# Abstract

Diabetes mellitus is one of the metabolic disorders prevailing all the over world. About 381.8 million people are affected by diabetes mellitus (DM) during 2013 and it is estimated to increase to 80% by 2035. Nephropathy, retinopathy, neuropathy and cardiovascular diseases are common complications arise in the patients suffering from diabetes Type I and Type II. Continuous monitoring of glucose will give greater clinical acumen on glucose metabolism of patients than conventional intensified glucose monitoring. It benefits patients to plan their meals and insulin dosages to prevent hypo- and hyperglycemia. Diabetes is a major cause of chronic kidney disease (CKD). Chronic kidney disease increases the risk of cardiovascular diseases (CVD). CVD and CKD are strongly intertwined. Urea and potassium are the major markers used in the diagnosis of chronic kidney disease and cardiovascular disease, respectively. Continuous monitoring of urea and potassium will help to initiate appropriate medical intervention to decelerate the progression of chronic kidney disease and cardiovascular disease. Conventional invasive blood withdrawal procedure has potential risks like infection, pain and discomfort to the patients. Moreover, invasive techniques deter periodic blood sampling as it requires for frequent vascular puncturing. At present, no medical device is available for continuous monitoring of blood analytes non-invasively.

Present investigations aim at developing a non-invasive technique for monitoring blood analytes (glucose, urea and potassium) which have great potential to use as a point of care diagnostics. Interstitial fluid bathes the cells of the body and it is ultrafiltrate of plasma. It contains ions like potassium, sodium, etc., and neutral molecules like glucose, urea, etc. Analytes (glucose, urea and potassium) level in interstitial fluid equilibrates with blood with lag time of 0 – 15 minutes. Reverse iontophoresis is a process in which a small current is applied through the skin to enable the transdermal extraction of interstitial fluid. Reverse iontophoresis is a non-invasive method and it is suitable for developing an integrated system to extract and analyze the extracted analyte. It enables frequent analyte sampling in high risk patients like elderly and pediatric with more comfort than conventional methods.

In the present work, investigations are conducted on non-invasive monitoring of blood glucose, urea and potassium using reverse iontophoresis (RI). As part of experimental investigations, *in vitro* models are developed. *In vitro* investigations are conducted to optimize the reverse iontophoresis parameters current density and time of extraction. With these optimized parameters, the *in vivo* investigations are conducted on human subjects. A dedicated instrumentation suitable for extraction of analytes is developed.

Screen printed electrochemical glucose sensors suitable for revere iontophoresis applications are developed using mediated carbon ink. Glucose oxidase is immobilized on screen printed sensor using crosslinking method. Electrochemical and material characterization studies are conducted on the developed sensors. The obtained results confirm that the suitability of developed sensor can be used for serum glucose measurement as well as for reverse iontophoresis. Screen printed potentiometric urea biosensors are also developed to monitor the blood urea level non-invasively using reverse iontophoresis. The extraction and sensing system consists of a reverse iontophoresis electrodes, a working electrode, and a reference electrode. Urease enzyme is immobilized in the polypyrrole matrix on the working electrode using cyclic voltammetry. The electrochemical and material characterizations are conducted on screen printed sensors. The sensitivity, selectivity and sensing range of sensors show that they have a potential application in reverse iontophoresis applications.

The *in vitro* models are used to evaluate the developed (urea and glucose) sensors. They are further validated by this conducting the clinical investigations on 15 human subjects. A correlation between blood analyte (glucose and urea) level and transdermally extracted analytes (glucose and urea) is established. It is attempted to integrate both the sensors (glucose and urea) and evaluated their performance on human subjects.

The effect of potassium present in the stratum corneum of skin during reverse iontophoresis is investigated by conducting *in vivo* studies on human subjects. Tape stripping technique is used to detect the presence of potassium in stratum corneum. Reverse iontophoresis investigations with and without passive diffusion are also conducted to analyze the effect of potassium in stratum corneum. Skin impedance is

measured during reverse iontophoresis in order to study the effect of reverse iontophoreteic current on skin properties.

*The* c*linical investigations are conducted on human subjects to validate the performance of the developed sensors (glucose and urea) with the approval of Institute Human Ethical Committee (IHEC), IISc, Bangalore. Non-invasive monitoring of blood analytes (glucose and urea) on human subjects is successfully demonstrated with the indigenously developed sensors through reverse iontophoresis.*