Abstract

Preamble

This thesis focuses on developing novel non-enzymatic methods for the electrochemical determination of the renal dysfunction marker, creatinine, in human body fluids (urine and serum) by studying and optimizing its condition-dependent interactions with transition metal ions (cobalt and copper) and an organic reagent, 2-nitrobenzaldehyde, using glassy carbon and platinum electrodes.

Background

Creatinine (2-amino-3-methyl-4*H*-imidazol-5-one) is a nitrogenous waste that is produced in the muscle cells due to the catabolism of creatine {2-[Carbamimidoyl(methyl)amino]acetic acid}. This waste product is typically excreted from the body via urine. However, when a patient suffers from any renal disease, decreased glomerular filtration rate (GFR) disrupts normal creatinine levels in body fluids. Therefore, the significance of creatinine lies in the area of healthcare, as it is the most reliable renal function marker.

Historically, urea and blood urea nitrogen (BUN) were used as kidney function markers, but they have limitations due to non-renal factors like diet and urea cycle enzymes. Low molecular weight proteins (LMWPs) such as β2-microglobulin (B2M), β-trace protein (BTP), and cystatin C have also been explored as alternative markers. However, these proteins face challenges, including dependence on non-GFR factors and lower serum concentrations, making detection more complex. Thus, creatinine remains the most reliable and widely accepted renal function marker. Its significance is unlikely to be replaced soon due to its well-established role and the limitations of alternative markers.

There are two clinically practised traditional methods, employed to measure creatinine levels for renal function assessment: the Jaffe method and the Enzymatic method. The Jaffe method involves a chemical reaction between creatinine and picric acid in an alkaline medium, producing an orange-red complex. In contrast, the Enzymatic method utilizes a multi-enzyme, multi-step reaction to form a dye. Both methods rely on colorimetry for creatinine detection. However, these traditional approaches are limited by

interference from other components in body fluids. Hence, the development of alternative, precise, interference-free, cost-effective and robust creatinine sensors is an exciting area of research.

While the traditional methods were colorimetry-based, several other transduction systems have been explored for creatinine determination in recent times. Electrochemical methods are claimed to have an edge over all other transduction systems, due to their high sensitivity, ease of operation, robustness, variability of output signals and possibility of miniaturization, so that the sensor can be further designed into a hand-held point-of-care-testing (POCT) device. Moreover, researchers have also aligned their approaches to developing enzyme-free creatinine determination methods to mitigate the disadvantages (high cost and short-term stability) of enzymatic processes.

The biggest challenge that emerges while designing non-enzymatic electrochemical creatinine determination protocols is the electrochemical inactivity of creatinine. To counter this challenge, the inherent coordinating property of creatinine with transition metal ions to form electro-active complexes has been favourably utilized. While methods based on creatinine-transition metal complexes found utmost prominence, the usage of molecular-imprinted polymers has also been gaining attention.

However, the domain of creatinine sensor development still remains an open area of much more work as the demand for a widely accepted alternative method that can address the shortcomings of the traditional method has not yet been satisfactorily met. In this thesis work, we have attempted to develop some novel approaches for creatinine determination. The contents of this thesis have been divided into the following 7 chapters-

Chapter 1: ***A general introduction to creatinine, its importance and its detection techniques***

In Chapter 1, a brief introduction to creatinine, and its importance in healthcare and research, while highlighting the necessity of its quantification in human body fluids, have been presented. This chapter also comprises a critical overview of the traditional creatinine detection method and some of the latest reported alternative methods with different transduction systems. Furthermore, the foundation of the approaches we have undertaken in this thesis work to determine creatinine in urine and serum has been discussed.

Chapter 2: ***Materials and Methods***

In Chapter 2, the details of all the chemicals and reagents used in the entire study have been presented, along with the chemical structures of some important molecules. The chapter also contains the details of all the instruments used to carry out this thesis work and a brief description of each of the electrochemical techniques used.

Chapter 3: ***A novel method for electrochemical determination of creatinine in human urine based on its reaction with 2-nitrobenzaldehyde using a glassy carbon electrode***

Chapter 3 marks the beginning of the experimental sections of this thesis. This chapter presents a unique approach to quantifying creatinine using the DPV technique, as the oxidation of creatinine by 2-nitrobenzaldehyde under basic conditions has been explored to accomplish its detection. The oxidized product yields a redox peak whose intensity varies quantitatively with creatinine concentration. The optimization of reaction time, pH and 2-nitrobenzaldehyde concentration for reaction efficiency has been elaborated here. Furthermore, the designation of all the redox peaks and the proposal of a plausible mechanistic reaction pathway, supported by electrochemical and spectroscopic findings, have been presented. This chapter also comprises the interference study of the system in the presence of some other urinary components (glucose, ascorbic acid, uric acid, urea and dopamine) and the successful demonstration of the reliability of this system in human urine. To the best of our knowledge, this is the first reported work on creatinine detection based on its prior metal-free chemical transformation to electrochemically active species.

Chapter 4: ***Deciphering the complexation processes of creatinine-cobalt and creatinine-cobalt-2-nitrobenzaldehyde: Morphological, spectroscopic and electrochemical analysis***

While we developed a metal-free creatinine detection method based on its oxidation by 2-nitrobenzaldehyde (discussed in Chapter 3), we were curious to discover how the reaction pathway changes in the presence of a transition metal ion. This inquisitiveness has been addressed in Chapter 4. Cobalt ion was selected for this study, due to creatinine's inherent coordinating property with cobalt ions and also, due to the ability of the nitrite group to form chelate and bridging compounds with cobalt ions. As different conditions-dependent pathways of reactions can be undertaken when any two of the three components (cobalt ion, creatinine, and 2-nitrobenzaldehyde) react, bringing

these three components together opens up multiple reaction possibilities. Chapter 4 deals with the exploration of the reaction between creatinine, cobalt ion and 2-nitrobenzaldehye, under some controlled conditions. The preparation of a creatinine-cobalt complex, in the absence of 2-nitrobenzaldehyde, and how the addition of 2-nitrobenzaldehyde to the complexation pathway of creatinine-cobalt yields another complex of creatinine-cobalt-2-nitrobenzaldehyde, has been highlighted in this chapter. The characterizations of the two new complexes by SEM, P-XRD, FTIR, Raman, DRS, EDX, CV, DPV and EPR techniques and the establishment of differences in their physiochemical properties (morphologies, coordination spheres and oxidation state of the central metal ions) have also been presented.

Chapter 5: ***A highly selective method for electrochemical determination of creatinine in human serum based on its coordination reaction with Co^{3+} in the presence of 2-nitrobenzaldehyde***

Establishing the complexation of cobalt with creatinine and 2-nitrobenzaldehyde (discussed in Chapter 4) enabled us to develop a new method to determine creatinine using the DPV technique. Chapter 5 focuses on the development, optimization and demonstration of this method. The different voltammogram responses for the buffer systems (pH 7.4) containing a mixture of cobalt ions and 2-nitrobenzaldehyde, in the presence and absence of creatinine, have been elaborated in this chapter. The identification of a creatinine concentration-dependent redox peak and the optimization (reaction time and concentrations of the reagents) of the system for the reaction efficiency have also been presented. Furthermore, this chapter comprises a plausible mechanistic pathway of the reaction involved, with electrochemical and spectroscopic evidence, and the designation of all the redox peaks. The interference in the system by some other serum components (glucose, albumin, uric acid, ascorbic acid and urea) and the successful demonstration of the reliability of the system in deproteinized human serum are also significant parts of this chapter.

Chapter 6: ***Insight into the creatinine-copper interaction through electrochemical, UV-vis spectrometric and impedimetric study and feasibility of molecularly imprinted creatinine sensor design***

While copper has been the most commonly chosen transition metal to develop creatinine sensors, some ambiguities have been noticed in the voltammograms of copper and in the trend of how the voltammogram changes in the presence of creatinine. The ambiguity can be addressed by obtaining stable voltammograms of copper and by correctly designating the oxidation state of copper with which the creatinine interaction predominantly occurs. This chapter focuses on these aspects as electrochemical and spectroscopic analyses have been carried out to study the creatinine-copper interaction. The importance of voltage sweep directions in order to get stable voltammograms of copper has also been marked out. The electrochemical analyses were carried out with both bare and copper-deposited electrodes to resolve the ambiguities found in the literature. The feasibility of electrochemically fabricating an MIP-based creatinine sensing platform has also been demonstrated. Microscopic and electrochemical analyses, utilizing the creatinine-copper interaction, enabled us to validate the formation of an electrochemically fabricated MIP-modified electrode.

Chapter 7: ***Conclusions and Future Scope***

In this chapter, we have summarized the key findings and outlined the future direction for further development.