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Electrical double layer modulation of hybrid room temperature ionic liquid/aqueous buffer interface for enhanced sweat based biosensing

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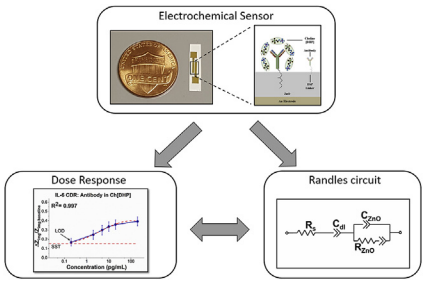
HIGHLIGHTS

- Leveraging kosmotropic and kaotropic behavior of RTILs for electrochemical detection of biomolecules.
- Correlation between surface charge modulation using zeta potential to non-faradaic impedance measurements.
- Enhancement in sensitivity for cortisol and IL-6 using RTIL/sensor interface.
- Identifying and matching the appropriate RTIL to biomolecule for maximizing non-faradaic interfacial response.

GRAPHICAL ABSTRACT



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ABSTRACT

We have investigated the role of kosmotropic anionic moieties and chaotropic cationic moieties of room temperature hydrophilic ionic liquids in enhancing the biosensing performance of affinity based immunochemical biosensors in human sweat. Two ionic liquids, 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM[BF_4]) and choline dihydrogen phosphate (Choline[DHP]) were investigated in this study with Choline[DHP] being more kosmotropic in nature having a more protein stabilizing effect based on the hofmeister series. Non-faradaic interfacial charge transfer has been employed as the mechanism for evaluating the formation and the biosensing of capture probe antibodies in room temperature ionic liquids (RTILs)/aqueous human sweat interface. The charge of the ionic moieties were utilized to form compact electrical double layers around the antibodies for enhancing the stability of the antibody capture probes, which was evaluated through zeta potential measurements. The zeta potential measurements indicated stability of antibodies due to electrostatic repulsion of the RTIL charged moieties encompassing the antibodies, thus preventing any aggregation. Here, we report for the first time of non-faradaic electrochemical impedance spectroscopy equivalent circuit model analysis for analyzing and interpreting affinity based biosensing at hybrid electrode/ionic liquid-aqueous sweat buffer interface guided by the choice of the ionic liquid. Interleukin-6 (IL-6) and cortisol two commonly occurring biomarkers in human sweat were evaluated using this method. The limit of detection (LOD) obtained using both ionic liquids for IL-6 was 0.2 pg mL^{-1} with cross-reactivity studies indicating better performance of IL-6 detection using Choline[DHP] and no response to cross-reactive molecule. The LOD of 0.1 ng/mL was achieved for cortisol and the cross-reactivity studies indicated that cortisol antibody in BMIM[BF_4] did

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not show any signal response to cross-reactive molecules. Furthermore, improved sensitivity and LOD was achieved using ionic liquids as compared to capture probes in aqueous buffer.

1. Introduction

Human sweat contains a wide range of ions, proteins such as interleukin-6 (IL-6), and hormones such as cortisol. These biomolecules are biomarkers that can provide health information [1] and therefore, this biological fluid is suitable for rapid non-invasive biosensing. IL-6 is a cytokine secreted by lymphoid and non-lymphoid cells. IL-6 is an important inflammatory biomarker that can be useful in monitoring immune response for cancer treatment [2]. IL-6 also impacts basal glucose intake [3] and increases cortisol secretion during psychological stress [4,5]. The physiological levels of IL-6 in sweat range between 5 and 15 pg mL^{-1} and show a good correlation to plasma levels [6]. Therefore, IL-6 levels can be monitored non-invasively using sweat based biosensors. Cortisol, a hormone, is a generic biomarker for stress and is expressed in sweat in the concentration range 20–140 ng mL^{-1} [7]. The increased physiological stress is a growing concern and monitoring of cortisol serves as the most valuable biomarker [8] for stress-related disorders. Furthermore, cortisol levels vary as per the circadian rhythm and environmental conditions which necessitates continuous monitoring of cortisol [9]. Sweat being a non-invasive fluid would be the most suitable biological fluid for real-time continuous monitoring of these biomarkers. Among the existing methods for non-invasive biosensing from human sweat, electro-analytical techniques, particularly, non-faradaic impedance measurements have high sensitivity, fast response time and can be miniaturized for low cost diagnostic assessments of biomolecules [10].

Electrochemical impedance spectroscopy (EIS) is a label-free method that utilizes very small voltage perturbations to measure biomolecular interactions and hence, is more robust as compared to other electrochemical techniques in detecting biomolecules without the requirement of a redox label or tag. EIS measures changes in impedance across measurement electrodes in biosensor systems due to biomolecular binding with in the electrical double layer (EDL) at the electrode-solution interface [11]. Furthermore, EIS based measurement is most attractive to human sweat based biosensing as modulation to the EDL associated with biomolecule binding can be captured with low power bioelectronics interfaces thus enabling wearable biosensing. In this article, we have investigated methods of enhancing the modulation of EDL in order to enhance the analytical performance of a human sweat based biosensor.

In an affinity mechanism based biomolecule sensing system measured using EIS, the changes in impedance resulting from the interaction between the capture probe and target analyte within the EDL can be modeled as a Randle's equivalent electrical circuit. The modulation of EDL resulting from bimolecular interactions at the electrode-solution interface may be attributed to changes in terms of charge-transfer resistance (R_{ct}) or double layer capacitance (C_{dl}). Hence, to accurately identify these biomolecular interactions vis-a-vis non-specific interactions, it is necessary to have a stable and compact EDL formed at the electrode-solution interface. Although an EDL is formed upon voltage bias at the electrode-aqueous solution interface, it is primarily dependent on the ionizable content within the buffer which is subject to a number of microscale electro-kinetic mechanisms such as electro-osmosis and electro-convection, thus, resulting in charge screening effects and producing reduced signal response at the electrode interface.

One strategy that our group has adopted to enhance the EDL stability and obtaining resolution in the modulation to the EDL is the use of room temperature ionic liquids (RTILs). These non-aqueous highly polar solvents exhibit excellent capacitive properties and form compact EDLs [12] that enhance the signal response in addition to enhancing protein stability. Effective affinity based biosensing can be achieved when there is a stable bio-functionalization of the affinity based capture probes required for the detection of the target biomolecules. RTILs are known to stabilize proteins and enzymes [13]. We have previously shown a stable and reliable detection of proteins in human sweat for up to 1 week using capture probe antibodies in RTILs as compared to capture probes in aqueous buffers that lack stability over time [12]. This enhanced performance in RTIL is primarily due to the protein stabilization property of RTIL and formation of compact EDL resulting in high charge density. However, no characterization on the modulation of EDL due to biomolecular binding at the electrode/ionic liquid interface has ever been performed. Here, we report for the first time using electrochemical equivalent circuit analysis of the tuning of compact EDL at the hybrid ionic liquid interface that results in enhanced biosensing performance. Most of the biosensing platforms that use RTILs do not leverage these electro-chemical properties of RTILs and rely mostly on amperometric or potentiometric techniques which often require the use redox labels in conjunction with the RTILs. These techniques with redox molecules require higher input voltages and have slower response time, and the measurement mechanism is faradaic in nature. Faradaic impedance spectroscopy requires high concentration of redox labels and is dependent on the electron transfer kinetics between the electrode and redox probe. Whereas, non-faradaic impedance spectroscopy is a label-free technique as it does not involve any redox probe and therefore, eliminates any complexity in the electrochemical system [14]. Non-faradaic EIS measures capacitive changes in the electrical double layer at the electrode-solution interface with a fast response time [14–16].

Table 1 summarizes various biosensing platforms using RTILs and the corresponding method of electrochemical detection. The stable biosensing performance using RTILs may be influenced by the properties of RTILs such as size, hofmeister series or charge density. The interactions between the anionic moiety of the RTIL and protein affect the stability of the immunoassay. 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM[BF_4]) and Choline dihydrogen phosphate (Choline[DHP]) that fall at the center and more kosmotropic region of the hofmeister series respectively have shown to enable biomolecule stabilization [13]. EDL modulation mediated through the use of these two RTILs independently was investigated for biosensing of two well established biomarkers in human sweat, IL-6and cortisol.

In this work, we provide a comprehensive analysis on the gold/ZnO and aqueous/RTIL buffer hybrid EDL interfaces and its impact on the electrochemical biosensing of proteins and metabolites in sweat.

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