



Chapter 7



Conclusions and Future Scope

7.1 Overall conclusions

This thesis commences with an introductory Chapter 1, followed by Chapter 2, which outlines the essential chemicals, instruments, and fundamental principles of the employed electrochemical methods. The core experimental findings are presented in Chapters 3-6. This concluding chapter provides the overview of each preceding chapter, and the key findings, conclusions, significance, drawbacks and possible future scopes are also highlighted.

7.1.1 Chapter 1

In this introductory chapter, the significance of creatinine as a renal function marker, the traditional creatinine determination methods and the need to develop alternative creatinine determination methods, have been discussed. The key findings from this chapter are:

- ❖ The limitations of alternative biomarkers ensure creatinine's continued dominance as the primary indicator of kidney function.
- ❖ Traditional creatinine determination methods, widely used in clinical settings, have significant drawbacks due to interference, driving the need for innovative and more accurate detection techniques.
- ❖ Creatinine has an inherent property to coordinate with transition metal ions.
- ❖ Researchers have turned to non-enzymatic electrochemical techniques as a viable alternative for creatinine analysis, capitalizing on their advantages.
- ❖ Due to creatinine's electrochemical inactivity, its ability to form complexes with transition metal ions is often leveraged for non-enzymatic electrochemical detection.

7.1.2 Chapter 2

In this chapter, the chemicals and instruments used in this thesis work have been detailed, and the basic working principle of electrochemical methods have been described.

7.1.3 Chapter 3

In this chapter, the reaction of creatinine with ethanolic 2-nitrobenzaldehyde (2-NBA) and NaOH has been investigated from an electrochemical perspective, and a unique

approach for urinary creatinine determination has been presented using the DPV technique. To the best of our knowledge, this is the first reported work on electrochemical creatinine determination based on its chemical transformation to electro-active species in non-enzymatic and metal-free conditions. The conclusions drawn from this chapter are:

- ❖ A reaction time of 10 minutes, pH of 6.6 and 2-NBA concentration of 7 % (w/v) are the optimum conditions.
- ❖ The LOD of the system is 0.5 mM, with an excellent R^2 value of 0.99 in the linear detection range of 1–25 mM.
- ❖ The system exhibits high selectivity, as for the selected concentration ranges, interference in the presence of ascorbic acid and dopamine was determined to be less than 0.55 %, less than 3.15 % in the presence of urea and glucose and less than 8.5 % in the presence of uric acid.
- ❖ The method was successfully demonstrated in human urine and creatinine recovery percentage (96.02–105.14 %) infers a remarkable sensitivity of the system.

7.1.4 Chapter 4

This chapter focuses on the creatinine-cobalt complexation and explores how introducing 2-NBA in the creatinine-cobalt complexation pathway yields another new complex of creatinine-cobalt-2-NBA with different physiochemical properties. The conclusions drawn from this chapter are:

- ❖ Two new complexes, $[\text{Co}(\text{CR})_3(\text{H}_2\text{O})]\text{Cl}_2$ (% yield = 73.18%) and $[\text{Co}(\text{CR})_3(\text{H}_2\text{O})_2(2\text{-NBA})]\text{Cl}_3$ (% yield = 86.34%) have been synthesized. [CR = creatinine]
- ❖ With spectroscopic analysis, cobalt coordination with the endocyclic nitrogen of creatinine and chelation with the nitro group of 2-NBA have been determined.
- ❖ The complexes are amorphous, water-soluble, ionic, electrochemically active, conducting and paramagnetic.
- ❖ Striking differences in their morphologies (rod-shaped and fibrous, respectively) and oxidation state of the cobalt ion (+2 and +3, respectively), have been established.

7.1.5 Chapter 5

This chapter presents a new approach for developing a serum creatinine determination method, harnessing the coordinating properties of cobalt with creatinine and 2-NBA in a DPV-based detection scheme. The conclusions drawn from this chapter are:

- ❖ Conversion of Co^{2+} -2-NBA complex to a Co^{3+} complex in the presence of creatinine triggers a voltammetric response.
- ❖ A reaction time of 5 minutes, cobalt chloride solution concentration of 0.5 M and 2-NBA concentration of 7 % (w/v) are the optimum conditions.
- ❖ The LOD of the system is 9.5 μM , with an excellent R^2 value of 0.99 in the linear detection range of 50–600 μM .
- ❖ The method exhibits negligible interference ($< 7\%$) in the presence of uric acid, urea, ascorbic acid, and glucose within the tested concentration ranges, but albumin significantly impedes detection.
- ❖ Deproteinization of serum by ACN treatment ensures the mitigation of interference by albumin.
- ❖ The method was successfully demonstrated in deproteinized serum and creatinine recovery percentage (94.65–99.22 %) infers a remarkable sensitivity of the system.

7.1.6 Chapter 6

In this chapter, the ambiguities in the literature regarding the electrochemical response for creatinine-copper interaction have been addressed, and the interaction has been utilized to confirm an MIP platform fabrication for creatinine sensing. The conclusions drawn from this chapter are:

- ❖ The redox peaks of copper obtained by recording voltammograms of a solution containing Cu^{2+} ions are inhibited by creatinine due to creatinine-copper complexation.
- ❖ Stable and well-resolved voltammograms of copper enabled us to conclude that creatinine interacts preferably with Cu^{1+} ions in aqueous medium.
- ❖ At a DC voltage of 0.15 V, the kinetics of the redox process of copper is determined to be the quickest.

- ❖ The hyperchromic shifts in the electronic transitions in creatinine in the presence of Cu^{2+} ions vouches for the coordination of the ion with the endocyclic nitrogen of creatinine.
- ❖ A new charge transfer peak in UV-vis spectra of a mixture containing creatinine and Cu^{1+} ions substantiated the creatinine- Cu^{1+} complexation.
- ❖ The redox peaks of copper obtained with Cu^0 deposited Pt electrode intensify in the presence of creatinine, but the current degrades quickly in subsequent voltammogram runs
- ❖ Creatinine can be successfully imprinted in conducting polymer polypyrrole, which can be validated by SEM analysis, impedimetric studies and by utilizing the creatinine-copper electrochemical interaction. This hinted at the possibility of developing an MIP-based creatinine sensor.

Hence, it can be stated that, in this work, we endeavoured to make some contributions to the area of creatinine sensor development as we undertook the non-enzymatic approach to develop robust, stable, accurate and cost-effective electrochemical creatinine determination methods. To accomplish our aim we primarily focussed on two aspects: a) the chemical transformation of creatinine to electro-active species in a non-metallic condition, and b) the interaction of creatinine with metal (cobalt and copper) ions, and our research yielded some interesting insights and discoveries.

7.2 Significance

Despite our methods being developed in an enzyme-free condition, they offer quick, selective, and highly sensitive creatinine determination in urine and deproteinized serum. Unlike most other electrochemical approaches, no tedious electrode modification is required as a bare glassy carbon electrode can provide the sensing platform in both creatinine determination processes. The methods are applicable across the entire pathological range, exhibit better analytical performance than many contemporary methods and are promising new tools in clinical practices for creatinine determination.

7.3 Drawbacks

The drawbacks of this work are as follows:

- ❖ Our creatinine determination methods lack extensive validation through diverse sample populations, encompassing various ages, body weights, genders, ethnicities, and health statuses.
- ❖ The reaction time required for our creatinine determination methods presents a challenge for their adaptation into point-of-care testing (POCT) devices.
- ❖ Although clinical labs can easily accommodate this step, the necessity for deproteinization in serum creatinine analysis prolongs the process, rendering it more challenging for incorporation into POCT devices.
- ❖ The chelation of cobalt with 2-NBA was determined in the aqueous medium by electrochemical and spectroscopic analysis but the cobalt-2-NBA complex could not be isolated.
- ❖ The Cu⁰-deposited Pt electrode sensing platform exhibits poor reusability due to degradation observed in the successive voltammogram runs.

7.4 Future Scopes

There are several potential directions for further investigation as pointed below:

- ❖ The electrochemical responses in urinary creatinine and serum creatinine determination protocols can be studied with electrodes modified with conducting polymers (polypyrrole, polyaniline, carbon nanotubes) and other electroactive materials like rGO or their composites.
- ❖ The development of creatinine sensors based on creatinine's coordinating ability with ions of cobalt, chromium, nickel, etc. has been underexplored and must be studied.
- ❖ The serum creatinine determination method based on creatinine-cobalt-2-NBA complexation can also be studied in the presence of other metal ions to develop a ratiometric sensor, promising higher selectivity.
- ❖ In the serum creatinine determination method based on creatinine-cobalt-2-NBA complexation, integrating the deproteinization step creatinine determination into a single step can streamline the process and reduce overall time.

-
- ❖ The time of the chronopotentiometry run and the concentrations of the pyrrole and creatinine must be optimized for the fabrication of the MIP platform.
 - ❖ Integrating Cu⁰ with MIP sensors promises a fruitful research avenue, ripe for future advancements.
 - ❖ The creatinine determination methods can be further developed into POCT devices through integration with electronics.