care (POC) detection. This has led to investigations on a new class of electrochemical creatinine sensors that have robust stability and are not reliant on the use of enzymes but still exhibit excellent selectivity and sesitivity.

3.2.2. Non-enzymatic creatinine sensors

Studies on non-enzymatic electrochemical creatinine sensors are limited in comparison with those conferring to enzymatic creatinine sensors. Though limited, these electrochemical sensors exhibit robust storage stability and repeatability unlike their enzymatic counterparts. They can be fabricated easily according to the needs and requirements of the sensing parameters to result in relevant clinical detection ranges with least interferences. In such non-enzymatic sensors, the sensing is generally achieved via the interaction of creatinine to specifically synthesised nano-particles deposited at the electrode surface directly [22], or with a supporting media [69], or in the form of a composite [70]. Also, an artificial lock and key mechanism can be utilized using Molecularly imprinted polymers (MIPs). Studies have shown the flexibility and clinical applicability of these non-enzymatic creatinine sensors that will be discussed in further detail in the coming sections.

4. Non-enzymatic electrochemical detection of creatinine via metallic centres

In recent years, we have observed studies developing creatinine biosensors based on the interaction of the analyte with various metallic centres. The mechanism of the interaction of creatinine with metallic centres, typically starts via the physical adsorption of creatinine at the active sites of such metallic centres, forming a metal-creatinine complex followed by a change in the amperometric signal. This change in the signal can be attributed to two inherently different behaviour of the creatinine-metal complex; (i) the complex can either agglomerate at the electrode surface, consequently decreasing the electroactive surface area of the electrode and resulting a decrease in the current signal [23], or (ii) cause an electrocatalytic reaction involving desorption of a soluble reaction product, thereby exposing fresh layers of the metallic centre at the electrode and leading to an increase in the current signal. However, this electrocatalytic process depends on several factors such as favourable electronic states of the redox centre, and unfilled d-orbitals in the case of transition metal centres. It can be safely summarized that the affinity of the metal-creatinine complex at the electrode surface plays a major role in predicting the relationship between the amperometric signal and creatinine concentration. In other words, the peak current varies proportionally or inversely based on the complex's tendency to either leave the electrode as a soluble complex or cause an agglomeration decreasing the electroactive surface area.

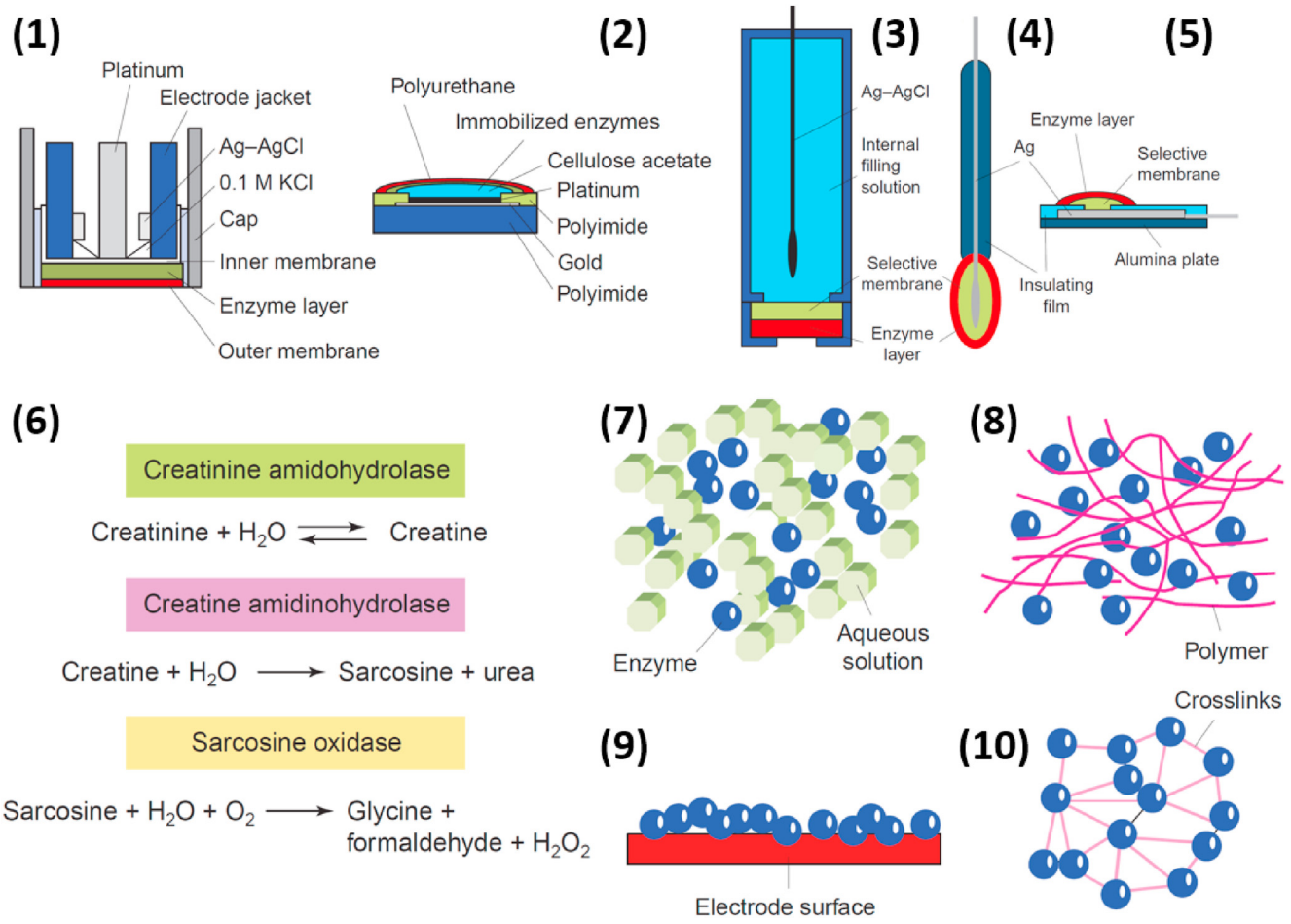

Fig. 4. (1,2) Macroscale and microfabricated three-layer enzymatic creatinine sensing electrodes [61]; (3) Single enzyme immobilized macroscale Ag–AgCl electrode with gas-sensitive membrane for the detection of ammonium (NH_4^+) or hydrogen (H^+) ions; (4) A similar single enzyme based NH_4^+ sensitive sensor with Ag wire as the electrode; (5) Solid-state planar electrode showing an alumina insulating layer that shields an Ag electrode, which is again modified with an immobilized enzyme layer and an inner selective membrane; (6) Schematic representation of the three-stage enzymatic catalysis of creatinine; (7–10) Basic methods of enzyme immobilization employed in creatinine biosensors; (7) Gel entrapment; (8) Polymer entrapment; (9) direct non-immobilized deposition; (10) Crosslinking. Reprinted with permission from Ref. [5].

It has been observed that at biological pH, creatinine exists as the amino tautomer of creatinine which acts as an acid in an aqueous medium (pKa = 4.89 at 20 °C) [71]. Hence, most creatinine biosensors with metallic centres working in a biological pH around 7–8 are based on the chelating ability of the amino tautomer of creatinine, resulting in complexation via the delocalization of π electrons around the three endocyclic N atoms. Several tautomeric forms have been proposed for creatinine (Fig. 5) in which the C=N bond is assumed to be either exocyclic (1,2) or endocyclic (3,4) to the five membered ring [71] and (5) shows the protonated form. Additionally, the methylene group adjacent to the carbonyl group with the α-hydrogen can act as a nucleophilic donor and undergo nucleophilic reaction (refer Fig. 3).

Based on ^{1}H NMR and chemical transformation data, the endocyclic C=N bond tautomers are preferred over those with exocyclic C=N bonds [5] with structure (1) being the most probable. In water solution creatinine shows acidic properties (pK_a, = 4.89 at 20 "C) [71]. In acidic medium, it is protonated, resulting in the formation of (5). Muralidharan et al. [72] suggested that the metabolism of creatinine might be connected with its complexation to different metal ions. In the past two decades, studies have emerged aiming at POC detection of creatinine using screen-printed carbon electrodes with various surface modifications, including electrodeposition, drop-casting, etc, which generally employ a complexation interaction of creatinine with a metallic centre [22,23]. It has also been observed that the peak potential

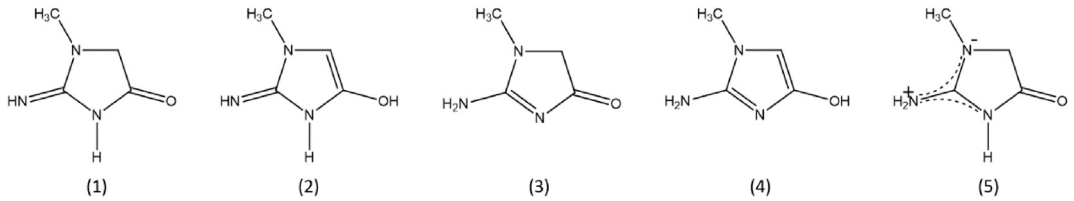

Fig. 5. Various tautomers of creatinine with exocyclic C=N bond (1–2) and endocyclic C=N bond (3–4) (tautomers with endocyclic C=N bonds are more stable than the ones with exocyclic C=N bonds; with structure (3) being the most stable), (5) Protonated form of creatinine in acidic medium.

shifts when the scan rate is varied, which can be explained by considering that the such complexation occurs with a mechanism based on the weak adsorption of the reactant onto the electrode surface [73]. Owing to the presence of several bonding sites in molecules of both creatinine and creatine, they are able to coordinate via different donor groups depending on the reaction conditions. The complexation ability towards a number of metal ions with Ag(I), Hg(II), Cd(II), Zn(II), Co(II), Ni(II), Cu(II), Pt(II) and Pd(II) was already studied [74–78]. Thus, the binding property of creatinine in its various forms with redox-active metal or metallic centres ranging from noble metals to transition metals can be exploited to enable detection. A general trend of electron delocalization is observed amongst all complexes of creatinine with main group and transition metal ions, wherein the π electrons are delocalized around the three endocyclic N atoms, which indicated that these three N-atoms in most cases acts as donors during complexation via chelation. Canty et al. [74] first obtained phenylmercury(II) organometallic compounds with both creatine and creatinine. In acidic water-ethanolic solution, creatinine reacted with $PhHg[(OH)NO_3]_{1/2}$ to form a 1: 2 creatinine: PhHg complex [79]. For instance, Kumar et al. developed an accurate urine creatinine biosensor using Iron (III) receptor membrane coated electrode surface where the redox current is dependent on the formation of Fe (III)-Creatinine complex [69]. Viswanath et al. prepared RGO/AgNPs (Fig. 10.3) composite film, which was used to fabricate a novel electrochemical sensor for creatinine in phosphate buffer (PB) solution with a detection limit of 0.743 pM [70]. The peak currents of RGO/AgNPs composite decreased with the gradual addition of creatinine, which was attributed to the binding of creatinine with the silver nanoparticles. This is because the nitrogen centres of creatinine have greater affinity to act as binding sites for the Ag + ions according to the Pearson's classification [80,81]. In addition, Metal NP-modified electrodes have added advantages in electroanalysis due to enhanced electron transport, large effective surface area and control over the electrode microenvironment [82,83].

4.1. Copper based metallic centres

Copper (Cu), oxides of copper (CuO and Cu_2O), and a combination of both in various morphologies such as nanoparticulate layers electrodeposited on carbon and platinum-based electrodes, drop-casted on SPCE (Screen Printed Carbon Electrode) and even encapsulating and trapping the Cu metallic centres in polymers, have been proven to be effective for electrochemical amperometric detection of creatinine [22,23,84]. Since it shows various coordination numbers [85], it can undergo complexation via chelation with ligands such as creatinine. Cu metal and cuprous oxide (Cu_2O) is generally preferred over cupric oxide (CuO) due to better affinity of creatinine for complexation. Such copper and oxides of copper based creatinine biosensors generally work on the formation of a complex via chelation which either brings about an increase or decrease in the peak current depending upon the mechanism of the complexation. This complexation typically undergoes via a redox-active site through a simple process of physical adsorption. Several studies have exploited unique approaches to the complexation process of creatinine with Cu metallic centres to develop novel creatinine biosensors. For instance, Raveendran et al. [23] have developed a disposable non-enzymatic creatinine sensor free from interference, with a detection limit of 0.0746 μM and linear detection range from 6 to 378 μM, by electrodepositing copper on screen-printed carbon electrodes. The real sample detection was performed in serum and its estimation is based on the formation of a soluble copper-creatinine complex, which was established using the pseudo peroxidase activity of the copper-creatinine complex. It was found that the peak current increases linearly with the increase in creatinine concentration, which is explained as the electrochemical oxidation of copper to form cupric ions that then form a soluble copper-creatinine complex (refer Fig. 6). It was proposed that due to the formation of this copper-creatinine complex, the fresh copper surface is exposed and on increasing the concentration of creatinine, more amount of Cu undergoes oxidation. The sensor exhibited a linear response at lower concentrations and was found to deviate from linearity at higher concentrations. This is due to the dependence of the complexation process on adsorption, which at lower concentration is effective. However, at higher concentrations, the adsorption sites reach saturation thereby leading to deviations from the linear response. This trend in non-linearity at elevated concentrations of the analyte has also been reported in past studies [86–88]. Interference studies were performed using spiked buffer solution containing 62.5 μM creatinine, spiked with glucose- 6 mM, urea- 6 mM, ascorbic acid- 125 μM, dopamine-62.5 μM, uric acid- 125 μM. The sensor was found to be free from these interfering agents except uric acid. A simple pre-sampling process that involved incubating 4 mL solution with 0.6g of PbO_2 powder decisively removed the said interference.

In a recent study by Chi-Hao Chen and Meng Shan Lin [22], a novel non-enzymatic and specific amperometric detection scheme for urine-creatinine was developed. This study was based on the principle that upon the formation of a soluble copper–creatinine complex on the copper electrode surface, an oxidative current arises due to the regeneration of the surface oxide layer, which was monitored and found to be proportional to the concentration of creatinine. To explain the generation of the oxide layer, the group proposed “a potential assisted surface oxide regeneration process” (PASOR) [89]. The group proposed that even though this increase in oxidative current and decrease in reductive current decreases with an increase in creatinine concentration, it is similar to those of a typical electrochemical reaction. However, their assertion stays valid that creatinine is not an electroactive species and thereby a direct charge transfer between the electrode and creatinine is improbable. Hence the observed oxidative current could be a direct result of a potential aided fresh Cu_2O layer regeneration due to the formation of Cu-creatinine complex which in a way “eats away” layers of Cu_2O exposing new layers with the further introduction of creatinine (refer Fig. 8.1 to 8.4). This study observed a linear range 1.8 μM to 108 μM ($R^2 = 0.997$) with an estimated detection limit of 6.8 μg/dL (S/N = 3). The coefficient of variation for 21 successive detections of 0.2 mg/dL creatinine was found to be 1.8% indicating good stability during detection. Nevertheless, like most non-enzymatic creatinine sensors this study also suffered major interference from amine-based agents, uric acid in particular. However, coating the electrode surface with a Nafion film, managed to lower the interference of the uric acid from 298.1% to 5.0%, and effectively minimized the interferences from glucose and other amine-based agents to less than 5%. This Nafion/Cu electrode was used to analyse real samples (dilution factor = 100) and was found to have a suitable correlation (R = 0.980) with the results determined via a standard addition method using a bare Cu electrode as the HPLC detector. This study did not, however, provide a direct comparison between the results of the said scheme with that of the results determined using an accepted clinical creatinine detection method based on techniques like Jaffe's method [29]. Recently, a study was reported whereby instead of drop casting Nafion as a cover layer, the gold electrode itself was modified with a film composed of Nafion mixed with graphene quantum dots dispersed in the presence of Cu^{2+} (introduced as $Cu(NO_3)_2.3H_2O$) [90].The Au/Nafion-GQDs-Cu electrode also exploited the electro-oxidation of Cu to Cu^{2+} and the subsequent Cu(II)-creatinine complex formation, thus demonstrating an inverse relationship between the oxidative peak

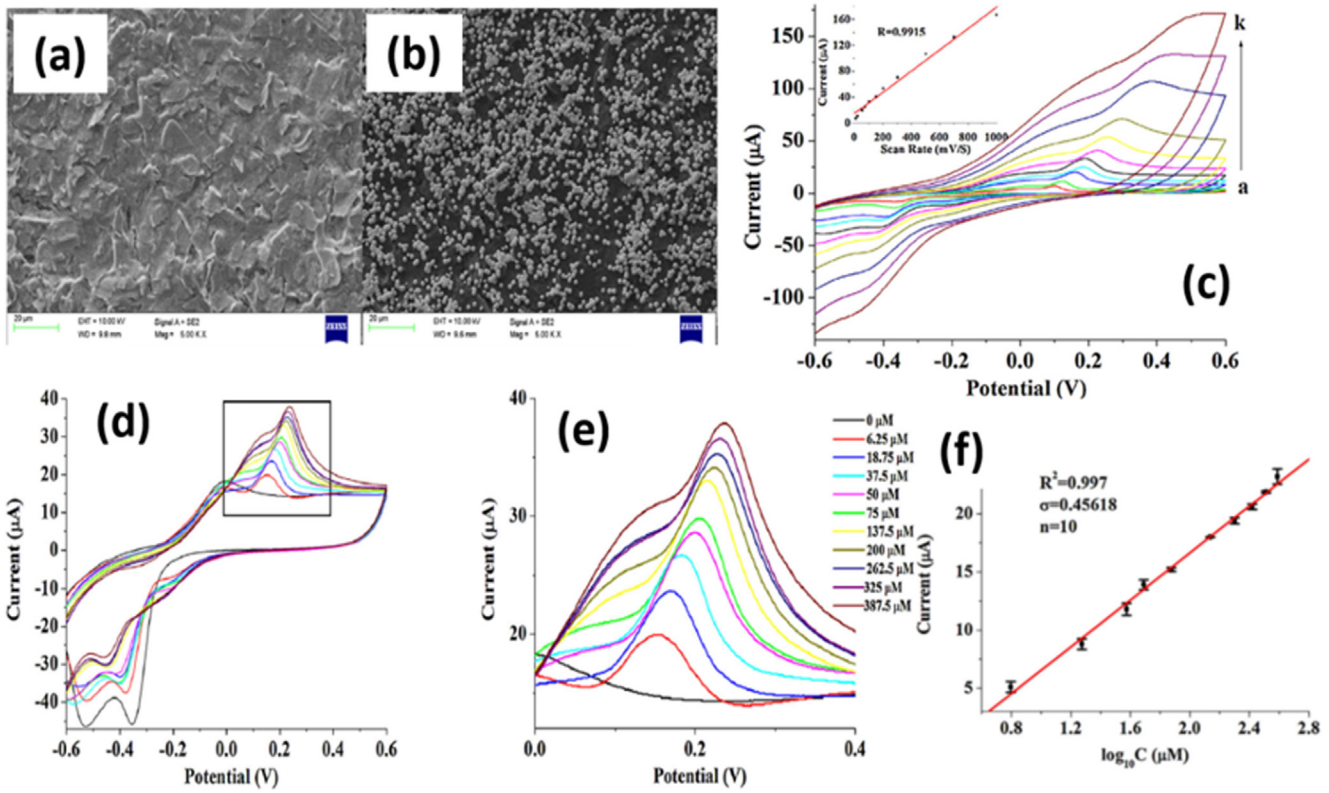

Fig. 6. SEM (Scanning Electron Microscopy) images of (a) SPCE; (b) Cu/SPCE; (c) CVs on Cu/SPCE in 0.1 M phosphate buffer (PB) with creatinine concentrations of 62.5 μM at different scan rates (a → k: 10, 20, 50, 70, 100, 150, 200, 300, 500, 700, 1000 mV/s)(Inset: plot of peak current versus scan rate); (d) CVs on modified electrode in 0.1 M PB with different concentrations of creatinine at a scan rate of 100 mV/s; (e) Enlarged CVs showing the increase on peak current with increase in creatinine concentration; (f) Plot of peak current versus concentration. Reprinted with permission from Ref. [23].

current and creatinine concentrations in the linear range of 1 μM–450 μM in buffer solution. The complexation proceeds through an adsorption mechanism and thus the amount of Cu and the electroactive surface area of the modified electrode greatly affected the signal reproducibility. The typical CV obtained in this study exhibited two peaks during the forward scan which were attributed to the oxidation of Cu to Cu^{2+}. The relative higher stability of Cu^{+} and the higher charge density of Cu^{2+} favoured the complexation of creatinine with Cu^{2+}. A 5% increase in signal strength was observed due to interfering agents (urea, uric acid, glucose, potassium ions and sulphate ions). The Nafion-GQDs-Cu based Au electrode was used to analyse creatinine concentration utilizing Square Wave Voltammetry (SWV) in pre-treated urine samples spiked with known concentrations of creatinine and was found to have a confidence limit of 95% with the analysis results obtained using HPLC (refer Fig. 8.5 to 8.7). The ease in the fabrication of such nanocomposites further sheds light on the potential of non-enzymatic electrochemical creatinine sensors. The use of polyethyleneimine with phosphotungstic-acid multilayer modified electrodes has also been reported to increase the current response for catalytic creatinine detection.

Microfluidic devices also present immense advantages including low cost of fabrication and suitability for POC diagnostics. Boobphahom et al. reported a paper-based analytical device (PAD) with an integrated composite electrode for non-enzymatic creatinine sensing. The paper-based microfluidic sensor was fabricated by dispersing an ultra-thin film of CuO/IL (ionic liquid) using the HP D300 dispenser onto the surface of ERGO (electrochemically reduced graphene oxide) modified carbon electrodes printed on paper substrates (refer Fig. 7.1to 7.2). The study reported that the presence of GO enhanced the current response. However, a concentration beyond 0.9 mg mL^{-1} generated multiple layers of GO, thus reducing the signal strength. Additionally, optimization studies for CuO and IL concentrations were conducted and optimum values were found to be 5mgmL^{-1} CuO and 3mgmL^{-1} IL, respectively. The analytical performance of the developed sensor was tested using amperometry in PBS (pH 7.4) and the sensor showed a linear range of 0.01 mM to 2.00 mM (LOD = 0.22 μM) with insignificant interferences from glucose, uric acid, urea, ascorbic acid. Similar to previous reports, a sensing mechanism based on the oxidative peak current generation (proportional to creatinine concentration) due to oxidation of copper to cupric ion which then chelated with creatinine to form a soluble Cu(II)-creatinine complex, was reported. The use of an automated disperser is an effective way to generate a highly reproducible fabrication process which in turn leads to repeatable results. Moreover, the use of paper provides an adsorption medium that aids in structural support and acts as an effective reservoir for copper ions. The steady and controlled release of copper ions from the copper source is crucial for a repeatable signal response. Such PADs have also been implemented using modifications with metallic ions other than copper, as will be discussed in further sections.

75 mg/dL (d) of creatinine in a 10-fold diluted urine sample, respectively); (5) Schematic representation of Au/Nafion-GQDs-Cu electrode for creatinine quantification, (6) Cyclic voltammograms using the Au/Nafion-GQDs-Cu electrode and sequential additions of creatinine. (a) blank, (b) creatinine addition; (7) Square-wave voltammetric responses of creatinine using Au/Nafion-GQDs-Cu electrode based on the interactions of creatinine with Cu^{2+}

released from the oxidation of Cu thus decreasing the characteristic Cu/Cu^{2+} anodic peak.

Reprinted with permission from Refs. [22,90].

Fabrication techniques that utilize metallic centres distributed in specialized polymer matrix have also been reported. A particularly unique study by Kalasin et al. [84] proposed an electrochemical-creatinine sensor using cuprous oxide nanoparticles (Cu_2O NPs) encapsulated and entangled by PAA gel-Cu(II). Their sensor showed a detection range of 200 μM to 100 mM with good specificity for creatinine in artificial and human urine against urea, glucose, ascorbic acid, glycine, and uric acid. The exceptional aspect of this study is the use of both Cu (I) and Cu (II) with the synergic reinforcement of acrylic polymer to bring about an enhanced kinetic control of creatinine sensing (refer Fig. 9.1 to 9.8). The group observed two peaks during the forward scan and proposed an absorptive mechanism of creatinine with Cu^+ and Cu^{2+} ions which were stripped from the Cu_2O NP. A one-electron transfer mechanism between Cu(I)−creatinine and Cu(II)−creatinine complexes was also proposed to explain the shifting of anodic peaks. They attributed this to the reduction of the modified SPCE by creatinine, which requires further investigation considering that creatinine has been widely reported as being electro inactive [22]. Furthermore, they optimized the size of Cu_2O NP and the gel volume fraction to attain reasonable signal magnitude with reliable storage stability.

The group also reported an IoT enabled device for remote creatinine sensing in saliva by using the aforementioned strategy with an additional Nafion perfluorinated membrane [93]. This reported sensor also used the same composite fabricated utilizing Cu_2O NPs encapsulated and entangled by PAA gel-Cu(II). The additional Nafion perfluorinated membrane acted as an acid catalyst and induced the oxidation of cuprous nanoparticles (refer Fig. 9.9 to 9.11). The analytical performance of the reported sensor was tested using both DPV and EIS. The group achieved a linear sensing range of 1−2000 μM in PBS with a selectivity efficiency of 97.2% using DPV. However, the remote creatinine detection was achieved by measuring the normalized impedance changes (ΔZ/|Z|) of the modified electrode which was integrated with a portable readout module with a detection range of about 10−500 μM. A more recent study reported by the same group utilized a wearable sensor and machine learning to predict the levels of environmental heat stress using a network of PVA-Cu^{2+}−PEDOT:PSS ionically cross-linked onto cuprous oxide nanoparticles incorporated into a carbon black coated nylon fabric. This fabric absorbs sweat from the epidermal layer and the group proposed a diffusion induced polarization and electron transfer sensing mechanism wherein the creatinine molecules diffuse toward the cuprous ions trapped at the thiophene-associated rings of PEDOT, thus eventually losing an electron and becoming Cu^{2+} [94] (refer Fig. 9.12 to 9.14). The analytical performance was assessed utilizing DPV and demonstrated a linear response in the range of 0.4−960 μM and high specificity in artificial sweat against interfering species such as glucose, urea, uric acid, and NaCl. Overall, this nylon-based hybrid wearable sensor addresses the needs of the rapidly developing telemedicine market and aims to provide low-cost non-enzymatic electrochemical schemes for POC creatinine detection.

Alternatively, the utilization of conducting polymers in association with copper metallic centres are also reported. Cu^{2+} impregnated activated carbon nanofibers decorated with electropolymerized methylene blue dendritic fibres (PMB) was used as the working electrode for quantitative detection of creatinine in saliva with an achievable detection range of 0.5−900 ng/mL and a detection limit of 0.2 ng/mL [95]. The proposed sensing mechanism was based on the electrostatic interaction between the cationic PMB and the polarized tautomeric form of Creatinine in aqueous solution, thus resulting in a Cu-Creatinine complex. Most reported copper based electrochemical creatinine sensors utilize either the (i) the diffusion based physical adsorption of creatinine at the Cu centres which proceeds via a PASOR process generating more Cu^{2+} centres or (ii) the soluble Cu(II)-creatinine complex formation that decreases the net amount of active Cu centres. This results in either a linear or an inverse relationship between creatinine concentrations and the corresponding peak current, respectively.

4.2. Other metallic centres

Creatinine has excellent chelating abilities and has proven to form complexes of various structures with metal centres under different reaction conditions. However, this chelating ability is still an untapped resource that needs to be exploited further for creatinine sensing via a non-enzymatic electrochemical pathway. Though studies on copper-based metallic centres are widely available, research on other metal-based non-enzymatic creatinine sensors are limited. Silver and iron based metallic centres have also been investigated in the past few years and were proven viable options for application as non-enzymatic creatinine sensors. A study by Viswanath et al. [70] investigated a graphene-oxide/silver nanocomposite for non-enzymatic trace level detection of creatinine. In this study, GO/Ag was electrochemically reduced to RGO/Ag and was then drop casted on polished GCE (Glassy Carbon Electrode). The sensor exhibited good repeatability and reproducibility with a very low detection limit of 0.743 pM and a linear range from 10 pM to 120 pM. It was able to perform well under the interferences of other biomolecules such as glucose, ascorbic acid, uric acid, urea, and creatine. However, a decrease of 2% and 3% in the relative activity of the sensor was observed in the presence of 10 μM Urea in phosphate buffer solution (PBS). The proposed sensor showed a decrease in peak current with a subsequent increase in creatinine concentration. This observation was due to the adsorption of creatinine on the metallic centre of the RGO/Ag composite. Creatinine has nitrogen centres that act as binding sites for Ag^+ ions, which are known to show good affinity towards such nitrogen binding sites. As creatinine gets adsorbed at the Ag metallic centre, the net electro-active surface area starts to decrease, which continues to decrease with the subsequent addition of creatinine, leading to a decrease in the peak current (refer Fig. 10.1 to 10.4). A recent study by Fekry et al. reported a carbon paste electrode (CPE) modified with silver nanoparticles (Ag NPs), multi walled carbon nanotubes (MWCNT) and folic acid (FA) for detection of creatinine in diluted urine and serum utilizing DPV [96]. The sensor demonstrated good repeatability ($n = 15$, RSD = 5.2%) and stability (for two weeks with 95% signal retention) over a linear range of 10 nM to 200 μM with a detection limit of 8 nM. The developed sensor suffered interferences due to uric acid, which was selectively removed by incubating the sample solution with PbO_2 powder. The group proposed an adsorption-based charge transfer mechanism for the linear increase in peak current with creatinine concentration, whereby folic acid acts as an adsorption site for creatinine followed by a chelation process with the Ag metallic centre and subsequent electro oxidation of creatinine involving the transfer of two electrons. Similarly, Fe based interactions with creatinine have also been exploited for the development of sensitive electrochemical creatinine biosensors. Sriramprabha et al. utilized a supramolecular nanocomposite of Fe_2O_3 integrated polyaniline (PANI) for the quantitative detection of creatinine in serum [97]. The DPV based sensing scheme demonstrated a linear detection range of 1 μM−13 mM while the utilized detection potential from 0 to 0.5 V removed non-specific signals for uric acid and dopamine in PBS with a pH of 6. Given the acidic conditions, the O in creatinine is present as the keto form that

undergoes nucleophilic interaction with the Fe^{3+}. Furthermore, the large number of protonated NH^{+} present at the surface PANI interacted with the secondary amine of creatinine, resulting in increased redox current due to enhanced charge transfer through the PANI network. However, the physiological pH of human serum being about 7.4 is slightly alkaline and the sensor could be further improved by substituting PANI with other conducting polymers that possess high charge transfer capabilities in neutral pH, thus enabling effective creatinine quantification in biological fluids.

Numerous approaches have been investigated to further stabilize the current/signal response of the sensor via employing techniques such as incorporating the metallic centres in fibre membranes. This improves the surface charge transfer capabilities as well and ensures that the metallic centres adhere firmly to the electrode for enhanced signal stability and reproducibility. Kumar et al. [69] reported a non-enzymatic electrochemical creatinine sensor using disposable carbon screen-printed electrodes layered with cotton fibre membranes that are coated with reaction mixture of ferric chloride and sodium chloride ($FeCl_3$ and NaCl solution). The group presented an inversely proportional relationship between creatinine concentration and peak current. This observation was explained as a decrease in redox current due to the formation of the Fe(III)-creatinine complex which results in diminished availability of the electro-active Fe^{3+} ions (refer Fig. 10.4 to 10.12). This study reported robust and repeatable sensing capability against the interference of a range of concentrations of albumin with coefficient of variation ranging from 0.5% to 7.1%. However, the linear range of the sensor included only the healthy creatinine levels and was unable to detect and quantify creatinine levels of patients with advanced stages of renal disease. In a similar study, Fava et al. [92] reported a screen printed carbon black working electrode layered with paper adsorbed Fe^{3+} ions as the active sensing site. The Fe^{3+} ions form a complex with creatinine and the remaining free Fe^{3+} ions get reduced to Fe^{2+} during the cathodic scan, thus generating a reduction peak current that varies inversely with creatinine concentration. This study is unique as it utilizes the unreacted Fe^{3+} and the corresponding reduction peak current as opposed to the general trend that considers the oxidation peak. The proposed sensor exhibited linear response in the creatinine concentration range of 0.10–6.5 mM with 9.5% change in signal strength due to inferences (glucose, uric acid, dopamine, ascorbic acid, and caffeine) and 98%–110% recovery in creatinine spiked human urine. Additionally, implementation of alternative matrices which can serve as a protective layer against interference can effectively reduce the variation in signal strength as the proposed mechanism uses highly reactive Fe^{3+} which is expected to result in considerable non-specific interactions. Furthermore, paper based microfluidic devices utilizing Fe^{3+} based detection of creatinine offer immense advantages such as multiplexed monitoring, low sample volume and rapid detection [98]. A recent study by Fava et al. reported a disposable paper-based multiplexed working electrode for the sequential analysis of glucose, creatinine, and uric acid [91]. The detection mechanism of creatinine was identical to their previous report [92], whereby the creatinine present in the sample reacted with paper adsorbed Fe^{3+} to form the Fe(III)-creatinine complex. Consequently, the intensity of the cathodic peak current due to the reduction of Fe^{3+} to Fe^{2+} decreased proportionally with increase in the creatinine concentrations. The feasibility of the developed microfluidic device was investigated in diluted urine samples and exhibited high repeatability ($n = 5$, RSD = 6.69%) and specificity against urea, ascorbic acid, dopamine, and caffeine (RSD = 8.25%) with an LOD of 0.084 mM in the linear range of 0.106–4.500 mM. Hence, the Fe(III)-creatinine complexation was proven to be highly effective and suitable for POC detection of creatinine.

In addition to synthetic polymers like PANI, natural fibrous polymers such as inulin have also been effectively utilized as a matrix for the development of creatinine biosensors based on biocomposites with metallic centres. Metallic oxide nanoparticles with semiconductor properties such as TiO_2 and SnO_x have been investigated for chemical and biological applications [99]. A recent study utilized carbon paste working electrodes modified with TiO_2-inulin bio-composite for quantitative detection of creatinine in human urine [100]. The group proposed a Ti^{4+} co-ordination with creatinine and inulin matrix, leading to the electro oxidation of creatinine present in its hydroxylamine form and resulting in the transfer of two electrons^{-} at the electrode surface. DPV was utilized to investigate the analytical performance of the TiO_2-inulin based sensor with a linear dependence on creatinine concentration in the ranges of 0.05 mM to 12 mM (LOD = 90 μM) and 0.05 mM to 12 mM (LOD = 90 mM) and a 95% confidence level with results obtained via Jaffe's method.

Various forms of metallic centres including ionic compounds and nanoparticles have been used for electrode surface modification of creatinine biosensors. Quantum dot based creatinine biosensors have also been reported in literature. Quantum dots (QD) are utilized for their enhanced optoelectronic and catalytic properties for a wide range of applications [101]. Hooshmand et al. [102] reported a CdSe quantum dot/ionic liquid-mediated hollow fibre-pencil graphite electrode for simultaneous electrochemical quantification of uric acid and creatinine in serum and urine. This interesting approach achieved selective determination of creatinine in the presence uric acid by employing polypropylene hollow fibre membranes as a solid/liquid microextraction membrane impregnated with ionic solutions while the CdSe QD was involved in sensing and improving the charge transfer characteristics of the electrode (refer Fig. 10.13 to 10.17). The amount of QD, buffer pH, equilibration time, amount of ionic solution and scan rate were optimized and a linear response for creatinine detection in the range of 0.297–2.970 mM was observed. The creatinine detection was performed in diluted urine and serum with a 98.67% and 95.25% recovery rate, respectively, thus demonstrating the potential of microextraction based creatinine sensors.

To summarize, non-enzymatic electrochemical creatinine sensors based on metallic centres mentioned in above sections have numerous inherent advantages over enzyme (and multi-enzyme) based sensors and are gaining much attention in the past few years. The research on such metallic centre based creatinine sensors though limited showed particularly promising results with versatile detection mechanisms including a prominent complexation of creatinine with transition metal ions with vacant d orbitals such as Cu^{2+}, Fe^{3+}, Ti^{2+} etc, wide detection ranges, excellent selectivity (via a proper sample pre-treatment, Nafion, Polymeric methylene blue based cover layer, and use of microextraction techniques using hollow polymeric fibres), and far superior storage stability/shelf-life than their enzymatic counterparts. Table 2 presents a comparison of currently available metallic centre based non-enzymatic creatinine biosensors, mentioning the various electrode modifications used and their analytical performances. Unquestionably, enzyme-based sensors inherently exhibit outstanding selectivity; however, its high cost and poor storage stability combined with the intrinsic stringent sensing conditions prove to be a major impediment when it comes to its implementation for non-centralized detection including POC setting. In comparison, non-enzymatic sensors like the ones based on metallic centres, are low cost and the inherent excellent storage stability can be successfully exploited to develop POC tests that will help at-risk patients to perform routine quantitative analysis of the creatinine levels in blood/serum and/or urine.

Table 2
Comparison of metallic centre based non-enzymatic creatinine biosensors.

Ref	Metallic centre	Modification[1]	Bare Electrode [2]	Method of modification	Signal	Sensing Machanism	Real Sample Analysis	Detection Limit (μM)	Detection Range (μM)	Studied Interfering Agents[4]	Removal of Interfering agents	Repeatability [5]	Reproducibility [5]	Stability[6]
[22]	Cu_2O	ED layer	Pt	Electro deposition	Increases	PASOR	Tenfold diluted urine	0.4896	1.8–108	Glu, AA, Ur, Cr, Ac, Sc, Am, Cl, UA	Anionic cover layer (Nafion)	1.8% (n = 21)	–	–
[23]	Cu	gMP (1 μm)	SPCE	Electro deposition	Increases	Electro oxidation of copper to cupric ion	Serum incubated with PbO_2 powder	0.0746	6–378	Glu, AA, Ur, Dp, UA	Incubating with PbO_2 powder	4.1% (n = 10)	4.8% (n = 10)	–
[69]	Fe	$FeCl_3$/NaCl in CFM	SPCE	Drop casting	Decreases	Fe(III)-creatinine complexation	Urine	–	884–21.6 x10^3	Alb	–	#0.5% to 7.1%	–	–
[70]	Ag	NC Film	GCE	Drop casting	Decreases	Agglo-meration of Electrode surface	Urine	7.43x10^{-7}	10x10^{-7} - 120x10^{-7}	Glu, AA, UA, Ur, Cr	N/A	4.3% (n = 5)	3.8% (n = 5)	88% 50 days
[84]	Cu_2O/ Cu(II)	PAA gel-Cu^{2+}/Cu_2O NPs	SPCE	Drop casting	Decreases	Decrease in active site and higher adsorption	1:75 Diluted Urine	6.5	200 –100x10^3	Ur, Glu, AA, Gly, UA	Potential based isolation of signal via DPV	–	1.6–1.9% (n = 6)	96% 28 days
[90]	Cu^{2+}	Nafion/Graphene QD/ Cu^{2+} film	Au	Drop casting	Decreases	Decrease in active site and higher adsorption	Pre-treated Urine	–	1.0–450	Ur, UA, Glu, K^+, So_4^{2-}	–	–	–	–
[91]	Fe	Fe^{3+} based μPAD	CB	Drop casting	Decreases	Fe(III) creatinine complexation	Diluted Urine	84	106 –4500	Ur, AA, Dp, Caf	Potential based isolation of signal via DPV	6.92% (n = 4)	–	–
[92]	Fe	Paper adsorbed Fe^{3+} CB microcell	SPCB microcell	Drop casting	Decreases	Fe(III) creatinine complexation	NaCl treated Urine	43	100–650	Glu, UA, Dp, AA, Caf	Potential based isolation of signal via DPV	4.58% (n = 10)	7.76% (n = 3)	–
[93]	Cu_2O/ Cu(II)	Nafion/PAA gel-Cu^{2+} encapsulated Cu_2O NPs composite	SPCE	Drop casting	Increases	Electro oxidation of copper to cupric ion	Artificial Saliva	0.3	1-500 (using EIS)	Ur, UA, Glu, Gly, AA	Nafion PEM	–	–	~96% 30 days
[94]	Cu_2O/ Cu(II)	PVA- Cu^{2+}–PEDOT: PSS/Cu_2O NPs composite	CB/Nylon fabric	Dip coating	Increases	Electro oxidation of copper to cupric ion	Artificial Sweat	0.06	0.4–960	Glu, Ur, UA, NaCl	Nafion PEM	2.6% (n = 5)	–	95.5% 30 days
[95]	Cu_2O/ Cu(II)	Cu^{2+}/ACF/PMB film	ACF	Drop casting	Increases	PMB based adsorption and electro oxidation of creatinine	Saliva, CSF	0.2 x10^{-3}	0.5x10^{-3}- 900 x10^{-3}	Dp, AA, UA, Chl, Ur, Glu	PMB PEM, washing step	1.0% (n = 185)	–	96% 180 days
[96]	Ag	Ag/MWCNT/FA composite	CPE	Grinding	Increases	Ag-creatinine chelation and electro oxidation of creatinine	Spiked Urine	0.008	10x10^{-3}- 200	Glu, Gly, Urea, AA, Dp, UA	Incubating with PbO_2 powder	5.2% (n = 15)	3.7% (n = 5)	95% 30 days
[97]	Fe	Fe_2O_3/PANI SM Composite	GCE	Drop casting	Increases	Fe(III) creatinine complexation	Serum	144 x10^{-3}	1–13x10^3	AA, UA,Ur, Dp,Alb	Potential based isolation of signal via DPV	3.4% (n = 100)	2.7% (n = 10)	97% 60 days
[100]	Ti	MWCNT Inulin TiO_2 bio composite	CPE	Grinding/ Homogenization	Increases	Complexation of Ti^{4+} with hydroxyl-amine tautomer of Creatinine	Urine	90 x10^3	0.5x10^3- 12x10^3	–	–	3% (n = 10)	2.7% (n = 5)	90% 240 days
[102]	Cd/Se	CdSe NS on PolyP-HF encapsulated PGE	PGE	Dip casting, sonication	Increases	CdSe-creatinine interaction and microextraction via PolyP-HF	Urine Serum	0.299 x10^3	0.4x10^3- 8.84x10^3	–	–	1.96% (n = 3)	–	97.6% 20 days
[150]	CuO	CuO/IL/ERGO	Paper basedSPCE	Inkjet dispensing	Increases	Electro oxidation of copper to cupric ion	Treated Urine	0.22	10–2000	UA, Ur, AA, Glu	–	2.1% (n = 5)	3.8% (n = 5)	–

Abbreviations.
[1] CFM= Cotton fibre membrane, gMP = Granular Mesoparticles, ED = Electrodeposited, NP= Nanoparticles, NC Film = Nanocomposite film, CB= Carbon Black, SM=Supramolecular, NS= Nanostructures, PolyP-HF= Polypropylene Hollow Fibres, IL= Ionic Liquid, ERGO = Electrochemically Reduced Graphene Oxide.
[2] SPCE= Screen-printed Carbon Electrode, GCE = Glassy Carbon Electrode, CPE= Carbon Pate Electrode, ACF = Activated Carbon Fibre, PGE= Polished Graphite Electrode [3] PBS= Phosphate Buffer Solution, AU = Artificial urine.
[4] Glu = Glucose, AA = Ascorbic Acid, Ur = Urea, UA= Uric Acid, Dp = Dopamine, Cr= Creatinine, Ac= Acetaminophen, Sc= Sarcosine, Am= Ammonium, Cl= Chloride, Gly = Glycine, Chl = Cholesterol, Caf = Caffeine.
[5] RSD (Relative Standard Deviation) is shown in percentage, #Coefficient of Variation is provided instead of RSD.

5. General concept of MIPs based sensors

It is well established that enzyme-based sensors provide excellent sensitivity and selectivity due to its "lock and key" mechanism, however, suffer extremely due to its innate poor storage stability and high cost. Thus, a new goal arose, which was to attain the sensitivity and selectivity of enzyme-based sensors without its drawbacks of storage and cost. One such approach was to create synthetic receptors that mimic the mechanism of affinity interactions like antibody-antigen formation with the added benefit of being inert to environmental stimuli and a reasonably low production cost. These synthetic receptors or molecularly imprinted polymers (MIPs) are artificial equivalents of the biological systems and operate by a "lock and key" mechanism to selectively bind with the target molecule. MIPs have high physical and chemical stability towards various external degrading factors, solvents, metal ions, and acid treatments. Also, the duration of synthesis is less in comparison with other sensors [103]. MIPs can be utilized to develop a range of sensors including chromatographic, FET-based, capacitive and electrochemical. It is important to be aware of a few parameters that are significant in the application of MIPs in sensors. These include: (i) imprinting factor (IF), which is the ratio of the template bound to the imprinted polymer as compared to nonimprinted polymer (NIP), (ii) binding capacity (BC) also defined as the percentage of the target molecule absorbed from the test solution with respect to its initial concentration, and (iii) response time (RT) which is the timespan required for the signal to grow to 63.2% of its final magnitude after the application of the stimuli.

5.1. Synthesis protocol of MIPs

MIPs can be synthesised via numerous techniques [104–107] which results in various distinct guest-host polymer characteristics; some of these synthesis methods include: (a) template based synthesis from monomers [108,109], (b) phase inversion via polymer precipitation via solvent evaporation or by addition of incompatible solvents [110,111], and (c) soft lithography or surface stamping [112,113]. However, all such techniques follow a common framework as listed below (refer Fig. 11):

1. The target molecule is bonded to a functional group of the host polymer either covalently or non-covalently.
2. The covalent/non-covalent bond between the target molecule (also called the template molecule) and the host polymer is broken to release the template molecule from the host polymer leaving behind an "imprinted" site/cavity that will bind specifically to the template molecule.
3. When the MIP is exposed to a complex sample containing the target molecule, the imprinted site will then bind selectively to the target molecule, thereby achieving a "lock and key" equivalent mechanism.

5.2. MIPs based non-enzymatic electrochemical creatinine sensors

In the past two decades, MIPs based electrochemical creatinine sensors have gained much interest, owing to its enzyme like selectivity and sensitivity and robust storage stability which remains unaffected with changing environmental and sensing conditions. Table 3 shows various MIPs fabricated via numerous methods, exhibiting different detection ranges using different detection mechanisms. Additionally, MIPs are known to exhibit low conductivity and electro-catalytic activity. However, these drawbacks can be overcome by addition of external modifications. Such MIPs based sensors can be of different types depending on whether the sensing is solely based on the interaction of creatinine and the imprinted cavity or if it uses the aid of additives such as nanoparticles to enhance the signal strength and/or facilitate electron transfer.

5.2.1. Creatinine sensors using MIPs deposited/grafted on an electrode

Over the past two decades, we have seen numerous creatinine sensors based on MIPs synthesised using a functional monomer and a crosslinker which exploits the H-bonding of the analyte (creatinine) and the functional polymer [114], and in some cases the combination of H-bonding and covalent bonding [115]. It has been proposed that the kinetics of the non-covalent binding of creatinine with the active sites of MIPs is directed by Langmuir model [116–118]. Creatinine sensors based on MIPs synthesised using various functional polymers such as Poly(ethylene-co-vinyl alcohol) (EVAL) [119], methacrylic acid [120], acrylamido methylpropanesulfonic acid [121], poly(melamine-co-chloranil) [122], b-cyclodextrin (b-CD) [123,124] and 4-vinylpyridine [123] etc. were reported. EVAL based electrochemical creatinine sensor using MIPs modified Au electrode was reported by Ref. [125], with 40 ng/mL of detection limit and linear range from 4.42 to 17.68 μM. MIPs grafted on Au electrodes which exploit the change in current [126]and capacitance [127] with change in creatinine concentrations were also reported.

Diouf et al. [126] developed a MIP for detection of creatinine using screen-printed Au electrodes. The Au-SPE was modified initially by carboxylic polyvinyl chloride which provides the active −COOH functional group that binds with creatinine, followed by crosslinking via polymerization of acrylamide and N,N′ methylenediacrylamide (refer Fig. 12). The group implemented both Differential Pulse Voltammetry (DPV) and Electrochemical Impedance Spectroscopy (EIS) techniques to quantitatively determine creatinine in human urine. The differential pulse voltammograms indicated a decrease in peak current with an increase in creatinine concentration, which is due to the increased filling of free MIPs active sites (cavity) with subsequent addition of creatinine. However, the Nyquist plot shows increased impedance with increase in creatinine concentration, which implies that a higher creatinine concentration brings about an increase in interfacial charge-transfer resistance (R_{ct}). Similar observations were reported in a study [121] wherein a template based MIP synthesised using the functional monomer methyl acrylate and cross-linker ethylene glycol dimethacrylate was deposited on carbon electrodes. They reported an increased R_{ct} with an increase in creatinine concentration, thus implying the accumulation of creatinine into the polymer matrix. In another study, Delaney et al. [127] reported a molecularly imprinted photopolymerization of acrylamidomethylpropanesulfonic acid (AAPS) and methylenediacrylamide (MDA) which was grafted onto the surface of gold electrodes coated with Hexadecane thiol monolayer. This reversible chemo sensor showed decrease in capacitance with increase in the creatinine concentration. It also exhibited a detection limit of 10 μM and robust selectivity against urea, glucose, NaCl and creatine.

Besides Au electrodes [125], although rarely, hanging mercury drop electrodes (HMDE) have also been used. Lakshmi et al. developed a creatinine sensor based on MIP modified HMDE [128]. The pre-anodized MIP modified HMDE instantaneously concentrates creatinine and oxidizes it. Differential pulse cathodic stripping voltammetry was used to obtain a linear response in the range of 0.0025–84.0 μgmL^{-1} with a detection limit of 1.49 $ngmL^{-1}$. The sensor exhibited good repeatability (RSD = 5%, n = 3) and robust selectivity as it was observed that the sensor showed absolutely no interference from creatine, urea, glucose, NaCl and cytosine

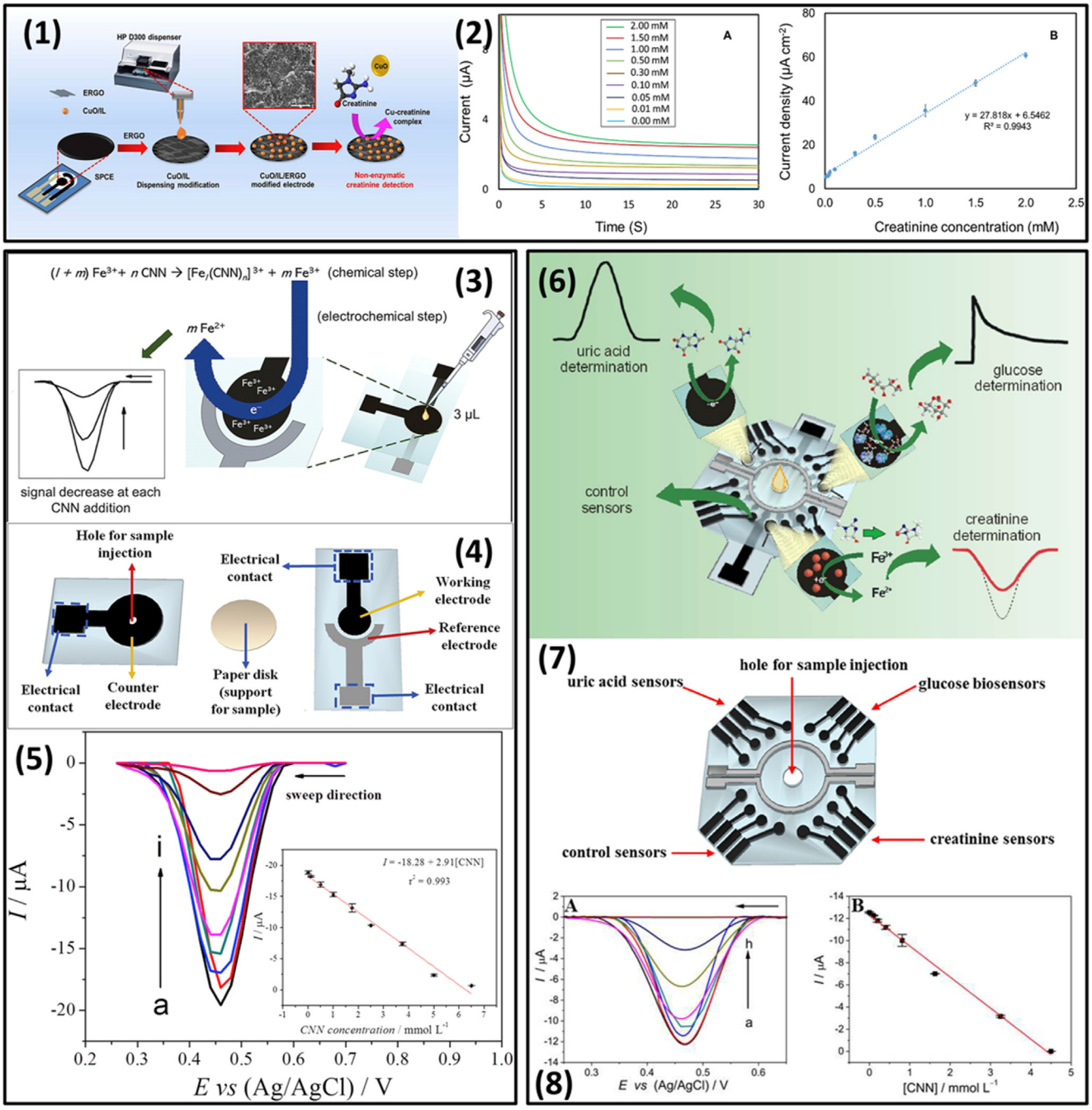

Fig. 7. (1) Schematics of the working principle of CuO/IL/ERGO composite paper-based microfluidic sensor for the non-enzymatic determination of creatinine in human blood serum; (2) (A–B) Amperometric response of the CuO/IL/ERGO/SPCE with different creatinine concentrations (from 0.0 to 2.0 mM) in 0.1 M PB (pH 7.4) and the corresponding calibration plot for creatinine detection. Reprinted with permission [150]; (3) Schematics of the working principle of paper adsorbed Fe^{3+} microcell for creatinine determination in urine; (4) Various components of the fabricated microcell; (5) DPV voltammograms for: a) blank; i) 6.5 mM creatinine with 0.1 M NaCl as supporting electrolyte (inset: calibration plots); (6) An electrochemical paper-based microfluidic device with multiplexed electrodes for biomarker determination in urine sample; (7) Illustration of components of the developed microfluidic device (8) (A–B) Differential pulse voltammograms using the multiplexed microfluidic device obtained by additions of creatinine standard solutions prepared in 0.1 M NaCl at concentrations of: a) 0.0 h) 4.500 mM and its corresponding calibration curve. Reprinted with permission [91,92].

phenylalanine with the exception of tyrosine and histidine. The Differential Pulse Stripping Cathodic Voltametry (DPSCV) signal was proposed to be a result of the heterogeneous charge transfer via an electron hopping mechanism between the redox centre present in the MIPs and the positively charged electrode. Since the carbonyl form of creatinine has a higher electronegativity than the imine form, which induces a keto-enol tautomerism, the creatinine is instantaneously oxidized to the corresponding radical at the modified electrode at +0.4V (refer Fig. 13), thus generating a DPSCV signal with changing creatinine concentration. Evidently, the pre-anodization prevents the use of supporting electrolytes but has been reported to catalyse electrochemical processes [129] due to the generation of oxygen containing functional groups like carbonyl, carboxylate and hydroxyl radical species in a very small quantity on the surface of electrode. During the preconcentration step, the creatinine is oxidized to give the creatinine radical, which

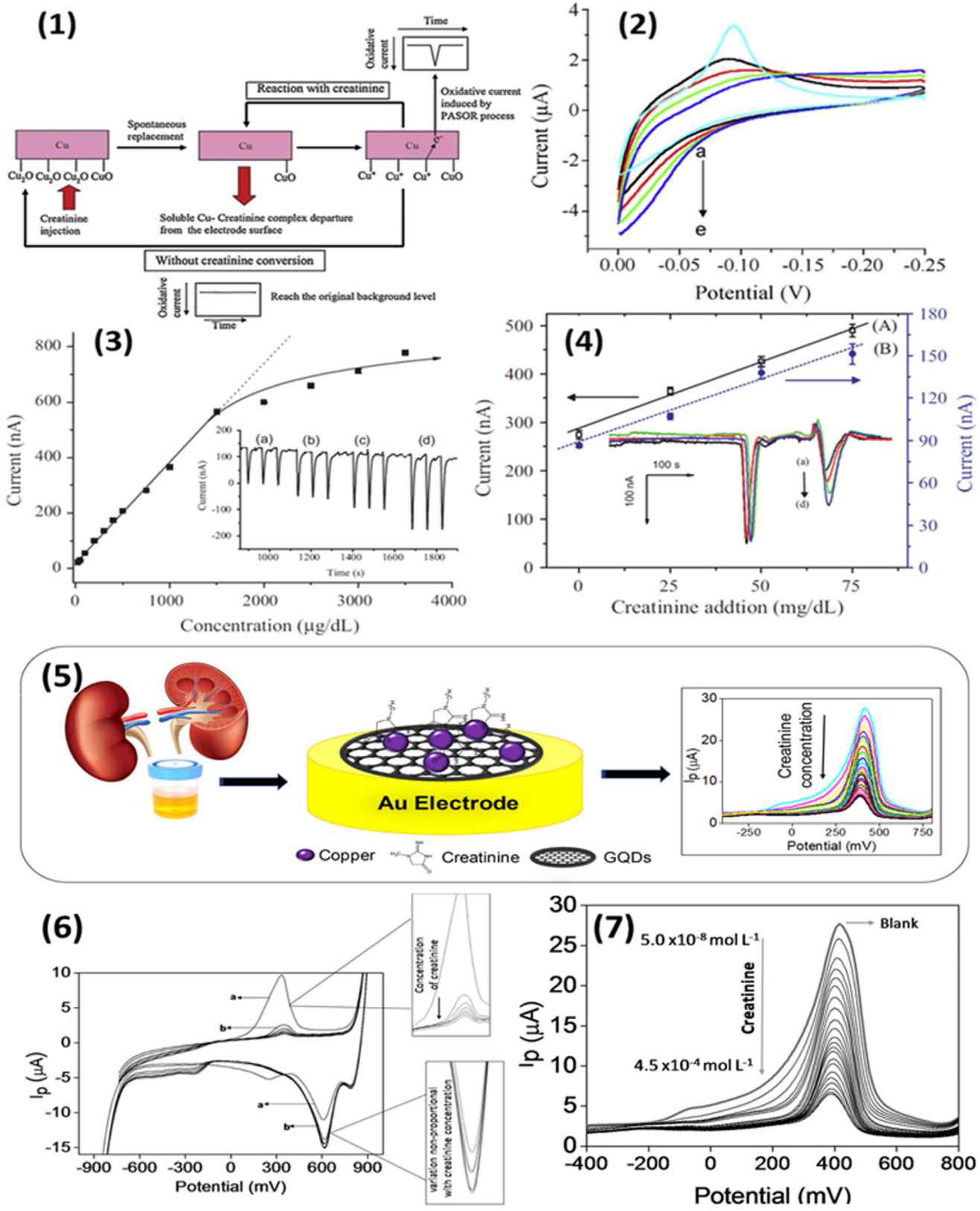

Fig. 8. (1) PASOR based mechanism for creatinine measurement on a copper based electrode; (2) Cyclic voltammograms of creatinine on a copper-deposited electrode in 50 mM phosphate (pH 6.5) and the concentrations of the creatinine used: 0 mg/dL (a), 5 mg/dL(b), 10 mg/dL (c), 15 mg/dL (d), and 20 mg/dL (e); (3) Calibration curve of the Cu based creatinine sensor (the inset diagram is the actual responses of sequential injection of 0.3 mg/dL (a), 0.4 mg/dL (b), 0.5 mg/dL (c), and 0.75 mg/dL (d) creatine); (4) Standard addition analysis of the real sample by using FIA integrated with Nafion®/Cu electrode (A) and HPLC integrated with Cu electrode (B) (Inset: actual chromatograms after addition of 0 mg/dL (a), 25 mg/dL (b), 50 mg/dL (c), and.

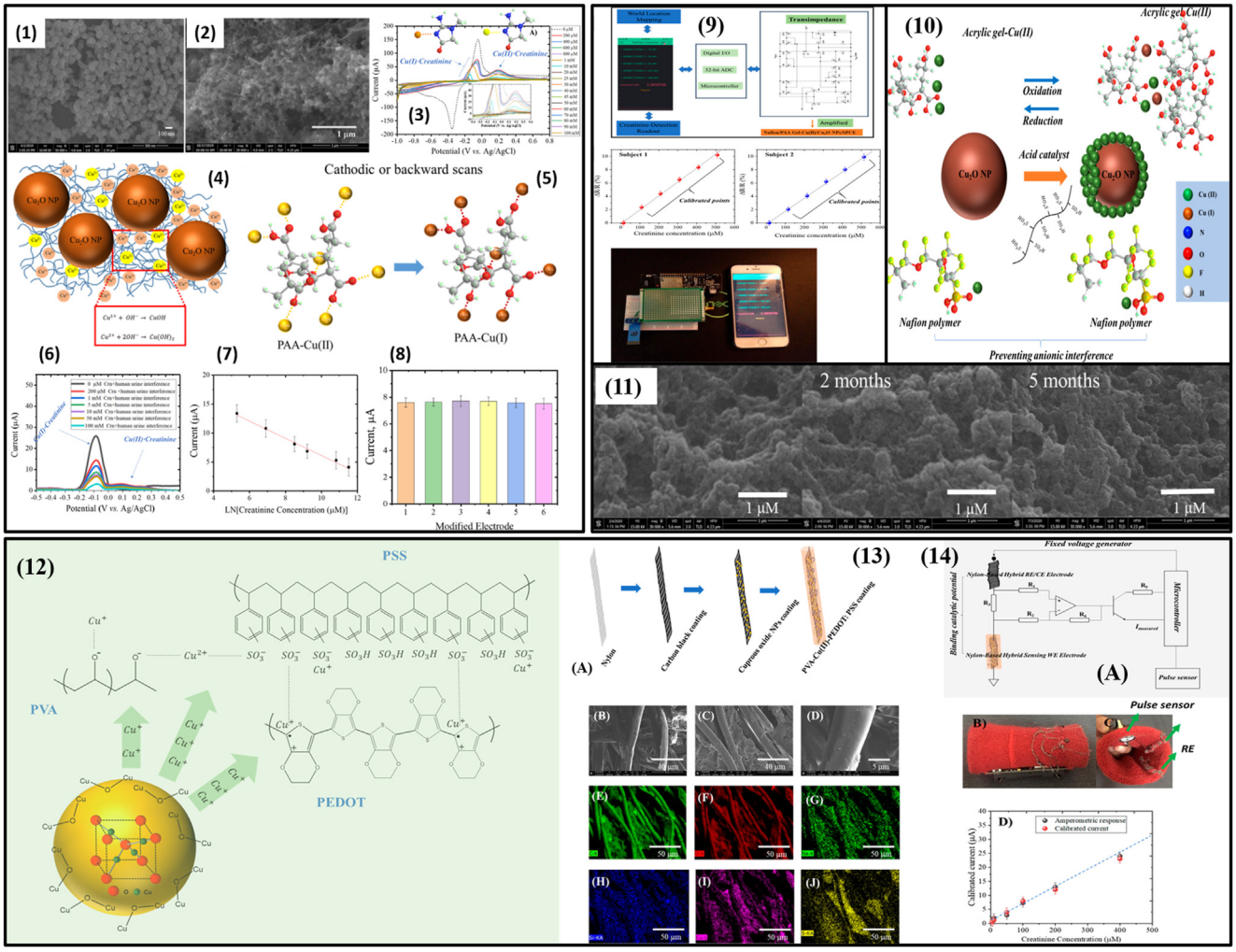

Fig. 9. (1) SEM image of synthesised bare Cu_2O NPs; (2) SEM image of PAA gel-Cu(II)/Cu_2O NPs/SPCE; (3) Cyclic voltammograms of creatinine detection in the concentrations range of 200 to 100,000 μM using PAA gel-Cu(II)/Cu_2O NPs/SPCE at a scan rate of 50 mV/s; (4) Anodic Stripping Cu^{+}/Cu^{2+} of Cu_2O NPs during Forward (Anodic) Scans; The Inset Area Represents Gel formation with PAA during Reverse (Cathodic) Scans; (5) The Transformation of PAA–Cu(II) to PAA–Cu(I) during Cathodic Scans; (6) Typical DPV voltammograms obtained using PAA–Cu(II)/Cu_2O NPs/SPCE electrode for sensing different concentrations of creatinine from 200 μM, 1 mM, 5 mM, 10 mM, 50 mM, and 100 mM spiked into the mixture of human urine and 0.1 M PB with 0.1 M KCl (dilution factor = 1:75); (7) Calibration plot of the anodic peak current vs spiked creatinine concentration. (Experimental condition: DPV parameters: E-pulse 25 mV, t-pulse 70 ms, E-step 5 mV, and equivalent scan rate 25 mV/s) and (8) Reproducibility test using 6 different PAA gel-Cu(II)/Cu_2O NPs/SPCE electrodes for 10 mM creatinine in 0.1 M PB and 0.1 M kC (B) Calibration plot of the anodic peak current vs spiked creatinine concentration; (9) (Top to bottom) Schematic design of IoT device for salivary creatinine detection using Nafion/polyacrylic gel–Cu^{2+}/Cu_2O NPs/SPCE, Calibrated fitting plots of the remote-sensing IoT device, Photograph of the modified sensor connecting to the smartphone; (10) Sensing mechanism of the sensors based on Cu^{2+}/Cu^{+} phase separation in the Polyacrylic Gel–Cu^{2+} in the Presence of Nafion ionomer–SO_3H as an acid catalyst; (11) SEM images of the modified electrode after 2 and 5 months (showing robust structural stability); (12) Schematic of the sweat creatinine detection platform based on the dissociation of Cu_2O NPs encapsulated by Poly Vinyl Alcohol (PVA), Poly(3,4-Ethylenedioxythiophene):Poly styrene sulfonate (PEDOT:PSS) crosslinked matrix; (13) (A) Fibre based electrode modification; SEM images of (B) carbon-black-coated nylon and PVA-Cu^{2+}-PEDOT:PSS/Cu_2O NPs carbon-black-coated nylon, SEM-EDS mapping of the modified nylon showing (E) C, (F) O, (G) Na, (H) Si, (I) Cu, and (J) S; (14) (A)Circuit design of the hybrid sensor; (B) side view and (C) inside view of the epidermal on-grip hybrid sensor for the determination of sweat creatinine; (D) Calibrated current response vs artificial sweat creatinine concentration (at V = 0.15 V). Reprinted with permission from Refs. [84,93,94].

gets stripped off via electronic repulsion during the cathodic stripping step. DPSCV based detection requires optimization of the creatinine oxidized during the pre-anodization step. In their study, the maximum uptake of creatinine was observed at 300 μgmL^{-1}, which was reported to form a thin film around the mercury drop during a period of 120 s.

In a similar study that utilize MIP based DPCSV detection of creatinine, Patel et al. reported a MIP sol-gel film on graphite electrodes [122]. They utilize pre-anodization which facilitates the formation of an active oxide film that contains electron-transfer mediating groups on the graphite electrode surface. This induces the electron hopping mechanism as mentioned before which generates the DPCSV signal. Moreover, the increase in the cathodic stripping peak current with increase in creatinine concentration was explained via Dahm–Ruff Model, wherein at the electrode surface the overall rate of binding phenomenon and charge transport dominates the rate of mass transport (diffusional flux) of the solution creatinine. This induces an increased cathodic current with subsequent increase of creatinine [130–132]. The DPCSV curve showed a broad peak attributed to the retarded heterogeneous electron transfer, but at +1.8V the MIP film instantaneously oxidizes creatinine to give the creatinine radical which was then readily stripped off during the cathodic stripping step.

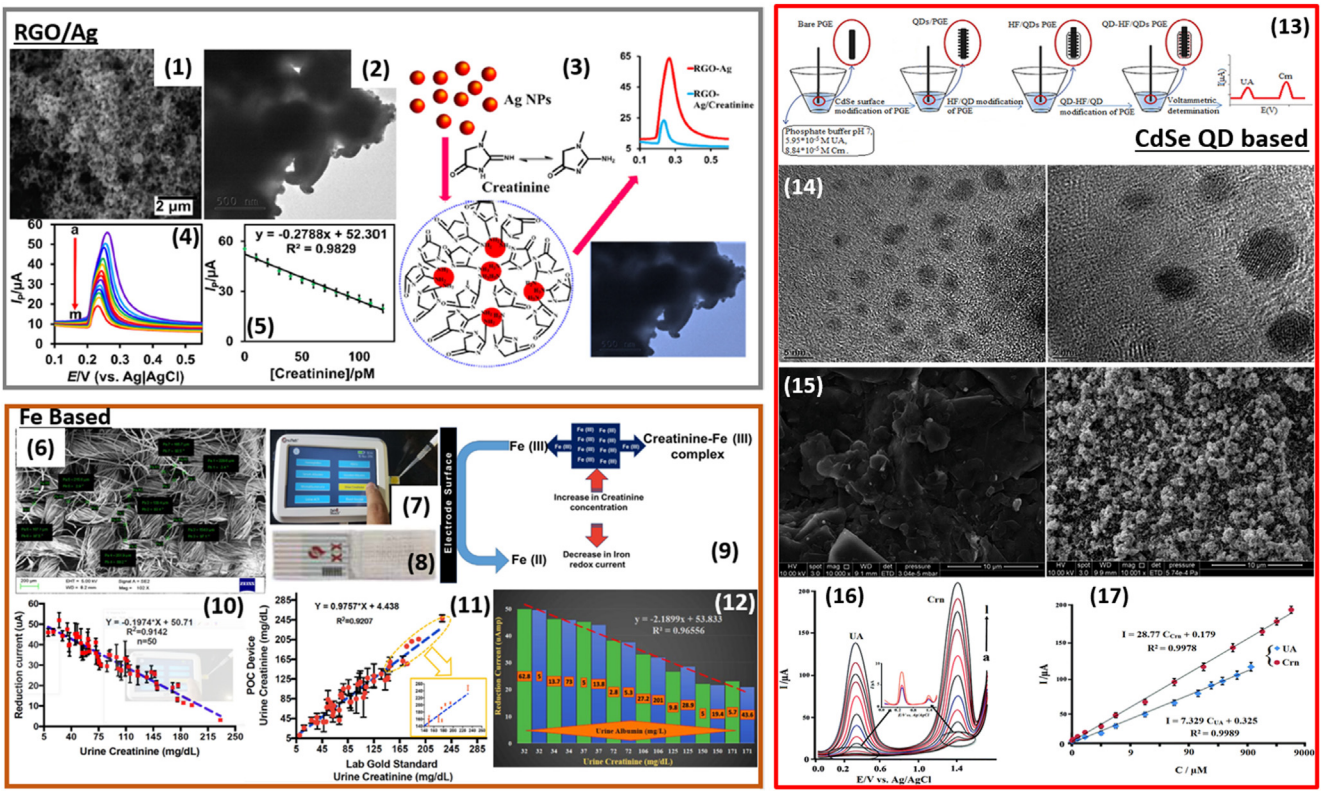

Fig. 10. SEM (1) and TEM (2) images of RGO/AgNPs showing the agglomerated surface after the adsorption of 1 nM creatinine**;** (3) Schematic representation of the mechanism of interaction of silver metallic centre with creatinine; (4) Square wave voltammograms at RGO/AgNPs in absence (a) and presence of creatinine from 10 pM to 120 pM (b to m) in PBS (pH = 7); (5) Calibration plot between I_p vs. [creatinine]; (6) SEM image of cotton membrane coated with $FeCl_3$+NaCl solution; (7) Fe(III) based POC device in test mode; (8) Disposable test strip; (9) Schematic representation of the mechanism of Fe(III) based electrochemical detection of creatinine; (10) Correlation between reduction current and urine creatinine concentration; (11) Correlation between the results obtained via the Iron-based POC device and pathology lab results and (12) Reduction Current Vs. Urine creatinine concentration for different values of Urine Albumin values; (13) Schematic steps for the modification of polished graphite electrodes (PGE) with CdSe QD and polypropylene hollow fibres for the development of a microextraction based creatinine biosensor; (14) TEM images of the synthesised CdSe QDs; (15) (Left to Right) SEM images of bare and CdSe QDs modified PGE; (16) (c) DPVs for different concentrations of UA and Crn in 0.1 M PBS (pH 7.0) at a scan rate of 10 mV−1 (a to l: 0.297−8840 µM); (17) Calibration plot showing the linear detection ranges for Uric acid and Creatinine. Reprinted with permission from Refs. [69,70,102].

Fig. 11. Schematic representation of a typical imprinting mechanism. The template binds with monomers or polymers, followed by the removal of the template consequently leaving behind the empty molecularly imprinted cavity that can then be used to selectively bind the target molecule. Reprinted with permission from Ref. [104].

5.2.2. MIPs based creatinine sensor enhanced with nanoparticle modification

MIPs based on the use of metal/metal oxide nanoparticles (NPs) with improved electron transfer capability have also been investigated in the past few years [30,133–136]. Evidently, the addition of NP is not limited to the generation of enhanced electron transfer rates but was also found to affect the morphology of the MIP film thereby influencing various parameters such as the loading capacity of the MIP. Selective interaction of some specific NPs (transition metal based) aid in the augmentation of the excellent sensitivity exhibited by MIPs based creatinine sensors. NPs also bring about an added benefit of crystalline structures whose properties such as magnetism can be exploited to fabricate MIP films with intricate morphologies, which distinctively affect its sensitivity due to its lock and key based sensing mechanism. The studies presented by Rao and Wen showed two similar examples of MIPs based on the use of NPs, such as Ni NPs or Fe_3O_4 NPs, together with polyaniline (PANI). In both cases, glassy carbon electrodes are modified with the MIP and nanoparticle-PANI composites by electro-polymerization, yielding similar Limit of Detection (LODs of 0.2 and 0.35 nM, respectively). The sensors were applied to detect creatinine in spiked samples, leading to recovery percentages close to 100% (refer Fig. 14). The creatinine sensor reported by Wen et al. was based on an electrochemical sensing platform exploiting the magnetic field-induced self-assembly of Fe_3O_4@PANI NPs [133]. The functional monomer (aniline) and the template molecule (creatinine) were self-assembled through the formation of N–H bonds, followed by a magnetic induced removal of the template

Table 3
Comparison of MIP based electrochemical creatinine sensors.

Ref	Synthesis[1]	Electrode[2]	Modification[3]	Signal[4]	Transduction[5]	Test solution[6]	Real Sample[7]	LOD[8]	Detection Range (μM)	Storage stability[9]	Selectivity[10]	Reproducibility[11]	Repeatability[12]
[30]	PI via Solvent Evaporation	GCE	AgNPs/POM/Rgo deposition	Current Increases	DVP	PBS (pH6.0)	Saliva serum	0.0151 nM	0.00005 −0.0015	90% 9days	NaCl, Ins,Ur, Fer, UA, Glo, Alb, Lys, His, GA, Arg, Tyr, CA	3.9% (n = 3)	4.4% (n = 5)
[121]	Template based Polymerization method	CPE	MIP using MA and EGDMA deposition	Impedance Increases	EIS	PBS (pH7.4)	–	20 ng/mL	0.18−5.9	–	Try, His, Ep, Ty, Ur, UA, AA, Cr	–	–
[122]	Grafting Polymerization	Au	MIP deposition	Capacitance Decreases	–	–	–	10 μM	–	Good 180 days	Cr, Ur, Glu, Alb, NaCl	–	–
[125]	PI via Solvent Evaporation	Au	EVAL MIPs deposition	Impedance Decreases	EIS	PBS (pH7.5)	–	40 ng/mL	4.42 −17.68	Good 14 days	–	–	–
[126]	Template based Polymerization method	Au	Au-SPE/PVC-COOH	Impedance Increases and Current Decreases	EIS and DPV	5 mM $[Fe(CN)_6]^{3-/4-}$	Urine diluted with PBS	0.016[a] or 0.081[b] ng/mL	0.0008 −8.84	Good 90 days	Ur, Glu	Good	4%
[128]	Template based Polymerization method	HMDE	MIP deposition	Current Increases	DPCSV	Aqueous (pH7.0)	Serum	1.49 ng/mL	0.022 −742.5	–	Tyr, Ur, Cy, Cr, NaCl, His, Phe, Glu	Good	5% (n = 3)
[133]	Template based MFI-SA	MGCE	Fe_3O_4@ [133]PANI NPs MIP deposition	Current Increases	CV and DPV	$K_3[Fe(CN)_6]$+ KCl or PBS (pH6.5)	Plasma Urine	0.35 nM	0.02−1	Good	AA, BR, Sr, Cr, UA	Good	2.3[a]-2.7[b] % (n = 3)
[136]	Template based MFI-SA	MGCE	Ni@PANI NPs MIP deposition	Current Increases	CV and DPV	$K_3[Fe(CN)_6]$+ KCl or PBS (pH6.5)	Urine	0.2 nM	0.04−0.8	93.5% 120 days	AA, Cr, UA, Ty, Dp	–	3.5% (n = 5)
[137]	Sol-gel Polymerization	ITOE	Ni NCs/GO Fe_3O_4 film	ECL Decreases	Potential sweep and PL	PBS pH(7.4)	Diluted serum and urine	0.5 nm	$5x10^{-3}$-$1x10^{3}$	>90% 180 days	NHS, NMP, Cr, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, NO^{3-}	–	3.41 (n = 5)
[138]	Template based Polymerization method	CPE	CuO coated MIP deposition	Current Increases	CV and FIA	PBS (pH8.0)	Urine	0.083 μM	0.5 to 200	80% 14days	AA, UA, Dp, NHS, Sr, Nr, Glu, NaCl	4.92% (n = 5)	1.94% (n = 30)
[140]	PI via Solvent Evaporation	GrE	MIP sol-gel drop casted on GrE	Current Increases	DPCSV	Aqueous (pH7.0)	Diluted Serum and Urine	0.37 μg/mL	10.87 −884.02	Good 80 days	Ur, Tyr, His, Cy, BA, Glu, Phe, Cr, NaCl	0.1% (n = 3)	0.9−2.1% (n = 3)

Abbreviations.
[1] PI = Phase inversion, MFI-SA = Magnetic field induced self-assembly.
[2] MGCE = Magnetic glassy carbon electrode, GCE = Glassy carbon electrode, CP= Carbon paste electrode, HMDE= Hanging mercury drop electrode, GrE = Graphite Electrode, ITOE= Indium Tim oxide electrode.
[3] POM = Polyoxometalate, MIP = Molecular Imprinted Polymer, EVAL = poly(ethylene-co-vinyl alcohol), PVC-C00H = carboxylic polyvinyl chloride, SPE= Screen printed electrode, MA = Methyl acrylate, EGDMA = ethylene glycol dimethacrylate.
[4] Effect on the signal with increase in creatinine concentration.
[5] CV = Cyclic Voltammetry· DPV = Differential Pulse Voltammetry, EIS = Electrochemical Impedance Spectroscopy, DPCSV = Differential Pulse Cathodic Stripping Voltammetry.
[6] PBS = phosphate buffer solution.
[7] PBS = phosphate buffer solution.
[8] a = LOD obtained via EIS, b = LOD obtained via DVP.
[9] Storage stability as the %signal strength retained after mentioned time (in days).
[10] AA = ascorbic acid, BR = bilirubin, Sr = sarcosine, Cr = creatine, UA = uric acid, Ur = urea, Ty = tyrosine, Dp = dopamine, NHS= N-hydroxy succinimide, Sr = serotonin, Nr = norepinephrine, Glu = glucose, NaCl= Sodium chloride, Ins = insulin, Fer = ferritin, Glo = globulin, Alb = albumin, Lys = lysine, His = histidine, GA = glutamic acid, Arg = arginine, Tyr = tyrosine, CA = citric acid, Try = tryptophan, Ep = Epinephrine, Cy = cytosine, NHS= N-hydroxy succinimide, NMP = 1-Methyl-2-pyrrolidinone, Phe = phenylalanine, BA = barbituric acid.
[11] RSD(Relative Standard Deviation) is shown in percentage.
[12] RSD(Relative Standard Deviation) is shown in percentage.

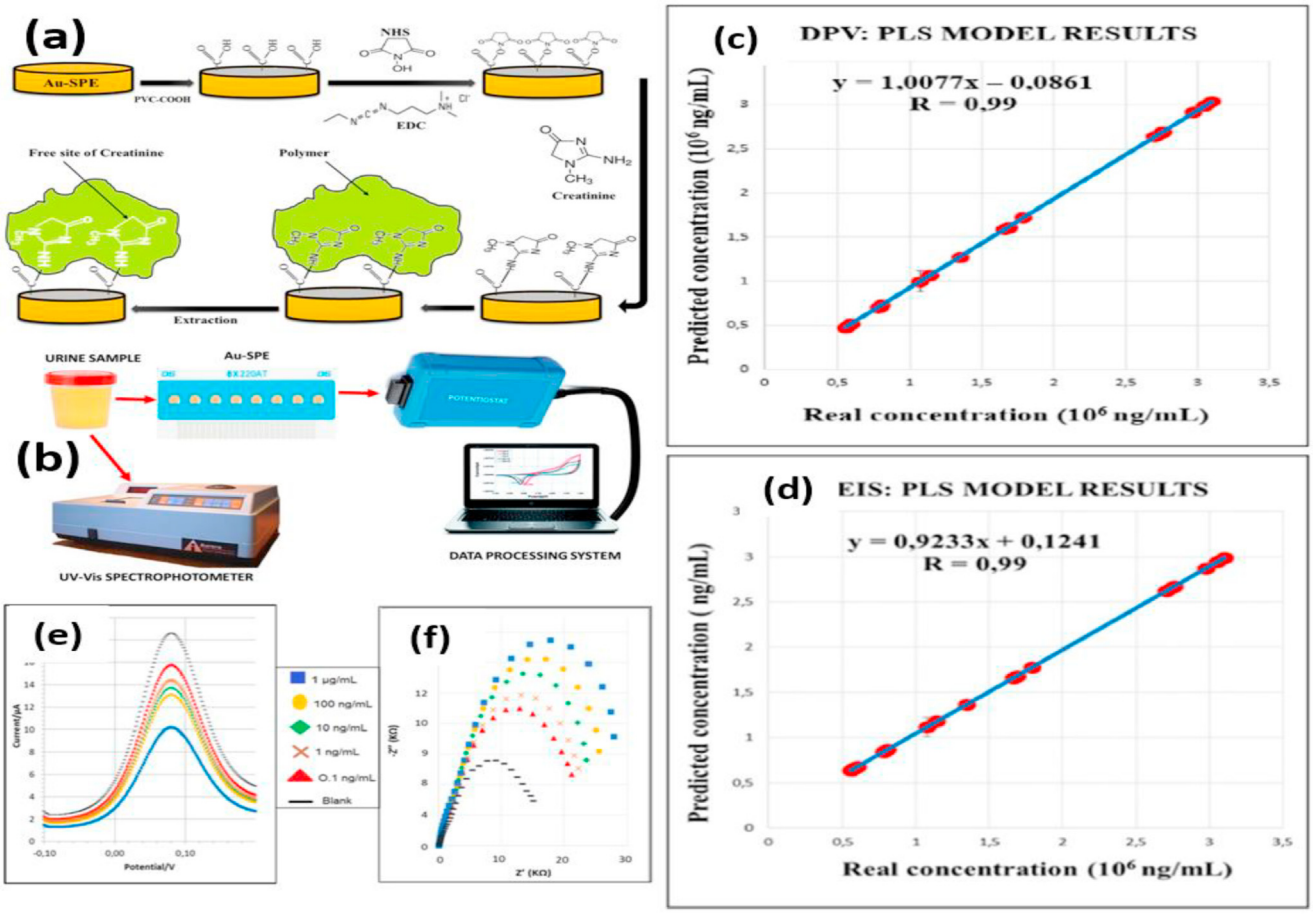

Fig. 12. Schematic representation of (a)Au-SPE/MIP procedures, (b) spectrophotometer and the portable device system used for electrochemical measurements, Results of PLS prediction of human urine creatinine levels via DPV (c) and EIS (d) methods versus Jaffe's method, (e) Differential pulse voltammograms of different concentrations of creatinine solutions (f) Nyquist plots of different concentrations of creatinine solutions. Reprinted with permission from Ref. [126].

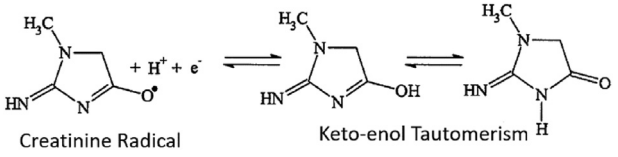

Fig. 13. Formation of creatinine radical via a keto-enol tautomerism. Reprinted with permission from Ref. [128].

via the magnetic glassy carbon electrode as shown in Fig. 14(a). In this case, the stable and hydrophilic Fe_3O_4@PANI serves two purposes. The polyaniline provides sites for H-bond formation to facilitate the binding of the target molecule and the Fe_3O_4 NPs promote electron transfer. However, it was observed that the amount of Fe_3O_4@PANI NPs controls the MIP film thickness and the increase in the film thickness resulted in deeper cavities from which the removal of the templates was difficult. This sensor exhibited good stability and reproducibility with the detection limit of 0.35nmolL^{-1} (S/N = 3) and was used for the clinical determination of creatinine in human plasma and urine samples with average recoveries of 90.8–104.9% and RSD lower than 2.7%. It is worth mentioning that the binding capacity (BC) of MIPs depend on the compatible conditions at which the target molecule and the cavity interact to form a bond (covalent or non-covalent). For creatinine sensors, the pH plays a major role. In acidic pH, creatinine exists pre-dominantly in its keto form, where the protonation of N atom is enhanced and the amount of negative oxygen ion is limited, which is reversed in basic medium. This can explain why Wen et al. [133] reported the absorption was low at pH 6.0 but showed increased absorption at pH 6.5. PANI is positively charged at higher pH and creatinine shows increased amount of negative oxygen ion and decreased protonation of N-atom, thereby promoting absorption. Babamiri et al. reported an ECL (electrochemiluminescence) based biosensor for creatinine detection. The sensor utilized Ni nanoclusters (Ni NCs) embedded GO-Fe_3O_4 modified ITO electrode in the presence of TPrA anodic coreactants which facilitated an increased ECL response [137]. The MIP film was fabricated using a sol gel polymerization method on ITO electrodes via the formation of surface hydroxyl groups. It was observed that the presence of GO-Fe_3O_4 greatly enhanced the ECL signal due to the increased surface area and enhanced electron transfer capability. The sensing mechanism was based on the consequent ECL quenching in the presence of creatinine. The introduction of creatinine into the cavities of the MIP hinders the contact of the Ni NCs with TPrA, thus leading to higher quenching which resulted in an inverse relationship between the ECL intensity and creatinine concentration. Under optimized conditions, the MIP-ECL based on Ni NCs/GO-Fe_3O_4/ITO sensor exhibited a linear response in the detection range of 5 nm to 1 mM (LOD = 0.25 nm) with minimal signal variation in the presence of creatine, N-hydroxysuccinimide (NHS), 1-Methyl-2-pyrrolidinone (NMP) (structure analogous compounds to the template creatinine) and co-existing ions (Na^+,

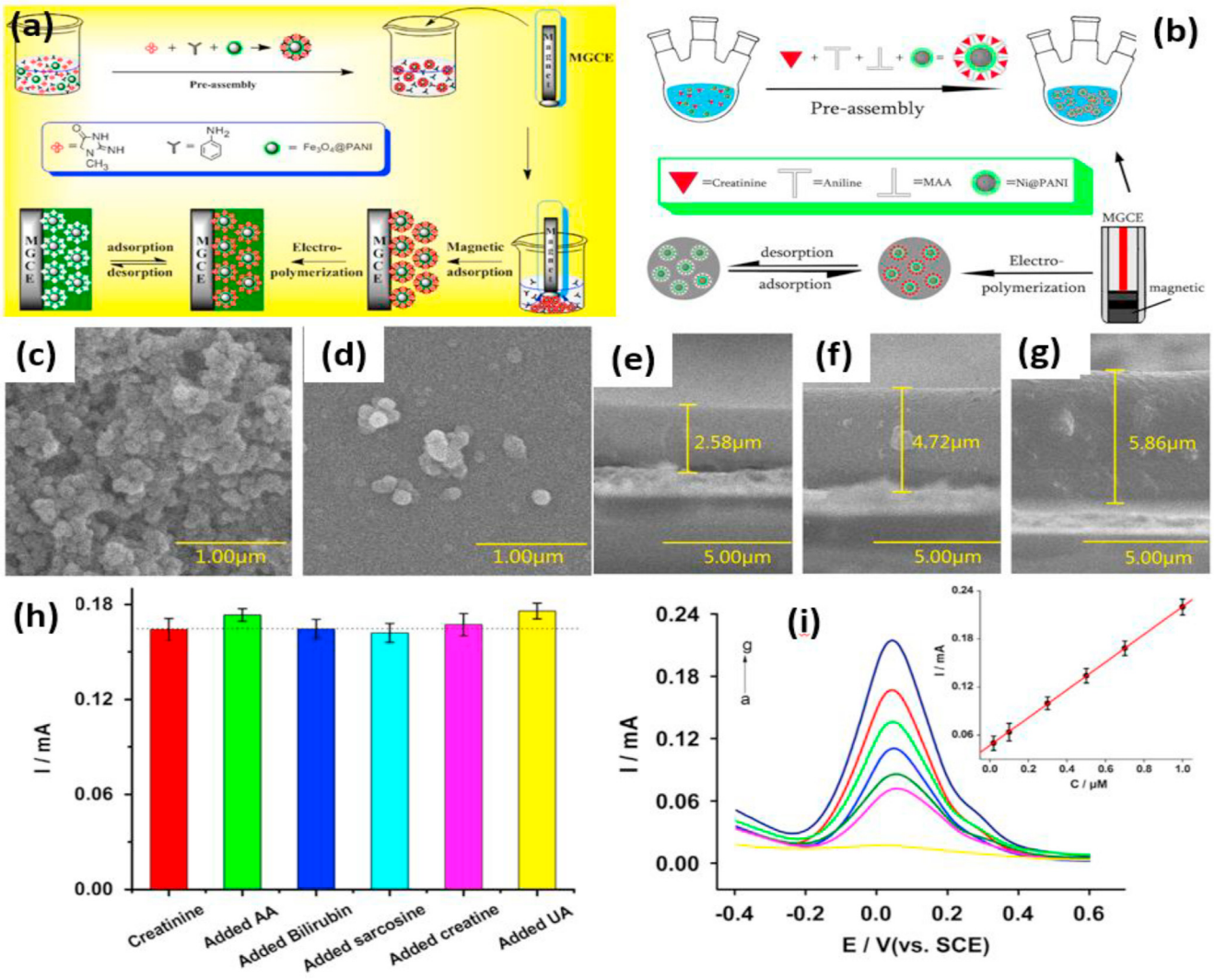

Fig. 14. Schematic representation for the preparation of the MIES (molecularly imprinted electrochemical sensor) (a) Fe_3O_4@PANI NPs; (b) Ni@PANI NPs; Fe_3O_4@PANI NPs MIPs film prepared with (c) and without (d) magnetic field; (e–g) SEM cross-sectional images showing the effect of increasing amount of Fe_3O_4@PANI on the film thickness; (h) The selectivity property of Fe_3O_4@PANI NPs MIP and (i) DPV current responses to different concentrations of creatinine:(a–g: 0, 0.02, 0.1, 0.3, 0.5, 0.7and 1.0 μM respectively). (The inset shows the resulting calibration curve). Reprinted with permission from Refs. [133,136].

K^+, Ca^{2+}, Mg^{2+}, Cl^-, NO^{3-}).

Another group also utilized a nanomaterial based MIP where the GCE was deposited with Ag NPs/polyoxometalate (POM) functionalized reduced graphene oxide (rGO) [30]. This also indicated that the addition of the Ag NPs caused an increase in surface area which enhanced the signal strength, similar to the observations reported by Wen [133] and Rao [136]. The MIP/AgNPs/POM/rGO coated GCE sensor was reported to have a linear detection range of 0.05–1.5 nM with a LOD of 0.0151 nM and showed good stability with negligible decrease in response after 2 weeks. Similarly, Rao et al. [136] presented a GCE based Ni@PANI magnetic molecularly imprinted polymer (MMIP) (refer Fig. 14b). The production of the MIP is quite similar to that of the study by Wen [133]. DPV was used to study the electrochemical response of the sensor, which reported two linear ranges of 0.004–0.1 μM and 0.1–0.8 μM. These two linear ranges are a result of different kinetics between the available active sites and the change in concentration of the analyte. The slope of the first linear region of calibration curve was high due to the higher number of available active sites as compared to the total number of the analyte molecules, which decreases with increase in the concentration of the analyte molecule, thereby causing a decrease in the slope in the second linear range.

The precision detection of MIP and NPs combination was further enhanced by implementing a flow injection analysis system in conjunction with the improved electron transfer provided by the metallic NP. Studies conducted by Amatatongchai et al. [134]. and Nontawong et al. [138] used these added advantages. Nontawong et al. [138] developed an amperometric flow injection analysis of creatinine using Au@Fe_3O_4 NP and CuO NP coated MIP deposited on a carbon paste electrodes (CPE) and carbon nanotube electrodes, which were then used to detect carbofuran and creatinine, respectively (refer Fig. 15). The sensor exhibited good selectivity against various interfering agents (ascorbic acid, uric acid, N-hydroxy succinimide, serotonin, dopamine, norepinephrine, glucose, NaCl) and achieved a dynamic linear range of 0.5–200 μM with a lower detection limit of 0.083 μM. The MIP was synthesised using a template based polymerization method, with creatinine as the template, methacrylic acid as monomer, N, N-(1,2-dihydroxyethylene)bis(acrylamide) as cross-linker, and 2,2-azobis (2-methylpropionitrile) as initiator. It was proposed that the CuO

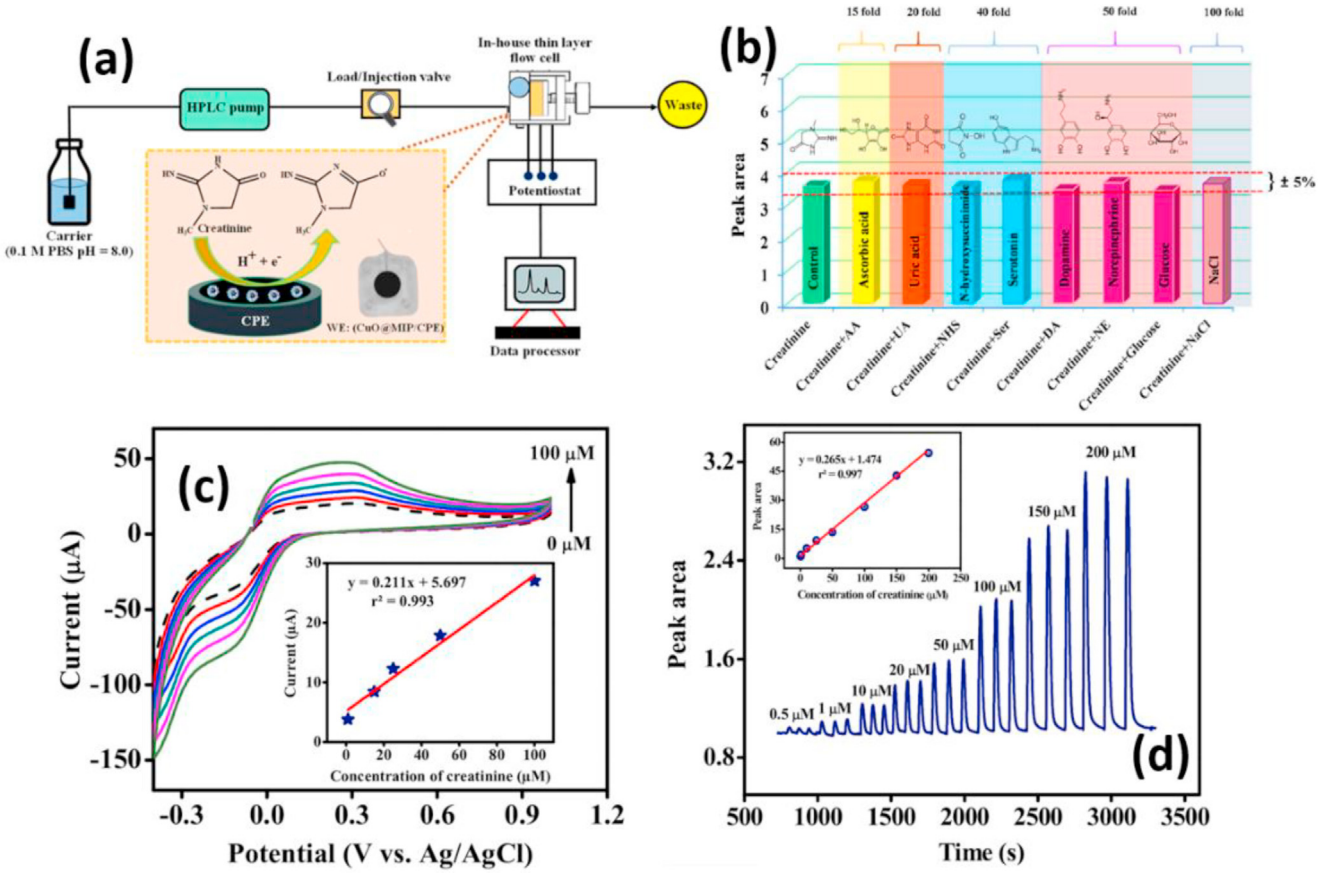

Fig. 15. (a) Schematic representation of the configuration used in the FIA system coupled with amperometric sensor on CuO@MIP/CPE and the corresponding creatinine measurement mechanism; (b) Selectivity of the CuO@MIP/CPE for creatinine determination; (c) CV plots at the CuO@MIP/CPE at creatinine concentrations ranging of 0–100 µM (1, 20, 30, 50 and 100 µM) in 0.1 MPBS (pH 8.0), scan rate: 0.05 V s−1(the inset figure shows the corresponding linear calibration curve) and (d) FIA gram of creatinine calibration range from 0.5 to 200 µM in to 0.1 MPBS (pH = 8.0), operating potential +0.35 V vs. Ag/AgCl, flow rate 0.6 ml/min(inset shows linear relationship between the signal of creatinine and the concentration). Reprinted with permission from Ref. [138].

provided increased surface area, which leads to higher number of adsorption sites and enhanced absorption and analyte diffusion, thereby decreasing response time. The flow rate of the system affects the reaction time between the CuO@MIP and creatinine. Higher flow rates results in lesser time to react and thus weaker signals as the analyte fails to bind with the active sites (imprinted cavities) [134,139]. Also, at low pH the dissociation of −COOH group of poly-methacrylic acid at the MIP surface is limited. Therefore, its interaction with the target molecule (creatinine) is less resulting in weaker current, which increases with increase in pH and was found to be maximum at pH = 8.0 (refer Fig. 14). Hence, we can safely say that the pH condition at the time of sensing is crucial for an optimum result. However, the role of pH is not only limited to sensing but also for the optimal synthesis of MIPs. A study conducted by Subat et al., in 2004 [135] used pH dependent binding and release of creatine with polymerizable Lewis acidic zinc(II) cyclen complexes co-polymerized with Ethylene Glycol Dimethyl Acrylate, that exhibited nearly absolute analyte recovery rate via the pH dependent release. The imprinting was carried out through the reversible binding of creatinine with the zinc metallic centres, similar to the binding observed in metallic centre based electrochemical creatinine sensors [23,69,70]. This strong interaction allows the high affinity absorption of creatinine from the solution to the MIP.

6. Highly selective non-enzymatic electrochemical methods for creatinine detection

6.1. Non-enzymatic potentiometric creatinine sensor

A very unusual non-enzymatic clinical creatinine detection scheme was proposed in 2005 by Hassan et al. [141]. The group reported a flow-injection analysis system with fast response, reasonable selectivity, low cost and interfacing with computerized systems, to develop a coated-wire and tubular type membrane creatinine sensor. Creatinine was reacted with tungstophosphate (TP), molybdophosphate (MP) and picrolonate (PC) anions were synthesised to give creatinine tungstophosphate (CTP), creatinine molybdophosphate (CMP) and creatinine picrolonate (CPC) ion-pair complexes, respectively. These were then dispersed in plasticized poly(vinyl chloride) matrix membranes which in acidic pH reportedly detects creatinine as a monovalent cation (due to complete ionization and dissociation). The group extensively reported the effect of the polarity of the solvent mediator, flow rate and the geometry of the sensor. Three polymeric membranes were produced using o-nitrophenyloctyl ether (o-NPOE), Dioctylphthalate (DOP) and dibutylsebacate (DBS), with o-NPOE displaying the best performance. As for the flow rate, it was optimized at 4 mL/min with a calculated sampling rate of 40 samples per hour. It was

observed that flow rates >3 mL/min, exhibited memory effect, long washing times and poor analytical frequency and flow rates <5 mL/min resulted in decreased response and narrower peak width. The tubular type showed much stable results owing to the geometry and the closeness to the reference electrode. However, the coated wire type showed faster detection due to larger surface area. A detection limit of 3.5 μM and a linear detection range of 1–5 μM was reported by using a tubular type creatinine-tungstophosphate ion-complex, with no interference from creatine and other organic and ionic species (including urea, thiourea, glycinate, Ca^{2+}, Ba^{2+}, glucose, fructose, citrate, NH^{4+}) (refer Fig. 16).

Non-enzymatic sensing of biological markers by employing neutral carriers like crown ethers which acts as ionophores have also been reported and are gaining significant attention [142,143]. Based on such neutral carriers, Kumar et al. [144] in 2011 reported another non-enzymatic potentiometric creatinine sensor. This sensor was fabricated by depositing β cyclodextrin (βCD) incorporated poly-3,4-ethylenedioxythiophene (PEDOT) on the surface of glassy carbon electrode via electropolymerization (refer Fig. 17). The modified electrode was investigated for creatinine detection via EIS and estimating the charge transfer resistance (R_{ct}). A decrease in R_{ct} value was observed with increase in creatinine concentration, which was explained as the H-bonding interaction of the amide H-atom of creatinine with the O-atom present at the exterior hydrophilic regions of βCD. This causes an agglomeration phenomenon resulting in the reduction of the charge transfer resistance (R_{ct}). The sensor showed stable potentiometric response at neutral and acidic pH (4.0–7.0) and showed unstable results in alkaline pH. This was expected considering the sensing mechanism is dependent on the interaction of the acidic H-atom of creatinine (which at alkaline pH is not available) with the O-atom of βCD. The PEDOT/βCD modified GCE electrode exhibited a fast response time of 60 s, a linear detection range of 0.1 mM to 0.1 M with a detection limit of 0.05 mM and robust shelf life of 1 month with 95% retention of initial response. The sensor also showed selectivity against glucose, urea and ammonium ion. However, uric acid and ascorbic acid showed considerable interference and was suggested removal by using a permselective membrane like Nafion®.

6.2. Non-enzymatic creatinine sensor based on surface modification via pre-anodization

It is well established that the electron-transfer activity of carbon-based electrodes can be greatly enhanced via several electrochemical treatments including pre-anodization. It has been proposed that anodization of carbon electrodes such as GCEs results in surface functionalization via formation of predominantly carbonyl groups and also hydroxyl groups in relatively lesser amount depending upon the nature of the electrolytic/buffer solution used [145–147]. A faster electron transfer was achieved by anodic oxidation of highly oriented pyrolytic graphite (HOPG) due to destruction of the basal plane, consequently increasing the degree of exposure of a large quantity of edge plane graphite at the electrode surface, thereby facilitating electron transfer [148].

In 2006, Chen et al. [149] reported a non-enzymatic electrochemical creatinine sensor for selective and quantitative recognition of creatinine by using a pre-anodized (at 2.0V for 400 s in pH 6.7 PBS) screen-printed carbon electrode. The pre-anodization step generates carbonyl and hydroxyl functionality at the surface of the carbon electrode. Creatinine was then preconcentrated (in the presence of interfering species such as urea, uric acid, ascorbic acid, ammonium ion and creatine) at the SPCE (decomposition potential of 1.8V against Ag/AgCl for 50 s), wherein the carbonyl group at the surface of the SPCE binds relatively strongly with creatinine as compared to other interfering species. A medium exchange process then followed this where the SPCE was washed by dipping it in an aqueous medium under stirring, removing specifically all other species bonded to the surface of the SPCE except creatinine (refer Fig. 18). Ultimately the creatinine concentration was analysed using SWV (Square wave voltammetry) based on the interaction of the active methylene group of creatinine (generated with the addition of HCl), which acts as nucleophilic donor and bind with the carbonyl functionality generated at the SPCE, and the subsequent oxidative stripping at 0.4V. It was reported that in absence of HCl or Cl^{-1} Creatinine (anhydrous), no Faradaic response was observed. This supports the earlier claim of the active methylene group of creatinine being responsible for the nucleophilic binding with the carbonyl functional group at the SPCE generated during the pre-anodization process. Interestingly, since Cl^{-1} is inherently present in urine, this sensor can be used directly without the need for the addition of external chlorine ions to generate the active methylene group in creatinine. It is worth mentioning that this nucleophilic interaction of the active methylene group of creatinine with a carbonyl group is similar to the Jaffe method where the nucleophilic reaction of active methylene group of creatinine with picric acid in an alkaline medium gives the characteristic red-yellow chromogen. This electrochemical sensor reported a detection range of 0.36–3.7 mM with a detection limit of 8.6 μM

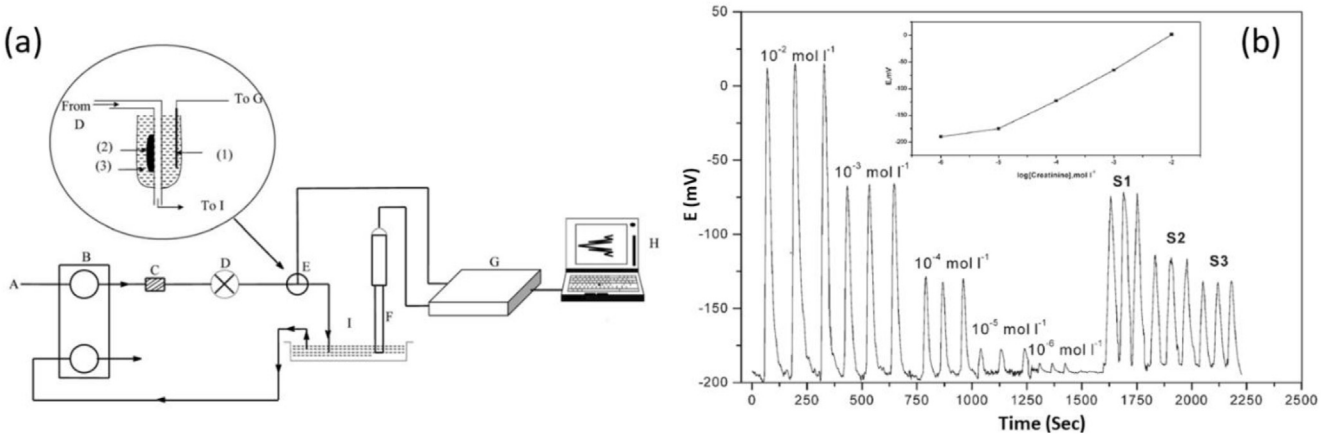

Fig. 16. (a) Schematic representation of the two channel FIA used for creatinine detection [A: Phosphate buffer solution pH = 4.5; B: Peristaltic pump; C: Pulse damper; D: Sample injection valve; E: Flow injection detector; (1) Ag/AgCl internal reference electrode; (2) membrane; (3) Internal reference solution; F: Reference electrode; G: Data acquisition system; H: Computer]; (b) Potentiometric response of various concentrations of creatinine and real human serum samples(S_1,S_2,S_3) using FIA with tubular creatinine-TP based detector (carrier solution, 10^{-2} M PBS pH = 4.5; flow rate = 4 mL/min; injection volume = 100 μL). Reprinted with permission from Ref. [141].

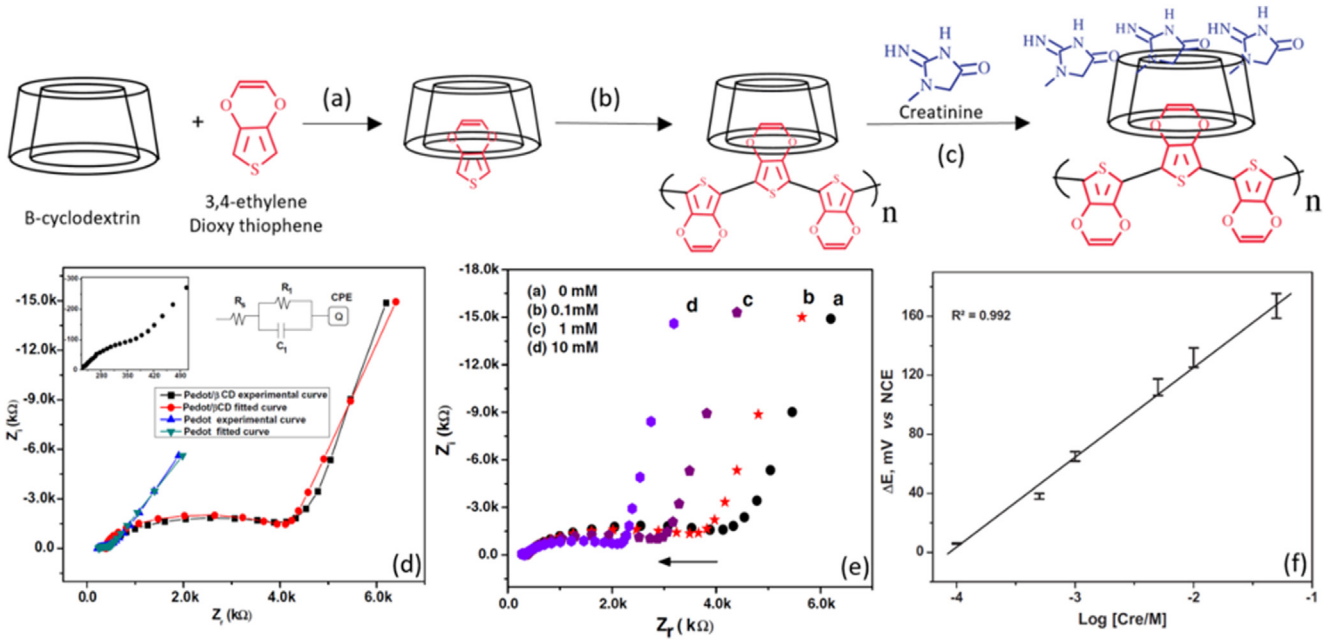

Fig. 17. Schematic representation of (a) Formation of inclusion complex of EDOT and βCD, (b) Formation of poly-3,4-ethylenedioxythiophene (PEDOT)/βCD inclusion complex via electropolymerization, (c) Interaction of creatinine with EDOT/βCD inclusion complex, (d) Nyquist plots of modified electrode with and without βCD (Frequency range 100 kHz to 50 mHz; bias potential: −0.1 V; amplitude: 5 mV; electrolyte: 0.1 M Tris-buffer. Insets show the Nyquist semicircle plot of PEDOT modified electrode and corresponding Randles circuit model), (e) Nyquist plots of PEDOT/βCD with different concentrations of solutions of creatinine, (f) Potential vs. log plot of creatinine concentration in 0.1 M Tris buffer solution (pH = 7). Reprinted with permission from Ref. [144].

(RSD = 3.42%) and was used to analyse two human urine samples which resulted in 97 and 102% recovery rate.

7. Interference removal via pre-treatment processes

Creatinine sensors have undoubtedly evolved significantly in the past two decade and have exhibited promising results. While non-enzymatic sensors provide relatively superior storage stability, they unquestionably suffer setbacks due to interference(s) from one or more interfering species present in the diagnostic bio-fluid, may it be urine, blood, or serum. Hence, to achieve clinically relevant detection results one must unquestionably employ certain approaches to reduce the interference and enhance the selectivity and specificity of the developed biosensor.

Owing to the wide range of sensing mechanisms and the materials used, several pre-treatment processes have been employed (as shown in Table 4). A typical pre-treatment process incorporated with non-enzymatic electrochemical creatinine biosensor aims to reduce the interferences via two main approaches. The first approach is to either reduce the concentration/amount of interfering agents in the sample. This can be done by employing centrifugation as in the case of using serum instead of blood, or diluting the sample in cases where the creatinine is present in relatively larger quantity than the interfering agents like in the case of urine samples [84]. The second approach is by hindering/retarding the contact and/or approach of the interfering agents with the surface of the working electrode while virtually unaffecting the creatinine molecule, thereby eliminating/reducing the chances of interferences. The use of permselective membranes is one such example. As creatinine is reported to be positively charged in biological pH, the use of proton exchange membranes such as Nafion® that can be drop casted or conductive matrices such as Methylene Blue that can be electrodeposited [95], can selectively restrict negatively charged interfering species while allowing creatinine to reach the active sensing site at the electrode surface with ease [22]. Microextraction techniques using hollow polypyridine fibres has also been proven to be an effective approach to achieve high sensitivity with minimized interferences [102]. Electrochemical analysis using specific potential windows has also been proven to be an effective way to segregate and avoid non-specific signal response [84,97]. Additionally, an optimization of pH, thickness of the modification layer and electrochemical sensing parameters like scanning potential range and scan rates can significantly aid in segregating the creatinine signal response from the signals corresponding to interfering agents [102].

8. Conclusions and outlooks

The detection of creatinine has long evolved since Jaffé reported the colorimetric method in 1886 [29]. The detection method and the underlying mechanism of creatinine detection have increased in complexity and have delivered better results. Setbacks due to the centralized nature of clinical creatinine detection based on Jaffé method have aided in the development of the modern creatinine biosensors. We have observed that enzymatic creatinine biosensors (amperometric and potentiometric) are the most widely studied owing to the specific nature of enzymes. Meyerhoff and Rechnitz [59] developed the first enzymatic creatinine sensor using an ammonium ion-selective electrode which had poor storage stability and clinically irrelevant detection range (0.52–9.6 m). Later in 1983, the three enzyme system was reported by Tsuchida and Yoda [62] which was widely used as the fundamental detection mechanism for enzyme based creatinine detection, upon which others have improved by optimizing various parameters like the enzyme immobilization step and the use of oxygen sensitive electrodes [63,64]. However, due to their inherently persistent poor stability [5], researchers investigated a new approach to creatinine detection, which led to the development of non-enzymatic creatinine biosensors.

Of all such non-enzymatic creatinine biosensors, electrochemical biosensors are considered the most promising due to the advantages such as fat response time, robust storage stability and

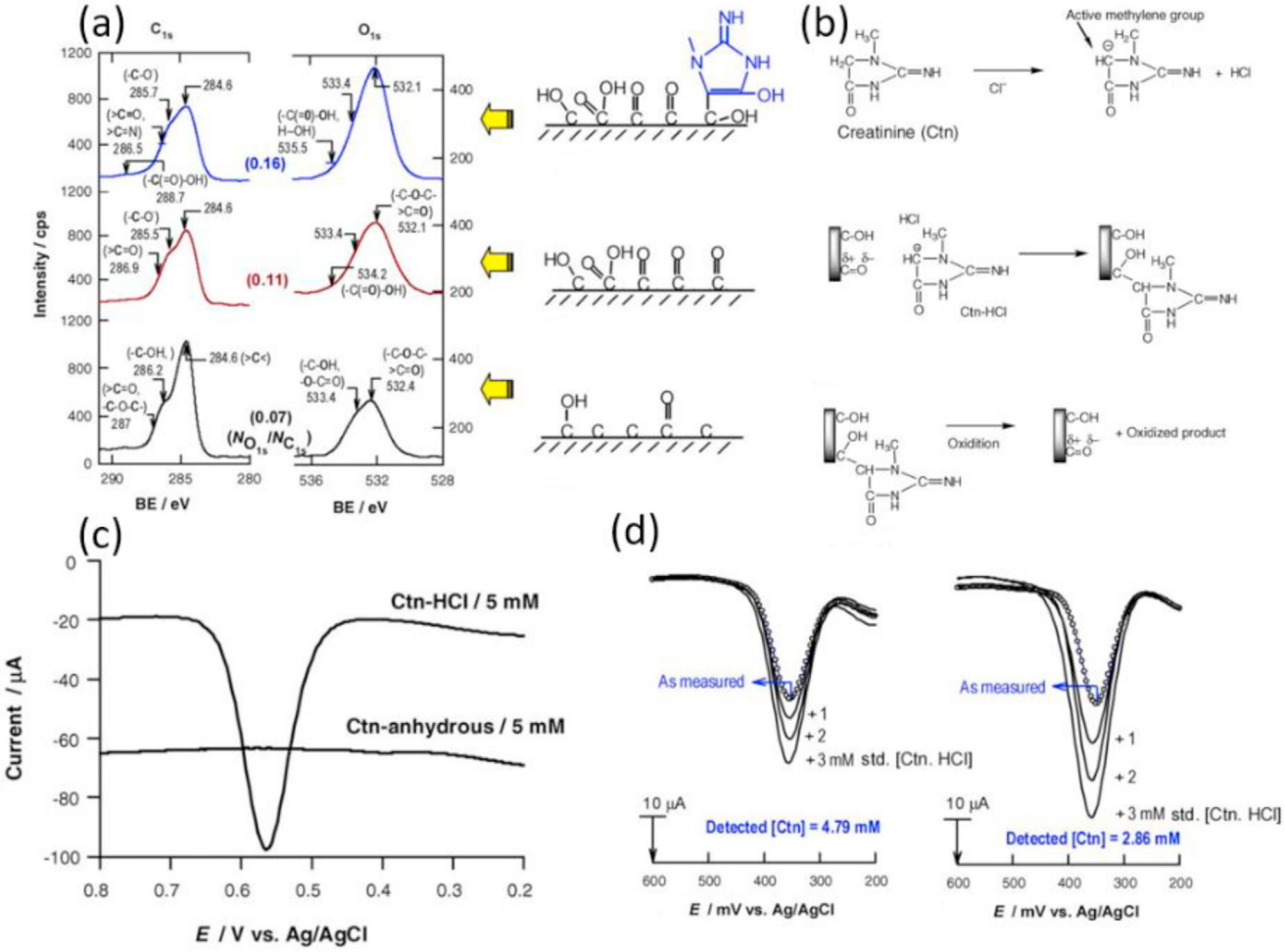

Fig. 18. (a) XPS responses and schematic representation of un-anodized, anodized SPCE and interaction of the generated carbonyl group with creatinine (bottom to top); (b) Top to bottom: Schematic representation of the formation of active methylene group, preconcentration of creatinine at 1.8V and the oxidative stripping at 0.4V; (c) SWV (Square wave voltammetry) responses of the anodized SPCE (in pH 6.7 PBS) with creatinine samples in the presence and absence of chloride ions and (d) Selective recognition of creatinine in two human urine samples which resulted in 97 and 102% recovery rate. Reprinted with permission from Ref. [149].

Table 4
Widely employed methods of reducing interference(s) in non-enzymatic electrochemical creatinine detection.

Ref	Modified Electrode[1]	Interfering Species[2]	Interference removal
[22]	Cu_2O electrodeposited on Pt electrode	Glu, AA, Ur, Cr, Ac, Sc, Am, Cl, UA	Coating with a Nafion® film on the electrode surface, the influences of all the interferences were decreased to less than 5%.
[23]	Cu electrodeposited on SPCE	Glu, AA, Ur, Dp, UA	Serum was incubated for 10 min with PbO_2 powder
[122]	MIP sol-gel drop casted on Graphite electrode	Ur,Tyr,His,Cy,BA, Glu,Phe,Cr,NaCl	Dilution of the urine sample

Abbreviations.
[1] SPCE = Screen printed carbon electrode, MIP = Molecularly imprinted polymer.
[2] Glu = glucose AA = ascorbic acid, UA = uric acid, Ur = urea, Cr = creatine, Ac= Acetaminophen, Sc= Sarcosine, Am= Ammonium, Cl= Chloride, Dp = dopamine, Ty = tyrosine, His = histidine, Cy = cytosine, BA = barbituric acid, Phe = phenylalanine.

suitability for label free detection at the point of care. Several methods were reported pertaining to the development of non-enzymatic electrochemical creatinine biosensors. For instance, the specific interaction of creatinine with metallic centres has been exploited to achieve sensitive creatinine biosensors. Chen and Lin [22], Raveendran et al. [23] and Kalasin et al. [84] used Cu-based metallic centres and achieved wide linear detection ranges from the order of µM to mM. These Cu-based sensors showed robust storage stability with negligible signal reduction and good reproducibility. However, they failed to reach absolute interference free detection and required additional treatments such as using permselective membranes like Nafion® and pre-treating the sample with PbO_2 powder to reduce oxidizable interfering agents. Other metallic centre based sensing schemes included use of Fe and Ag that demonstrated high achievable sensitivities and low limits of detection. Additionally, paper-based microfluidic devices for creatinine detection have also been developed to improve suitability for POC detection due to inherent advantages like ease of use, low-cost fabrication, improved sensitivity and multiplexing capability [91,150]. While these studies highlight the immense potential of metallic centre based electrochemical detection which exhibited inherent long term storage stability with clinically

relevant detection range and repeatable results, fundamental investigations are still required to understand the interaction of the active metallic sensing sites with creatinine. On the other hand, creatinine detection based on MIPs have also been widely studied. These sensors exhibited excellent sensitivity with detection limits as low as 0.015 nM [136], detected via exploiting various electrochemical techniques such as EIS, DPV and DPCSV. Creatinine sensors based on MIPs and NP combinations were also reported and exhibited an added advantage of enhanced electron transfer due to the presence of the NPs [133]. However, a general trend in reduced shelf life has been observed due to the delicate nature of the MIP modified electrode. The use of EIS based signal transduction as seen in most MIP based sensors can hinder repeatability since multiple variables can bring about a change in impedance such as the sample pH and ionic strength. While further research is required to improve performance, non-enzymatic electrochemical creatinine biosensors possess robust stability and POC amenability as compared to their enzymatic counterparts which evidently suffer from stringent sensing conditions and poor storage stability.

Evidently, non-enzymatic electrochemical creatinine detection has not been limited to metallic centres or MIP based sensors. Due to the various advantages of non-enzymatic biosensors, new and highly selective electrochemical creatinine detection techniques have surfaced. Non-enzymatic potentiometric schemes for creatinine detection were also reported which use ion association complexes of creatinine with tungstophosphate (TP) [141] or exploit neutral carriers that act as ionophores [144]. Pre-anodization as a tool for electrode surface functionalization to enhance selective binding of creatinine was also reported [149]. Novel and innovative modifications and electrochemical detection schemes can further extend the immense potential of non-enzymatic creatinine biosensors. This work presents for the first time a detailed review focused primarily on non-enzymatic electrochemical creatinine biosensors reported till date [22,70,128,138]. Considering the wide creatinine detection range and LOD in the order of nM offered by non-enzymatic electrochemical methods with the added advantage of material choices (especially metallic centres for specific integration with creatinine) and novel pre-treatment processes to remove interference, the full potential of sensors is yet to be harnessed. However, extensive studies are still required to enable successful mass adoption. Better control over the detection mechanism and stringent optimization is an absolute necessity. We thus hope that this review shall aid in the understanding the immense potential of creatine detection via non-enzymatic electrochemical approaches and serve as a tool to help in the development of more efficient and innovative creatinine biosensors in the future.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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