

POC device for non-enzymatic electrochemical detection of Chronic Kidney Diseases

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Abstract

The aim of this research study is to synthesize and characterization of the Graphene based nanocomposite material. Then fabrication of a miniaturized and disposable carbon strip-based sensing electrode utilizing the newly synthesized grapheme-based nanocomposite material was completed. Quantification of urea and creatinine with the fabricated strip to estimate the sensing ability of the synthesized nanocomposite using a potentiometric approach has been done. Finally, the Performance analysis of the developed sensor has been done with real sample and a standard protocol has been established for this chip based non-invasive electrochemical sensor.

Keywords: Kidney Diseases, serum creatinine, potentiometric

1. Introduction

Chronic Kidney Diseases (CKD) are progressing towards becoming a crucial health issue with a high economic burden to the present global health system. It has now become a health priority owing to its high prevalence, mortality, and morbidity due to the lack of proper screening and affordable treatment. CKD is characterized by steady and gradual deterioration of kidney function with an irreversible decrease in glomerular filtration rate which causes a gradual increase in blood urea nitrogen level (BUN) and serum creatinine. Blood tests for BUN and serum creatinine can diagnose kidney dysfunction because their levels in the blood partially reflect the state of kidney function. There are numerous diagnostic procedures like colorimetric, chromatographic, potentiometric, spectrophotometric, enzymatic electrochemical analysis, surface plasmon resonance which are available for quantitative

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analysis of BUN and serum creatinine levels. These conventional techniques involve comparatively longer sample preparation time, costly equipment, complicated enzyme immobilization method, high cost of an enzyme, and vital operating conditions which restrict their applicability for rapid onsite monitoring. Moreover, conventional diagnostic procedures involve the collection and use of blood samples. Repeated blood sample collection can cause discomfort and anxiety to the patients. In addition, frequent venous punctures can increase the risk of infection. Thus, in this present scenario, it is essential to establish an accurate, cost-effective, and non-invasive approach for real-time monitoring of urea and creatinine for clinical assessment of kidney dysfunction. Here, research work has been done to develop a portable device having the facility for insertion of a small, disposable chip. The chip will be fixed with graphene-based nanocomposite for the nonenzymatic electrochemical detection of urea and creatinine in a noninvasive way. Such an approach will make the CKD diagnosis user-friendly and affordable. In this work plan, firstly the nanocomposite material has been synthesized and characterized in detail by UV-VIS Spectroscopy, SEM, and EDS technique. The synthesized nanocomposite material has also been evaluated in terms of stability in phosphate buffer saline (PBS) before electrode preparation. Finally, a carbon strip-based sensing electrode has been fabricated using the nanocomposite for the detection of urea and creatinine in physiological fluids (urine, saliva, sweat). Electrode performance has been tested by measuring the CV and DPV of a mixture of urea and creatinine using the nanocomposite-based carbon strip by an electrochemical analyzer with a three-electrode electrochemical cell configuration. Then, a compact and portable device was developed by integrating the fabricated electrode with an electronic circuit module that can convert a micro-range current to a visible/ measurable output signal. After that, the developed portable device was tested with real samples in the laboratory and the result was correlated with other results of the same sample from standard laboratory techniques. Finally, a standard protocol has been established for non-enzymatic electrochemical sensing of urea and creatinine in a noninvasive manner with this developed handheld device.

2. Literature survey

Unlike blood-based methods, non-invasive techniques to analyze urea and creatinine using urine, saliva, and sweat samples cause reduce infection and user discomfort. Son Vu Hoang et al. developed an enzyme-based colorimetric POC device for automated urine testing [1]. Electrochemical detection-based POC devices have also been introduced. Enzymatic electrochemical sensor for urine urea analysis have been fabricated by Ahmad et al [2]. But,

all of the above approaches involve enzyme immobilization which affect the shelf life of the sensor in terms of storage and stability. Nguyen et al. fabricated a NiCo₂O₄/3D graphene based non-enzymatic electrochemical sensor with good sensitivity and fast response time for detection of urea in urine samples [3]. Diouf et al. developed Screen-printed electrode based sensor for creatinine estimation in urine [4].

In national level, Pandya et al. developed spectroscopic techniques to determine salivary urea and creatinine levels [5]. However, these methods are suffered from specificity problems because of interfering agents like ketoacids, amines or NH₄⁺ ions present in bio-fluids [6]. Indirect CRE detection by amperometric monitoring has been developed based on the interaction of Iron and creatinine by Kumar et al. [7]. Desai et al. demonstrated the enzymatic detection of CRE in spiked urine samples by CV and DPV analysis of cysteine [8]. In this context, we can say that the use non-invasive, non-enzymatic electrochemical system to detect urea and creatinine has not received much attention in India as POC device.

3. Study design

Firstly, the Graphene based nanocomposite material was synthesized and characterized according to the developed protocol. Atomic Force Microscopy and Raman Spectroscopy were utilized for detection and characterization of grapheme surface. FTIR Spectroscopy and UV-VIS Spectroscopy were employed to determine the functionalization of graphene. Then, the newly synthesized nanocomposite material was placed on a piece of carbon strip to fabricate a paper based electrode. One end of the nanocomposite embedded paper strip was connected with a copper wire to develop a working electrode. Then, an electrically integrated microfluidic channel was fabricated through which the sample was pumped to interact with the nanocomposite modified surface. After that, the electrode performance of the newly fabricated electrode was checked by using an electrochemical analyzer with three electrode electrochemical cell configuration. CV and DPV (The scan rate and the potential range will be 0.05 V/s and -0.8 to +1.0 V, respectively) were performed to check the electrochemical sensing ability of the nanocomposite using an electrochemical analyzer in combination with 3 electrode electrochemical cell. The Ag/AgCl (1 M KCl) and a Pt wire will be used as reference electrode and counter electrode, respectively. Position and height of the anodic peaks in the CV will be used for detection and quantification after analyzing the data using a computerized statistical program. The resulting electrical signal was recorded for quantification of urea and creatinine present in the sample. Initially, the urea and creatinine solutions of various concentration were prepared using phosphate-buffered saline (50mM; pH

7.2) in laboratory as source of study to examine the sensing performance of the developed carbon strip based device. After satisfactory performance the device was tested with real urine, sweat and saliva samples collected from hospital, pathological laboratory and common people. Then, the result was compared with the results from standard laboratory techniques to establish a degree of correlation. Finally, a protocol has been established for quantitative analysis of urea and creatinine in non-invasive approach. The device contained a replaceable fluidic chip. One end of the chip was to hold the nanocomposite based sensing electrode embedded micro channel. Other end of the chip was the sample collector. After fabricating the channel, planar electrodes (CE and RE) of suitable material were developed in a rectangular box where associated electronic module built. Then, the current was measured between WE and CE and its peak was directly proportional to concentration of analytes. The output current was then converted to voltage which was then amplified using a voltage amplifier. Finally, the concentration in terms of voltage was displayed on LCD panel after calibration.

4. Conclusion

CKD patients need to be under constant supervision for proper assessment of clinical diagnosis, validation of disease stage and continuous monitoring of treatment. But, repeated blood collection can cause discomfort and anxiety to the patient. Repeated venous puncture can also increase the possibility of infection. The proposed noninvasive, non-enzymatic electrochemical analysis of urea and creatinine can be used more frequently without any discomfort and risk of infection to the patient. The proposed diagnostic procedure is suitable to all age groups. It can monitor large populations from all economic group due to its low cost and relatively simple sample collection procedure. The proposed procedure can screen urea and creatinine level within short range of time. The proposed device is user friendly and light weight because it does not require any special equipment and trained medical personnel. This device is easy to store and transport. Moreover, this device has no chance of causing exposure to harmful chemicals. Due to the above advantages, in both public and clinical health scenario, this proposed device can be utilized as affordable, noninvasive and portable diagnostic procedure for screening of early stages of CKD. In developing countries like India where trained healthcare personnel are limited, the residential supervision of CKD biomarkers (Urea, creatinine) using portable and affordable sensor in point of care device is a promising approach for clinical diagnosis and personal healthcare. No existing literature or healthcare product is available as of today that resembles such type of noninvasive, enzyme free, potentiometric device. Hence, this research is highly relevant and will have strong

impact on both societal and health economy. Biomedical Industry having interest to promote Point of Care devices can also be benefitted by commercializing this developed device.

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