

Creatinine is the end product of creatine metabolism and its concentration in blood & urine is used as an indicator in the diagnosis of renal diseases, thyroid malfunction & muscular disorders (Julia *et al.*, 2002). Unlike urea, the concentration of creatinine in these body fluids is not influenced by the protein intake & hence creatinine is a more reliable indicator of renal functions (Kazmlerczak, 1991). On the other hand, in healthy individuals, the elimination of creatinine in urine occurs at an approximately constant rate & its concentration is used as a correction factor in the study of excretion of several substances (Iversen *et al.*, 1998). The normal human serum reference ranges of creatinine are 0.3-1.2 mg/dl (22.9-91.5 $\mu\text{mol/L}$) for new-borns, 0.2-0.4 mg/dl (15.3-30.5 $\mu\text{mol/L}$) for infants, 0.3-0.7 mg/dl (22.9-53.4 $\mu\text{mol/L}$) for children, 0.5 to 1.0 mg/dl (about 45-90 $\mu\text{mol/L}$) for women and 0.7 to 1.5 mg/dl (60-110 $\mu\text{mol/L}$) for men. Men have slightly higher values than women (Whelton *et al.*, 1994). In the body, creatinine is formed by a spontaneous and irreversible conversion from creatine and creatine phosphate, which is the source of high-energy phosphate bonds for the immediate reformation of ATP during muscular contraction. Formation of creatinine is fairly constant, about 2% of the whole body creatine being transformed per day. There is direct relationship between plasma creatinine level and muscle mass. Creatinine production is not affected by illnesses, such as sepsis and trauma, or dehydration, but may increase with increased dietary protein intake (Jacobsen *et al.*, 1980). Creatinine cannot be reutilized and thus is a waste product. It is freely filtered by the glomeruli and there is no significant tubular reabsorption. A small amount of creatinine is excreted by active tubular secretion and its amount increases with raised plasma creatinine concentration (Bauer *et al.*, 1982). The plasma creatinine level depends on the creatinine production rate and the rate of elimination in the glomerular filtrate. In steady state, the rates of creatinine production and elimination are equal and can be estimated as the product of urine creatinine concentration and urine flow rate. At constant creatinine production, the plasma creatinine level increases, when creatinine elimination via the kidneys decreases.

Measurement of creatinine in plasma or serum and urine is essential in the evaluation of renal functions. In clinical practice, there are two significant decision levels: Values above 140 μM indicates the necessity of performing other tests for assessment of renal function such as creatinine clearance test. Values above 530 μM are almost invariably associated with severe renal impairment. Creatinine values in plasma have been used directly to estimate the glomerular filtration rate (GFR). Plasma creatinine, however, is not usually measurably increased until there is at least 50% loss of renal function (Renkin

and Robinson, 1974). Creatinine clearance calculated from measured creatinine concentrations in plasma and in timed urine collections might thus be expected to indicate early dysfunction more sensitively (Bowers and Wong, 1980). A creatinine clearance test is used to evaluate the rate and efficiency of kidney filtration. It helps to detect kidney dysfunction and/or the presence of decreased blood flow to the kidneys. In patients with chronic kidney disease or congestive heart failure (which decrease rate of blood flow), the creatinine clearance test helps in monitoring the progress of the disease & evaluation of its severity. Any condition or disease that affects the glomeruli could decrease the kidney's ability to clear creatinine and other wastes out of the blood. During this, the blood creatinine level is increased and the creatinine clearance is decreased, because not as much creatinine can be excreted in urine. The less effective the kidney filtration, the greater decrease in creatinine clearance, which indicate congestive heart failure, glomerulonephritis, renal ischemia (blood deficiency), acute nephritic syndrome, acute renal failure, chronic renal failure, end stage renal disease, rapidly progressive glomerulonephritis, Wilm' tumor, acute tubular necrosis, rhabdomyolysis, pre-eclampsia, diabetic nephropathy, haemolytic-uraemic syndrome (HUS), bilateral nephrectomy, cardio vascular disease, hypoxic-ischemic encephalopathy (HIE), acute lymphoblastic leukemia (ALL) and acute pancreatitis (Jelliffe, 1973, Julia *et al.*, 2002). Based on their own data and data from the literature, authors argued that plasma creatinine is more sensitive than creatinine clearance in detection of changes in glomerular function. Rosano and Brown (1982), also found that plasma creatinine is a more sensitive marker of change in renal function.

Various methods have been used to determine creatinine like chemical (Jaffe, 1886), enzymic colorimetric methods (Miller and Dubos, 1937), high performance thin layer chromatography (HPTLC) (Brown, 1966), high performance liquid chromatography (HPLC) (Sadilek, 1965), liquid chromatography-mass spectrometry (LC-MS) (Takatsu and Nishi, 1993), liquid chromatography-tandem mass spectrometry (LC-TMS) (Keevil *et al.*, 2002), liquid chromatography-isotope dilution mass spectroscopy (LC-IDMS) (Harlen *et al.*, 2010), gas chromatography-mass spectrometry (GC-MS) (Siekman, 1985), capillary electrophoresis (Guzman *et al.*, 1990), isotope dilution mass spectroscopy (IDMS) (Bergman and Ohman, 1980), molecular imprinted polymer methods (Tsai and Syu, 1995) and optical methods with UV-absorbance (Fridolin *et al.*, 2010; Tomson *et al.*, 2011). Most of these methods are precise and suitable for many applications. However, these methods do not satisfy the requirements for a fast, accurate

and specific analysis as these are complicated, time-consuming, complex and usually required complicated sample pretreatment of collected samples, relatively large amounts of biological samples, delicate, bulky & expensive instrumental set-up and expertise handling specifically in case of chromatographic methods. Sensors and enzymatic biosensors have many advantages over these methods (Killard and Macolm, 2000; Lad *et al.*, 2008, Chou *et al.*, 2009). The goal of biosensor engineering in the clinical laboratories is to reduce cost, time, and complexity of routine analysis of biological fluids; enable near-patient testing of blood, urine, and saliva in medical centers; and ultimately enable home testing by individuals (Schenk *et al.*, 2007). The main requirements for a biosensor approach in terms of research and commercial applications are the identification of a target molecule, availability of a suitable biological recognition element and the potential for disposable portable detection systems. The biosensor is normally constructed by immobilizing a biologically sensitive material in intimate contact with a suitable transducing system to convert the concentration of the analyte into a quantifiable signal. With recent advances in electrochemical technology, enzyme based electrochemical biosensors were highly sensitive with change in substrate concentration with less response time and thus in high demand. The electrochemical biosensors were based on electrochemical species consumed/generated during biological interaction process of the biologically active substance/substrate where the electrochemical detector measured the signal produced by the interaction. The development of electrochemical creatinine biosensors have followed two paths; systems based on either potentiometric or amperometric detection (Killard and Macolm, 2000; Lad *et al.*, 2008, Chou *et al.*, 2009). Each system is characterized by certain advantages and disadvantages. However, these amperometric biosensors have significant problems involving low sensitivity & selectivity, elimination of interferences, limited electron communication, complexity of immobilization, long incubation time, high operational potential and low sensor stability which are still to be improved or solved. Unfortunately, potentiometric biosensors also suffer from interference by cations and endogenous ammonia that is present in blood and urine (Shih and Huang, 1999). To overcome these problems, the enzymes need to be immobilized directly/covalently onto the film-coated electrode. Therefore, it is highly desirable to design and prepare such a functional material for modification of the electrode surface that efficiently lowers the H₂O₂ oxidation potential and maintains the enzyme activity.

The emergence of nanotechnology offers great opportunities to improve the sensitivity, stability and anti-interference ability of the biosensing systems. Nanotechnology, a discipline which deals with the properties of materials at the nanoscale, is developing as a potent tool to enhance the performance of biosensors (Vaddirajua *et al.*, 2010). In recent years, with the development of nanotechnology, a number of novel nanomaterials have been fabricated and their novel properties are being gradually discovered and their application have also greatly advanced biosensors performance (Yadav *et al.*, 2011a,b; Devi *et al.*, 2011a,b). Biosensors represent an especially exciting opportunity for high-impact applications benefiting from “nano” attributes. Nanomaterials can contribute in either the bio-recognition element or the transducer, or both, of a biosensor. The effective biorecognition area, i.e. the area actually interacting with the analyte, is one of the important parameters, which determine the sensitivity of a biosensor. Nanomaterials, especially nanoparticles, provide a promising way to increase the bio-recognition area, because the high surface to volume ratio of nanoparticles provides a large number of sites available for molecular interactions. Nowadays, nanoparticle-enhanced biosensors show significant development. Researchers tend to integrate nanoparticles into the materials used for biosensor construction in order to improve the performance of the system in both existing and potential sensing applications. Nanobiosensors have generated a great deal of excitement due to their ability to detect a wide range of materials at incredibly small concentrations. As the field matures, it seems highly likely that the diagnostic techniques of today will soon become antiquated and a new class of low cost, robust, reliable, easy-to-use and ultrasensitive diagnostics will be available. This may even spur a dramatic increase in the number of point-of-care diagnostics, as well as diagnostic tools that can be used by patients on their own.

Carbon nanotubes (CNTs) have attracted much attention as an electrode material for electrochemical biosensors because of their excellent electrochemical properties, a large edge plane/basal plane ratio, rapid electron kinetics, semi- and superconducting electron transport, high tensile strength composites, and hollow core suitable for storing guest molecules (Iijima, 1991; Christopher *et al.*, 2010). The ability of CNTs to promote the electron transfer reactions of H_2O_2 suggest its great promise for amperometric glucose biosensors, because it can facilitate low-potential amperometric measurement H_2O_2 (Rahman *et al.*, 2009a). CNTs and conducting polymer with an expected synergistic effect have been explored for possible improvement in the electrical and mechanical

properties of polymers (Wei *et al.*, 2002), and hence CNT/polymer composite has been used to enhance the biosensor performance. The expected synergetic effect of conducting polymer–CNT composites has been a motivating factor to do research on sensors, exploiting the mechanical stability of CNT and the redox properties of conducting polymers. Conducting polymers contain π -electron backbone responsible for their unusual electronic properties such as electrical conductivity, low energy optical transitions, low ionization potentials and high electron affinity (Malhotra *et al.*, 2006). Among the various conducting polymers, polyaniline (PANI) is one of the most important polymers due to its environmental stability, high degree of processability, facile synthesis, reversible control of electrical properties by both charge-transfer doping & protonation and interesting redox properties associated with its chain heteroatom, PANI has been one of the most extensively studied electroactive (conductive) polymer (Chen and Hwang, 1994; Wang *et al.*, 1995). The dispersion of CNTs into PANI matrices for the fabrication of CNT/PANI composites has naturally generated interest among researchers. In recent years, the fabrication of PANI/CNT composites has received great attention, since the incorporation of CNTs into PANI could result in new composite materials with enhanced properties e.g. PANI fibres containing CNTs exhibited significant improvement in mechanical strength and conductivity (Mottaghitalab *et al.*, 2006). The introduction of CNTs to PANI composites enhances the electrical properties (the room temperature resistivity is decreased by one order of magnitude as compared to PANI) by facilitating charge-transfer processes, between the two components. The preparation of CNTs and PANI composites by chemical or electrochemical method has been reported recently (Rahman *et al.*, 2009b; Du *et al.*, 2009). The use of these composites in construction of electrodes has been shown to enhance charge density, electrical conductivity and electrocatalytic activity compared with pure conducting polymer materials. As both PANI and CNTs are excellent materials for the construction of electrochemical sensors and biosensors, the combination of these two materials is also expected to be an excellent platform for electrochemical sensing applications (Granot *et al.*, 2006).

Therefore the present work includes the following aims and objectives:

1. Preparation of carboxylated multiwalled carbon nanotubes (c-MWCNT)-polyaniline (PANI) composite film coated platinum (Pt) electrode.
2. Co-immobilization of creatininase (CA), creatinase (CI) and sarcosine oxidase (SO) onto c-MWCNT/PANI composite film coated Pt electrode.

3. Construction and testing of creatinine biosensor, employing Enzymes/c-MWCNT/PANI/Pt as working electrode.
4. Optimization of working condition of the creatinine biosensor.
5. Application of creatinine biosensor in determination of creatinine in serum & urine.
6. Evaluation of new amperometric method of creatinine determination.