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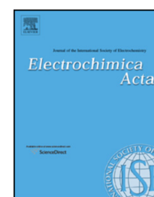

Redox cycling-based amplifying electrochemical sensor for *in situ* clozapine antipsychotic treatment monitoring

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ABSTRACT

Schizophrenia is a lifelong mental disorder with few recent advances in treatment. Clozapine is the most effective antipsychotic for schizophrenia treatment. However, it remains underutilized since frequent blood draws are required to monitor adverse side effects, and maintain clozapine concentrations in a therapeutic range. Micro-system technology utilized towards real-time monitoring of efficacy and safety will enable personalized medicine and better use of this medication. Although work has been reported on clozapine detection using its electrochemical oxidation, no *in situ* monitoring of clozapine has been described. In this work, we present a new concept for clozapine *in situ* sensing based on amplifying its oxidation current. Specifically, we use a biofabricated catechol-modified chitosan redox cycling system to provide a significant amplification of the generated oxidizing current of clozapine through a continuous cycle of clozapine reduction followed by re-oxidation. The amplified signal has improved the signal-to-noise ratio and provided the required limit-of-detection and dynamic range for clinical applications with minimal pre-treatment procedures. The sensor reports on the functionality and sensitivity of clozapine detection between 0.1 and 10 μg/mL. The signal generated by clozapine using the catechol-modified chitosan amplifier has shown to be 3 times greater than the unmodified system. The sensor has the ability to differentiate between clozapine and its metabolite norclozapine, as well as the feasibility to detect clozapine in human serum *in situ* within the required dynamic range for clinically related applications. This new biosensing approach can be further developed towards its integration in miniaturized devices for improved personalized mental health care.

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1. Introduction

One of the major challenges facing mental health clinicians is when patients discontinue care due to lack of optimized treatment. This problem has usually been demonstrated in mental health illnesses where chronic conditions require prolonged treatment with harsh and burdensome side effects. For instance, schizophrenia is one of the most complex psychiatric disorders. It is a lifelong illness that affects 1.1% of the population worldwide. The estimated direct and indirect costs of the illness exceeded $60 billion in 2002 [1]. Currently there is no cure for the disorder and lifelong treatment with antipsychotic drugs is recommended [2]. Approximately 30-50% of patients do not respond to primary, first-line antipsychotic treatment [2–4]. Clozapine (CLZ), a second-line antipsychotic, is the most efficacious medication currently available, providing effective treatment for patients who are unresponsive to other antipsychotics. It is also the only antipsychotic drug approved by the Food and Drug Administration (FDA) for treatment-resistant schizophrenia [2,5–10]. Despite its high efficacy, CLZ remains underutilized because of the required frequent and invasive blood draws to monitor adverse side effects such as agranulocytosis (decrease in the

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amount of white blood cells) [11,12]. Frequent CLZ testing for dose titration and efficacy monitoring has been shown to improve treatment outcomes and reduce the risk of toxicity [13–18]. However, this is often not implemented due to the frequent visits and supplementary blood draws required of patients, in addition to the weekly white blood cell counts over the first six months of treatment [19–25]. Miniaturized devices have the potential to revolutionize the way these illnesses are currently being treated, and to improve overall mental health care outcomes. For example, real-time monitoring of CLZ at the point-of-care will provide a rapid in-office means for physicians to monitor CLZ levels and adjust dosages accordingly to reach safe and effective blood levels. This approach will potentially reduce the cost and burden of monitoring, and increase the acceptance of CLZ treatment by patients and prescribers, leading to improved symptom control in patients [9,26,27].

Electrochemical biosensors are based on bioelectrochemical reactions of electro-active species being consumed or generated. By measuring the electrical signal from these reactions, oxidation/reduction of an analyte is detected [28,29]. Portable integrated electrochemical microsystems provide numerous advantages in clinical diagnostics, environmental monitoring and biomedical research fields. These translational technologies can be easily realized using microfabrication technology for the development of on-chip electrochemical microsystems where the sensing electrodes are integrated directly onto the microchip. This approach yields high magnitude signals and relatively low noise, with detection limits that are satisfactory for many practical applications [29,30]. Over the years, the electrochemical activity of CLZ [31,32] and its detection [33–57] were investigated. Still, only a small subset of studies showed electrochemical analysis of CLZ in blood samples [31,42,43,47,49]. Notably, these studies relied on extensive sample pre-treatment procedures prior to the electrochemical measurement; *e.g.* electrode rinsing and testing in a serum-free measurement cells, or deoxygenation with nitrogen. These pre-treatment procedures are mainly due to the increased background signal from electrochemical reactivity and non-specific adsorption of molecules in the serum, decreasing the signal-to-noise ratio and deteriorating the overall performance of the sensor. However, as these procedures add cost, time, and complexity to the system, the utilization for *in situ* real-time CLZ sensing at the point-of-care is impeded.

In this work, we present a new concept for *in situ* CLZ sensing based on an electrochemically-active biomaterial for CLZ oxidation amplification. We leverage the naturally derived polysaccharide chitosan, a versatile biomaterial [58,59], as a scaffold for subsequent modification with electrochemically-active catechol, resulting in a redox cycling system [60–64]. This allows for significant amplification of the generated oxidizing current of CLZ through a continuous cycle of CLZ reduction by the catechol moieties followed by re-oxidation at the electrode. The continuous redox reaction increases the total charge transferred by CLZ oxidation, amplifying the generated electrochemical current and improving the signal-to-noise ratio. This provides the improved sensitivity and detection range required for *in situ* CLZ analysis of clinical samples, *i.e.* blood levels between 0.350 μg/mL and 1 μg/mL for high efficacy and low toxicity [17]. In this work we study the functionality and detection limit of CLZ sensing with the amplification system. We also characterize selectivity with respect to the CLZ principal metabolite norclozapine (NorCLZ). Finally, we demonstrate the feasibility of the sensor to detect CLZ in human serum *in situ* within the therapeutic range. Therefore, the presented biosensing mechanism represents a promising candidate for integration in miniaturized analytical devices with minimal pre-treatment procedures for real-time *in situ* detection of CLZ.

2. Experimental

2.1. Materials

All chemicals were purchased from Sigma-Aldrich. Chitosan solution (1%; pH 5–6) was prepared by dissolving chitosan flakes in dilute HCl as previously described [65]. All other chemical solutions were prepared in phosphate buffer (PB; 0.1 M; pH 7).

Characterization of the biosensing mechanism in buffer solution was performed with microfabricated planar square gold electrodes. These electrodes (7.5 × 7.5 mm^2 electrode area; 20 × 8 mm^2 chip dimensions; 1 electrode per chip) were fabricated using standard microfabrication techniques. Briefly, 20 nm of chrome and 180 nm of gold were sputtered on 4 inch diameter silicon wafers insulated by 500 nm of PECVD silicon oxide, patterned using photolithography, and diced into chips. Prior to experiments, they were subjected to consecutive cleaning steps using acetone, methanol, isopropanol, piranha solution (25% H_2O_2/75% H_2SO_4), and deionized (DI) water. Human serum testing was performed with gold disk working electrodes (2 mm diameter; CH Instruments, Austin, TX; Polished before use).

2.2. Catechol-modified chitosan system biofabrication

Catechol-modified chitosan films were deposited following recently published protocols [63]. The gold working electrodes were coated with chitosan by immersion in solution and application of 6 A/m^2 cathodic current for 45 seconds using a three-electrode system with platinum foil as a counter electrode and a Ag/AgCl reference electrode with 1 M KCl electrolyte (CH instruments, Austin, TX). Subsequently, the chitosan films were functionalized by immersion in 5 mM catechol in PB and application of 0.6 V (*vs.* Ag/AgCl) anodic potential for 180 seconds, followed by immersion for 5 minutes in DI water to discard unbound catechol. Characterization of amplification dependence on the modification steps was performed by applying both electrochemical modification techniques (chitosan electrodeposition and catechol grafting) for electrodes immersed in either the modification solution or buffer alone, *i.e.* chitosan and catechol solutions were used for the catechol-modified chitosan electrode, chitosan and buffer (no catechol) solutions were used for the chitosan-modified electrode, buffer (no chitosan) and catechol solutions were used for the catechol electrode, and only buffer solutions were used for the unmodified electrode.

2.3. Electrochemical testing

All electrochemical tests were carried out using the three-electrode system described above and with a CHI660D single channel potentiostat from CH Instruments (Austin, TX), except of serum testing where Bio-Logic VSP-300 potentiostat was used (Claix, France; following reproducibility tests of CLZ in PB with the commercial gold disk electrodes). All voltages are denoted *vs.* Ag/AgCl. Catechol-modified chitosan films were initialized through cyclic voltammetry (range −0.4 V to +0.7 V, scan rate of 0.1 V/s, scan resolution of 0.001 V) in 25 μM hexaammineruthenium(III) ($Ru(NH_3)_6$; HARu) and 25 μM 1,1′-ferrocenedimethanol ($Fc(MeOH)_2$) prior to CLZ experiments. This cycle in the presence of oxidizing and reducing mediators decreased the observed background current. This may be attributed to the consumption of remaining unbound catechol molecules. Moreover, the initialization step prior to analyzing CLZ samples provides a means to validate the presence, amplification capability, and reproducibility of the biofabricated catechol-modified chitosan film. Reproducibility tests of the initialization solution with $Fc(MeOH)_2$ for different fabricated catechol-chitosan electrodes (N = 8) resulted averaged

anodic peak current and its corresponding potential of 240 ± 20 μA/cm^2 (Error 8.3%) and 0.3730 ± 0.0045 V (Error 1.2%), respectively.

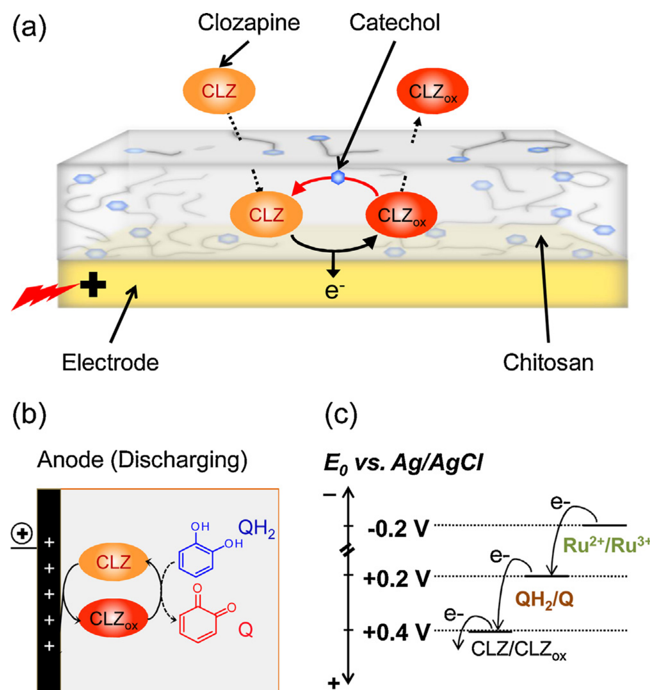
Next, the modified electrodes were immersed in buffer solutions and cyclic voltammetry data was recorded (range −0.4 V to +0.7 V, scan rate of 0.02 V/s, scan resolution of 0.001 V). The characterization of different modification steps used buffer solution with 25 μM CLZ and 25 μM HARu. Functionality testing used buffer solutions with 25 μM HARu and either 0.033 (0.1 μM), 0.101 (0.31 μM), 0.326 (1 μM), 1.01 (3.1 μM), 3.26 (10 μM), or 10.1 (31 μM) μg/mL CLZ. Selectivity testing used buffer solutions with 25 μM HARu and 25 μM of either CLZ or NorCLZ. The catechol-modified chitosan electrodes were stored for 3 days in PB with 0.01 M NaCl and cyclic voltammetry data was recorded in the presence and the absence of CLZ. The observed peak current for CLZ oxidation demonstrated a 13.5% decrease between day 0 and day 3 (Figure S1). CLZ-spiked human serum chronocoulometry testing (pre-conditioning step potential of -0.4 V, duration of 30 seconds, followed by step potential of 0.6 V, duration of 60 seconds, both steps used sample resolution of 0.5 seconds) used human serum (male type AB) with 25 μM HARu and either 0.163 (0.5 μM), 0.326 (1 μM), 1.63 (5 μM) or 8.15 (25 μM) μg/mL CLZ. Background signals were recorded using either buffer solution or undiluted human serum with only 25 μM HARu prior to each CLZ measurement. CLZ-spiked undiluted serum tests were factorized with 7% signal increase due to signal deterioration observed for subsequent measurements with the same electrode (data not included). All electrodes were rinsed with DI water between tests. Data analysis used the third cycle of each measurement to decrease the background signal observed for the first and second cycles. Sample volume was 1.5 mL for the microfabricated electrodes and 1 mL for the commercial electrodes.

3. Results and discussion

3.1. Redox amplifier characterization

The redox cycling system is comprised of grafted catechol moieties that can be inter-converted between oxidized (Q) and reduced (QH_2) forms (E_0 = +0.2 V). CLZ (E_0 = +0.4 V) can diffuse freely within the chitosan film. Following its oxidation upon the electrode under overpotential conditions, CLZ is reduced by the grafted QH_2 moieties, and it is electrochemically re-oxidized at the electrode (Scheme 1a and 1c). A continuous cycle of CLZ reduction in the presence of catechol followed by CLZ re-oxidation results from this use of CLZ as an oxidizing mediator (Scheme 1b). The continuous redox reaction is hypothesized to increase the total charge transferred by CLZ oxidation, amplifying the generated electrochemical current and improving the signal-to-noise ratio. To recover the redox cycling system to the reduced state, negative potential is applied in the presence of a reducing mediator, HARu ($Ru^{2+/3+}$, E_0 = −0.2 V).

Initially, the ability of the catechol-modified chitosan system to amplify the electrochemical current generated by CLZ was evaluated. Fig. 1a presents the electrochemical signal of CLZ in the presence and absence of the catechol-modified chitosan amplification system. In both cases, CLZ solutions yielded expected oxidation peaks [32] (+0.41 V and +0.465 V for the unmodified and modified electrodes, respectively) and a reduction peak due to the reducing mediator HARu (−0.24 V). The higher value of the oxidation peak potential may be due to the increased uncompensated resistance in the chitosan pores, forcing higher overpotentials compared to a bare unmodified electrode. A CLZ reduction peak (+0.36 V) was observed only for the bare electrode. We attribute its absence with the catechol-chitosan modification to the continuous reduction reaction of CLZ by catechol, which diminishes the expected



Scheme 1. CLZ as an oxidizing mediator in the catechol-modified chitosan system. (a) Schematic of the system with the diffusing CLZ. (b) Continuous oxidation of CLZ in the presence of catechol (Q) reduction. (c) CLZ acts as an oxidizing mediator of QH_2, and Ru^{2+} as a reducing mediator regenerating the Q. Electrochemical potential bar represents standard reduction potential of Ru^{2+}, Q and CLZ.

reduction of the oxidized form of CLZ by the electrode. Anodic current densities generated with the modified electrode both in the presence and absence of CLZ were amplified. CLZ oxidation peak recorded with the modified electrode was 3-fold higher than the peak current density with the unmodified bare electrode. Fig. 1b shows the effect of the individual modification steps on the amplification characteristics. The electrochemical response of analytical solutions in the presence and the absence of CLZ was recorded following electrode modification steps of only chitosan electrodeposition, only catechol oxidation, chitosan electrodeposition followed by catechol oxidation, and no modification. In order to evaluate the CLZ amplification magnitude, the background current density generated in the absence of CLZ ($j_{w/o\,CLZ}$) was subtracted from the current density generated in the presence of CLZ ($j_{w/CLZ}$). The significantly higher CLZ oxidation peak current density obtained with the catechol-chitosan system over all other cases are apparent.

To quantify and compare the ability of the system to amplify the signal generated by CLZ, an amplification factor (*AF*) is defined:

$$AF = \left. \frac{(j_{w/CLZ} - j_{w/o\,CLZ})_{\text{modified}}}{(j_{w/CLZ} - j_{w/o\,CLZ})_{\text{unmodified}}} \right|_{@\text{anodic peak}} \quad (1)$$

Table 1 lists the *AF*s for the four cases studied. The catechol-modified chitosan film demonstrated the highest *AF*, more than 3 times that of either a bare electrode or catechol alone and

Table 1
Amplification factor (*AF*) calculated for the different modification steps of the catechol-chitosan system.

Modification step	AF
Unmodified (bare)	1.00
Chitosan alone	0.30
Catechol alone	1.05
Catechol-modified chitosan	3.33

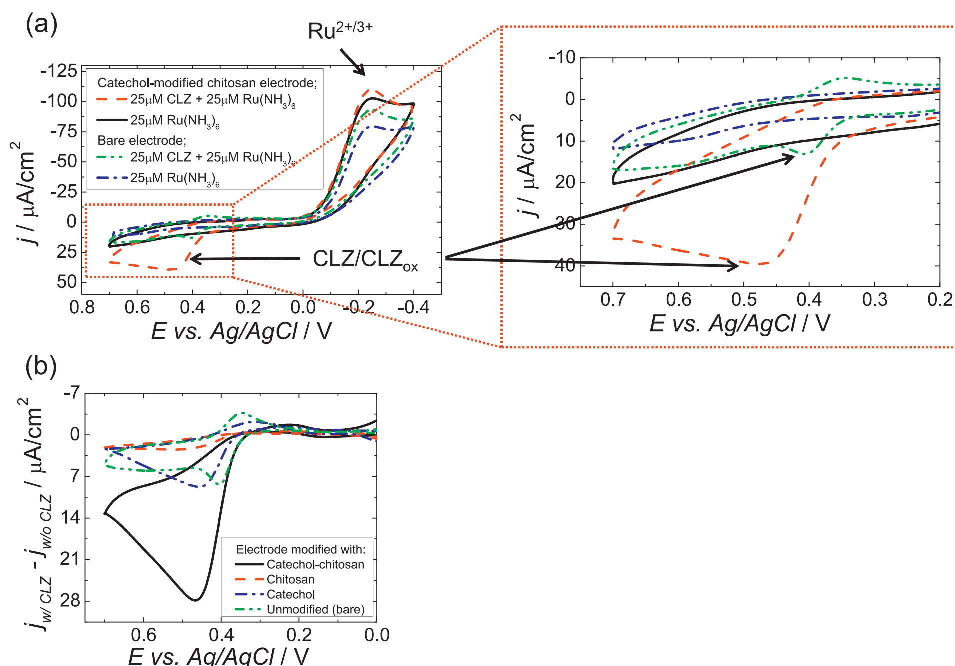


Fig. 1. (a) Cyclic voltammograms in the presence and the absence of CLZ with either bare unmodified (with CLZ–dash-dot-dotted green, without CLZ–dash-dotted blue) or catechol-modified chitosan (with CLZ–dashed red, without CLZ–solid black) electrodes. (b) Oxidation current density after background subtraction (signal in the absence of CLZ) for different modifications steps of the electrode: bare unmodified (dash-dot-dotted green), catechol (dash-dotted blue), chitosan (dashed red), and catechol-modified chitosan (solid black).

11-fold higher than chitosan-alone. The decreased electrochemical response of the chitosan-modified electrode likely results from the lower exposed surface area of the electrode due to the non-conductive chitosan coating. Furthermore, the porous structure of the chitosan may decrease the diffusion rate of CLZ towards the electrode, increasing the uncompensated resistance in the pores. Only the further functionalization of the chitosan matrix with the redox moiety catechol amplifies the charge transferred by CLZ oxidation, resulting in higher oxidation currents.

To characterize the biosensing performance of the redox amplifier, buffer solutions with known clozapine concentrations were used. Fig. 2a presents the background-subtracted oxidation response in cyclic voltammograms measured with the catechol-modified chitosan electrodes in the presence of 0.033, 0.101, 0.326,

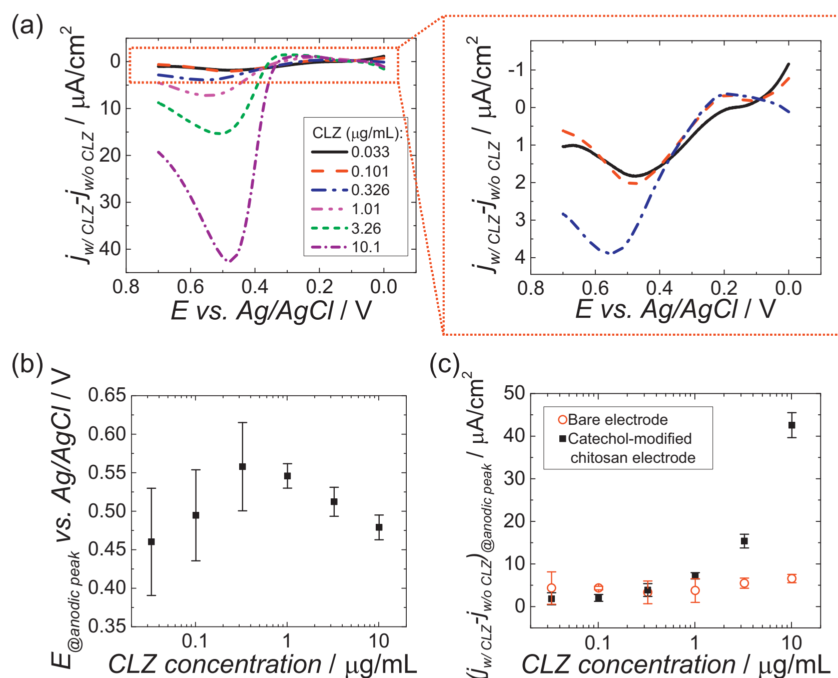


Fig. 2. CLZ detection in buffer solutions with 0.033, 0.101, 0.326, 1.01, 3.26, and 10.1 μg/mL CLZ. (a) Oxidation current density of cyclic voltammograms following background subtraction. (b) The effect of the CLZ concentration on the potential at the anodic peak. (c) CLZ dose response of the anodic current density peak for both unmodified (clear red circles) and catechol-modified chitosan (dark black squares) electrodes. All tests were repeated five times.

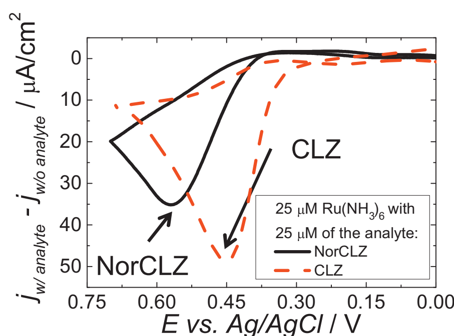


Fig. 3. Oxidation current density of cyclic voltammograms following background subtraction in the presence of either CLZ (dashed red) or NorCLZ (solid black).

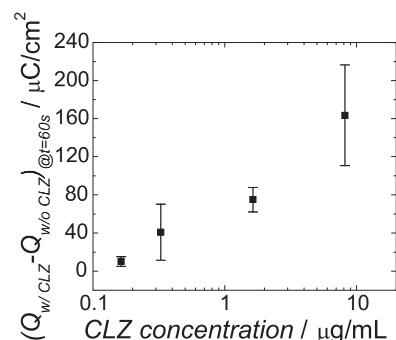


Fig. 4. *In situ* analysis of CLZ-spiked human serum. Total charge density transferred from chronocoulometry after 60 seconds following background subtraction for 0.163, 0.326, 1.63, and 8.15 μg/mL CLZ. Tests were done in triplicate.

1.01, 3.26, and 10.1 μg/mL CLZ. Higher CLZ concentrations resulted in higher oxidation current densities due to the increased charge mediated by CLZ oxidation. The influence of the CLZ concentration on the potential corresponding to the anodic oxidation peak was also evaluated (Fig. 2b). The data shows mixed trends with a maximum potential value of 0.56 ± 0.06 V at 0.326 μg/mL CLZ. These trends can be explained by the variation of the ratio between oxidized and reduced forms of CLZ near the electrode. For CLZ concentrations lower than 0.326 μg/mL, the rate-limiting factor in the system may be either CLZ diffusion between the catechol and the electrode or the reduction reaction of CLZ by catechol. This results in an increased concentration of the oxidized form of CLZ, therefore increasing the half-cell potential as described by the Nernst equation [66]. Conversely, at CLZ concentrations higher than 0.326 μg/mL, the limiting factor may be the oxidation reaction of CLZ by the electrode, which results in higher concentrations of reduced CLZ near the electrode and decreases the half-cell potential. Fig. 2c plots the anodic current density at the oxidation peak for different concentrations of CLZ measured with either modified or unmodified electrodes. Both the modified and the unmodified electrodes demonstrated a positive linear relation (analysis was done on a linear scale), with slope values of 40.0E-7 ± 2.7E-7 and 3E-7 ± 1E-7 A × mL/g × cm^2, intercept values of 25E-7 ± 5E-7 and 40E-7 ± 4E-7 μA/cm^2, and R-Square values of 0.99 and 0.91 respectively. The modified electrode yielded a measured detection limit of 0.1 μg/mL CLZ, compared to 3.26 μg/mL for the unmodified electrode. Note that recommended therapeutic CLZ blood levels are in the range of 0.35–1 μg/mL [17]. Hence, the catechol-modified chitosan electrode exhibits dynamic range and detection limit characteristics in line with clinical requirements, as opposed to the higher detection limit and lower sensitivity observed with the unmodified electrode.

The selectivity potential of the redox amplifier for CLZ and its principal plasma metabolite N-desmethylclozapine (norclozapine, NorCLZ) was tested. Monitoring NorCLZ can help in evaluating adherence, controlling dose adjustment, and minimizing the risk of toxicity [67]. Fig. 3 shows cyclic voltammograms of either CLZ or NorCLZ solutions measured with the catechol-modified chitosan electrode after background subtraction. The presence of the CLZ oxidation peak at lower potential (+0.45 V) than that of NorCLZ (+0.57 V) becomes apparent. This can be attributed to different standard reduction potentials of the two analytes. Performing electrochemical measurements sensitive to the difference between CLZ and NorCLZ will allow separate identification in samples. For example, in addition to cyclic voltammetry, chronocoulometry can be applied at potentials lower and higher than the standard reduction potential of NorCLZ. Such measurements will collect the oxidation charge generated by either CLZ alone or CLZ and NorCLZ together, respectively. As this test explored the potential selectivity of the sensor, a limitation of this approach would be cross-reactivity of CLZ with its metabolite or other electro-active species in serum with similar standard reduction potentials, which may hide CLZ signal. The concentration of either CLZ or NorCLZ may be another limitation. For example, the peak potential of CLZ oxidation is shifted towards 0.55 V when 0.326 μg/mL concentration is tested (Fig. 2b), overlapping with the NorCLZ oxidation signal. These limitations could be overcome by using intrinsic characteristics of the analyte such as its diffusion coefficient to differentiate the signals from the background. Examples include modulating the scan rate parameter of voltammetry-based techniques or modifying the biomaterial for increased selectivity.

3.2. Sensor for CLZ in situ human serum levels

A major challenge in the analysis of biological samples is the electrochemical reactivity and non-specific adsorption of molecules which cause fouling of the electrode. These molecules increase the background signal, decrease the signal-to-noise ratio, and deteriorate the sensitivity of the sensor. To validate that our approach is feasible in samples more complex than buffer solution, the sensing performance of the catechol-modified chitosan electrode system was characterized in human serum spiked with known CLZ concentrations. Potential sweep techniques (*i.e.* cyclic voltammetry and differential pulse voltammetry) demonstrated poor applicability for serum fluids testing with no dose response characteristics (Figure S2). The high background signal attributed to other electro-active species decreased the signal-to-noise ratio and deteriorated the CLZ oxidation signal. Utilizing potential step techniques allows accumulating electrochemical reactions at specific potentials. While CLZ re-oxidizes following catechol oxidation, other electro-active species, which are not being reduced by catechol, are consumed. Chronocoulometry measurement of the transient charge at an overpotential value slightly higher than the standard reduction potential of CLZ, will accumulate the charge continuously produced by its re-oxidation. Over time, the accumulated charge will differentiate CLZ from other electro-active species consumed during the electrochemical reaction, increasing the signal-to-noise ratio. Fig. 4 shows total charge integrated for 60 seconds for 0.163, 0.326, 1.63, and 8.15 μg/mL of CLZ-spiked human serum subtracted by the background signal measured with only human serum. Results demonstrated a positive logarithmic CLZ dose response characteristic within the range seen in buffer solution using cyclic voltammetry. This characteristic may be due to the exponential dependence between the electrochemical reaction and the applied overpotential [66]. The relationship demonstrated in serum is different than the non-logarithmic relationship demonstrated in buffer solutions (Fig. 2c). Such difference may be due to interfering electrochemical reactions of other electro-active analytes in serum that are absent in buffer. For example, ascorbic

acid may interact with the catechol-chitosan system or directly with CLZ, decreasing the overall amplification factor of CLZ [68]. Another reason could be non-specific adsorption of CLZ or other molecules in serum to the surface of the sensor, affecting the electron transfer kinetics. Such reactions may behave as the Langmuir adsorption model or Temkin adsorption isotherm [69]. As the measured CLZ range extends even beyond the required clinical range of 0.35–1 μg/mL, the feasibility of the catechol-modified chitosan electrode system to detect CLZ in clinical samples was demonstrated. With further investigation of the interference by CLZ metabolites and other electro-active species in the serum, this approach for *in situ* serum analysis will allow for treatment monitoring with only minimal, standard blood pre-conditioning steps (*e.g.* blood fractionation).

4. Conclusions

In this work, we present a redox cycling-based amplifier as a new biosensing system for electrochemical detection of CLZ. The amplified sensor demonstrated lower measured detection limit and improved sensitivity compared to unmodified sensors. Furthermore, the amplified system showed CLZ dose response characteristics with the potential ability to differentiate between CLZ and its metabolite NorCLZ due to their different standard reduction potentials. Finally, the ability of the characterized system to detect CLZ *in situ* in human serum within the required clinical effective range was demonstrated. This study satisfies the key challenges of low signal-to-noise ratio and minimal pre-treatment procedures in point-of-care systems for real-time *in situ* monitoring [70–72]. By improving real-time *in situ* monitoring of CLZ, treatment teams will be able to perform blood analysis at the point-of-care in a low cost, fast, and straightforward way that will ensure CLZ dosages within the effective range, decrease the patient's burden, and improve mental health care. However, further investigation is required to elucidate fundamental properties of the sensing mechanism. The working principle of this amplification system should be further modeled and critically investigated in comparison to other potential models. In addition, schizophrenia patients are commonly prescribed multiple antipsychotics and/or supplementary psychotropic medications [21]. The cross-reactivity of the sensor should be evaluated with other electro-active drugs with adjacent standard reduction potential to CLZ, such as the antipsychotic risperidone [73,74]. To overcome these potential challenges with maintaining *in situ* monitoring characteristics, other properties of the electro-active drugs, such as diffusion and charge, could be used to further differentiate between them. Another potential challenge is the high electrochemical reactivity of blood fluids. For example, ascorbic acid, which is an electro-active species naturally present in blood samples, can participate in the redox-based biosensing mechanism and decrease the signal-to-noise ratio and the sensitivity of the sensor.

Through the integration of the CLZ biosensing system with the advantages of miniaturized devices (*i.e.* cost-effective, easy to operate, high throughput, low sample volume, and fast reaction time), a new class of portable sensors can be developed for rapid in-office and in-home use that would allow physicians to monitor CLZ levels and adjust dosages accordingly. However, various challenges arise when translating sensing approaches such as the redox amplification system into the micro-scale regime. Sample preparation and mixing of fluids, physical and chemical effects (*e.g.* capillary forces, surface roughness, chemical interactions of construction materials with reaction processes), and low signal-to-noise ratio (due to lower sample volume and decreased amount of analyte) are among the most important problems to be overcome [75–77]. Optimizing major components in the system, such as chitosan thickness and porosity, and catechol density, will improve CLZ signal amplification and the overall performance of the miniaturized sensor. Furthermore, integrating other redox moieties will result in redox cycling systems with different electrochemical responses. These multi-functional redox systems will increase the range of the electrochemical activity generated by complex samples, improving the accuracy and the selectivity of the sensor.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.electacta.2014.03.045.

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