Programmable “Semismart” Sensor: Relevance to Monitoring Antipsychotics

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Mental health disorders are complex and poorly understood but would benefit from real-time chemical analysis capable of assessing a patient’s current status, personalizing a therapeutic action, and monitoring compliance. Here, an electrochemical sensor is reported for detecting the antipsychotic drug clozapine which is one of the most effective but under-utilized drugs for managing schizophrenia. This sensor employs a composite film of multiwalled carbon nanotubes (CNTs) embedded within a matrix of the aminopolysaccharide chitosan. Chitosan allows programmable assembly of the composite film at an electrode address while the CNTs confer electrocatalytic activities that displace interfering serum peaks from the voltage region where clozapine oxidation occurs. Using differential pulse voltammetry, high sensitivities (limit of detection $0.05 \times 10^{-6}$ M) are demonstrated for clozapine analysis in buffer. In serum, clozapine sensitivity is reduced by an order of magnitude but still sufficient for clinical analysis. Finally, the detection of clozapine from the serum of a schizophrenia patient is demonstrated without the need for serum pretreatment. In the long term, it is envisioned that the CNT-chitosan coated electrode could be integrated within a small array of other sensor types to enhance information-extraction to allow mental health disorders to be better managed and better understood.

1. Introduction

Portable devices that enable complex chemical analyses to be performed in real time promise to transform medicine by providing the information needed to tailor dosages in response to the patient’s current need. This potential has already been demonstrated by home-use glucose detection systems that enable diabetics to personalize their own treatment. As illustrated in Figure 1a, glucose analysis relies on an enzymatic biosensor with a coating that confers “smartness” by selectively recognizing the glucose signal in the presence of chemical interferents, and transducing this chemical “signal” into a device-compatible (i.e., electrical) output. Unfortunately, the success of portable glucose detection systems has been difficult to replicate for other analytes and many blood tests still require analyses in centralized laboratories with long delays. Importantly, there are several features of glucose analyses that are unique and we suggest that glucose detection may not represent the best paradigm for creating sensor systems for portable on-site analysis. In particular, the glucose signal is strong ($\approx 5 \times 10^{-3}$ M in serum;[1] the therapeutic problem is well-posed in the sense that diabetics only need information of the glucose “signal” to make decisions (i.e., to administer insulin); and biosensing enzymes are available that both filter-out the “noise” of chemical interferents and convert glucose into products that facilitate signal transduction (e.g., the enzymes generate redox-active products that can be transduced by electrochemistry into a convenient electrical signal).

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In contrast to glucose monitoring for diabetes management, there are many examples in medicine where the challenge is more complex: (i) the chemical signal may not be strong, (ii) the noise may be high, variable (both qualitatively and quantitatively), and contain useful information, (iii) biological recognition elements (e.g., enzymes) may not be available to filter/transduce information, and (iv) the problem may be ill-posed in that the measurement of a single chemical species may be insufficient to optimize a therapeutic action. We contend that in these cases, it may be better to have a set of “semismart” sensors that differentially filter the sample’s information, and then extract information from these multiple sensors using information processing approaches. Compared to a one-sensor-one-analyte approach, we believe a multisensor approach may provide a more complete and robust understanding of a patient’s status which may be especially important when a disease is complex and incompletely understood.\(^1\)

Our long-term goal is to create a portable device to assist in the management of schizophrenia and our initial focus is the detection of the antipsychotic medication clozapine. Clozapine is a second generation antipsychotic developed in the 1970s and after 40 years remains “one of the most clinically effective antipsychotics available”\(^4\) and a “treatment of choice for schizophrenic patients who are refractory to treatment, display violent behaviors, or who are at high risk of suicide.”\(^5\) Unfortunately, clozapine “is also the antipsychotic with the worst side effect profile, the highest risk of complications, and the most difficult to prescribe.”\(^6\) In fact, clozapine was once pulled from the market due to the adverse side effects but later reintroduced because of its therapeutic benefits.\(^7\) Nevertheless, clinicians are hesitant to prescribe clozapine and many consider it to be one of the most under-utilized therapeutically beneficial medications for mental health.\(^8\) One challenge to prescribing antipsychotics for this patient-population is compliance. Yet even when compliance is guaranteed in an in-patient setting, Figure 1b shows the serum levels cannot be controlled simply by dosage due to interindividual variation in clozapine’s metabolism.\(^7\) Thus, clozapine is a candidate for therapeutic drug monitoring (TDM) to enable a care-giver in a clinic, pharmacy or home to monitor compliance, optimize dosage, and minimize risks of side effects.\(^8\) Our short term goal is to create a device that allows simple, rapid and portable analysis for clozapine’s TDM.

There are substantial technical challenges to creating a highly-selective (bio)sensor for the detection of clozapine from blood samples. First, clozapine’s therapeutic concentrations are very low (over 3 orders of magnitude lower than glucose). Second, the clozapine “signal” exists in a noisy background. Finally, enzyme-based biosensors may be difficult to create because the enzymes that are known to react with clozapine (cytochrome P450 enzymes) are detoxification enzymes that have a broad substrate range and may not “filter” with high selectivity.\(^9\)

Even if it becomes technically possible to develop a highly selective clozapine sensor, we contend that this may not be the best approach to understand or manage schizophrenia. Specifically, we believe there is valuable but incompletely understood information in the “noise” and this information would be filtered-away by such a highly selective clozapine sensor. We cite four types of information that would be lost if this “noise” was filtered. First, information of clozapine’s metabolism could be valuable because one metabolite, N-desmethylclozapine, has biological activity through an unresolved mechanism(s).\(^4\) Second, information of other medications would be useful since schizophrenics are often treated for other maladies and there can be significant drug interactions (e.g., the antidepressant fluvoxamine inhibits clozapine’s metabolism).\(^10\) Third, information of a schizophrenic’s behaviors (e.g., cigarette smoking\(^11\) and coffee drinking\(^12\)) may require adjustments in clozapine dosages for optimal benefit. Finally, growing evidence indicates that schizophrenia is linked to oxidative stress\(^13\) and information of oxidative stress may be available through measures of...
other components\textsuperscript{14} (e.g., ascorbic acid\textsuperscript{15}, glutathione\textsuperscript{13d,16} or uric acid\textsuperscript{17}) or more global measurements (e.g., oxidative stress index\textsuperscript{18} of redox homeostasis\textsuperscript{19}). Thus, our long-term approach is to create a device in which multiple, semiselective electrodes collect, analyze, and transmit a large amount of information. We envision that such a device would allow individual therapies to be personalized (i.e., to adjust doses) and also to collect information to assist scientists and clinicians to better understand this complex disease.

As illustrated in Figure 1c, we envision a portable device composed of a small array of semismart sensors with each sensor interacting with the sample through mechanisms that can acquire relevant information. Importantly, we believe the sensors must be sufficiently sensitive to acquire information under physiologically relevant concentrations. For the case of clozapine, the therapeutic range is from $1.0 \times 10^{-6}$ to $3.0 \times 10^{-6}$ M (0.350–1 µg mL\textsuperscript{-1})\textsuperscript{\textsuperscript{20}} and thus a sensor must have high sensitivities to acquire information at these low concentrations. However, each sensor in the array does not need to be highly selective since its information will be combined with information acquired from other sensors in the array\textsuperscript{21} (i.e., clozapine’s level would be determined from the integrated information from multiple sensor types). In fact, as discussed above, a highly selective sensor would be undesirable as it would eliminate valuable information. Importantly, we believe that each semismart sensor in the array should have different but overlapping selectivities to capture the diverse information in the sample. We envision that these overlapping selectivities will be achieved by creating sensors that interact with the sample in different ways (i.e., through different mechanisms).

In previous studies, we developed our first sensor type by coating an electrode with a thin-film composed of catechol and the aminopolysaccharide chitosan. This catechol-chitosan film is redox-active but nonconducting, and thus possesses redox-capacitor properties that enable it to interact with samples through redox-cycling mechanisms.\textsuperscript{22} This redox-cycling: (i) amplifies output currents to enhance sensitivity;\textsuperscript{23} (ii) gates and partially rectifies output currents to confer unique selectivities;\textsuperscript{24} and (iii) allows cyclic potential inputs to be imposed to generate steady outputs that enable signal processing strategies to be employed to more fully extract information from a sample.\textsuperscript{25} Initial studies in buffered solutions demonstrated that clozapine redox-cycles with this capacitor film and the amplified outputs enable detection in the clinically relevant range.\textsuperscript{26} In clozapine-spiked commercial serum, however, the clozapine signal was altered qualitatively possibly due to redox-cycling interactions among clozapine, serum components, and the film. Through manipulations of the imposed input potential this sensor was shown to be capable of quantifying clozapine levels in this spiked commercial serum.\textsuperscript{27} However there is concern that the variable background of patient samples may interfere with such analysis. Thus, this redox-cycling sensor may be insufficient as a “stand-alone” sensor for clozapine detection. Nevertheless, serum-induced alterations to the signal likely reflect clozapine’s chemical activities and its interactions in the serum, and thus the signal contains relevant information of the patient’s status although this information is currently incomplete in the absence of additional information from other measurements. Thus, we envision the redox-cycling sensor would be capable of acquiring important information as one element in an array.

Here, we describe the development of a second sensor system that interacts with samples through an independent electrocatalytic mechanism and thus provides orthogonal information to that obtained from the redox-cycling sensor. As illustrated in Figure 1c, the electrocatalytic sensor is composed of an electrode coated with a film containing multiwalled carbon nanotubes (CNT) entrapped within a matrix of the aminopolysaccharide chitosan.\textsuperscript{28} We specifically report that: chitosan’s pH-responsive film-forming properties enable the CNT-chitosan film to be generated by electrodeposition;\textsuperscript{29} this film offers both sensitivity and selectivity for detecting clozapine from buffered samples and spiked serum; and this film can detect the presence of clozapine from serum samples obtained from schizophrenic patients without pretreatment of the sample.

### 2. Results

#### 2.1. Programmable Assembly of CNT-Chitosan Composite

It is well-known that CNTs offer unique properties for sensor applications\textsuperscript{30} and CNTs are often assembled at electrode surfaces using casting methods.\textsuperscript{31} Several groups have extended this approach by codepositing CNTs at an electrode surface using the pH-responsive film-forming aminopolysaccharide chitosan\textsuperscript{32} which is commonly used as an electroaddressable matrix for sensor applications.\textsuperscript{33} Figure 2a shows the mechanism for electrodepositing a CNT-chitosan composite film at an electrode address.\textsuperscript{34} First, CNTs are uniformly dispersed in a chitosan solution (0.9%) by ultrasonication for 10 min. Next, electrodes are placed into the CNT-chitosan suspension and biased to initiate the electrolytic reactions that result in the generation of a pH gradient adjacent to the cathode surface. The high localized pH at the cathode induces chitosan to undergo its sol-gel transition and coat the electrode with a thin hydrogel film with entrapped CNTs.

Figure 2b compares CNT assembly on chips patterned with gold electrodes. As an illustrative control, we prepared a cast film by dispersing CNT (1%) in dimethylformamide (DMF), dropping 20 µL of this CNT suspension onto the chip, spreading the viscous suspension and then evaporating the solvent. The first image in Figure 2b shows that CNTs were assembled over the entire chip surface for this “spread” control. The other chips in Figure 2b were prepared by electrodeposition from an aqueous chitosan solution. When a chitosan solution (without CNT) was electrodeposited, the second image in Figure 2b shows a transparent film was assembled on the electrode surface. When codeposition was performed from a CNT-chitosan suspension, the remaining images in Figure 2b show that CNT-assembly was confined to the electrode surface. These observations illustrate that chitosan’s electrodeposition allows the spatially selective assembly of a CNT-chitosan composite film at an electrode address.

To further demonstrate the spatial selectivity for chitosan’s electroaddressing, we used the test device in Figure 2c in which the gold electrodes were patterned on the sidewalls of a
fluidic device.\textsuperscript{[31b,32]} As illustrated by the schematic in Figure 2c, this device allows microscopic observation of the deposited film’s profile. In the first deposition step, a suspension of 5% CNT and 0.9% chitosan was introduced into the channel and a cathodic potential was applied to one of the electrode addresses (8 mA m$^{-2}$; 1 min). After flushing the channel with water, a second deposition solution containing only chitosan (0.9%) was introduced to the channel and a cathodic potential was applied to a neighboring electrode address (8 mA m$^{-2}$; 1 min).

The top-view images in Figure 2c show profiles of three electrode addresses; an uncoated electrode (no deposition), the electrode coated by chitosan deposition, and the electrode coated by codeposition of the composite CNT-chitosan film. These images demonstrate the spatial selectivity for codepositing individual hydrogel films based on chitosan’s electrodeposition. Using the magnified images, the film thickness (at the middle of the electrode) in the wet state was measured to be 84 ± 9 µm for the chitosan film and 256 ± 9 µm for the CNT-chitosan composite film.

The CNT-chitosan composite film was characterized by scanning electron microscopy (SEM). Figure 3a shows SEM images for the 1% CNT spread control film in Figure 2b. These images show an interconnected fibrous network with individual fiber diameters of 6–10 nm which is consistent with values provided by the supplier. Figure 3b shows SEM images for a control chitosan film that had been electrodeposited on the chip. These images show that the chitosan film surface is featureless. The SEM images for the codeposited CNT-chitosan composite film are shown in Figure 3c. These images show the composite has a wrinkled surface and porous microstructure consistent with observations from other studies.\textsuperscript{[29a,29b]} These images also indicate that the CNTs were uniformly distributed throughout the composite although the individual CNT fibers were less resolved compared to the control in Figure 3a.

Chemical characterization of the film-coated electrodes was performed using Raman Spectroscopy which is one of the most powerful methods for analyzing carbon nanotubes.\textsuperscript{[33]} The spectrum in Figure 4a for a sample of CNTs spread onto a gold electrode shows the characteristic D-band ($\approx$1340 cm$^{-1}$), G-band (1500–1600 cm$^{-1}$), and G$^\prime$-band (2450–2650 cm$^{-1}$).\textsuperscript{[33a,34]} The strong D-band and G$^\prime$-band indicate that the CNTs are multiwalled which is consistent with information provided by the supplier (i.e., multiwalled carbon nanotubes with diameters
of 6–9 nm). The spectrum for the electrodeposited CNT-chitosan composite film also shows the three characteristic peaks in the same frequency ranges. This observation indicates that the electrodeposition process does not alter the unique structure of carbon nanotube. The spectra in Figure 4b for the controls (uncoated and chitosan-coated gold electrodes) do not show the characteristic CNT peaks at these frequency ranges. Compared to the gold electrode, the Raman spectrum of the chitosan-coated electrode shows two weak peaks at 1150 cm$^{-1}$ (C–O–C stretching) and 1318 cm$^{-1}$ (acetyl; amide III).\(^{35}\) In summary, sonication allows CNT to be dispersed in the chitosan solution while chitosan’s pH-responsive film-forming properties enable co-deposition of CNT-chitosan composite films. Importantly, co-deposition allows rapid, reagentless, and programmable assembly at an electrode address. Further, the SEM images and Raman spectra indicate that co-deposition yields composite films with well-dispersed CNT and the unique chemical structure of the CNT is preserved.

### 2.2. Clozapine Analysis in Buffered Samples

In general, CNTs are attractive materials for electrochemical (bio)sensing because they offer strong electrocatalytic activities with minimal surface fouling.\(^{27a,27d,28a,28c,36}\) To optimize the fabrication of the CNT-chitosan composite film, we dispersed varying amounts of CNTs into a 0.9% chitosan solution and electrodeposited films at a constant current density (8 A m$^{-2}$) for 1 min. After deposition, coated electrodes were gently rinsed with water to remove nonspecifically bound material and then the film-coated electrodes were stored in a phosphate buffer solution (0.1 M, pH 7).

**Figure 5.** Carbon nanotubes (CNTs) enhance the electrochemical oxidation of clozapine (5 × 10$^{-6}$ M). a) Cyclic voltammograms (CVs). b) Differential pulse voltammograms (DPVs). c) Plot of anodic peak current (DPV) versus CNT content in the deposition solution. Analysis performed in 0.1 M phosphate buffer (pH 7).
phosphate buffer, pH 7.0) containing $5 \times 10^{-6}$ M clozapine. When the potential was swept into the positive direction, clozapine oxidation was observed near $+0.4$ V versus Ag/AgCl while reduction was observed during the negative potential sweep. As expected, films prepared with increasing levels of CNT were observed to offer larger redox peaks for clozapine. Also, the presence of CNT decreased the peak potential separation ($\Delta E_p$) from 60 mV (bare gold) to 0 mV (5% CNT-chit), which is attributed to the electrocatalytic properties of the CNTs.

We used a second electrochemical method to measure the oxidation of clozapine. Differential pulse voltammetry uses a series of small pulses superimposed onto a linearly varying potential and this method is commonly used to enhance sensitivity and selectivity of electrochemical analysis.[37] Figure 5b shows the differential pulse voltammograms (DPVs) for each of the coated electrodes. Consistent with the CVs of Figure 5a, the DPV oxidation currents were observed to increase with the increasing CNT levels but the current increases observed with DPV were larger than those observed for the CVs. To correlate our results, we subtracted the background current observed in the clozapine-free phosphate buffer from the current observed in the clozapine-containing buffer. Figure 5c shows a plot of the background subtracted peak oxidation currents from DPV as a function of the level of CNTs in the chitosan deposition solution. The increase in peak current with CNT level indicates that the incorporation of CNTs into the chitosan film enhances the sensitivity for clozapine detection. Further increase in CNT levels is constrained because higher concentration suspensions (above 5% in a 0.9% chitosan solution) became viscous pastes that could not be reproducibly electrodeposited. Importantly, the electrode coated with a composite film prepared from a 5% CNT-chitosan suspension offers 50-fold greater sensitivities for clozapine detection compared to a bare gold electrode.

To evaluate the sensitivity of a CNT-chitosan coated electrode for clozapine detection, we electrodeposited triplicate composite films from suspensions containing 5% CNT and 0.9% chitosan (8 A m$^{-2}$; 1 min). Each composite film-coated electrode was then placed into 0.1 M phosphate buffer (pH 7.0), clozapine was incrementally added to this buffer, and DPV measurements were performed to measure clozapine’s oxidation at each concentration increment. Results for one film-coated electrode are plotted in Figure 6a which shows the DPV responses to clozapine concentrations in the range of 0.05 to $5 \times 10^{-6}$ M. The DPV for the buffer (without clozapine), shows no peaks over the voltage range studied while a weak peak is observed around +0.17 V (vs Ag/AgCl) when $0.05 \times 10^{-6}$ M clozapine was added to the solution. This oxidation peak increased progressively with the addition of clozapine.

Figure 6b shows the background-subtracted anodic peak currents in DPVs versus clozapine concentration for the triplicate CNT-chitosan-coated electrodes. As seen from this plot, the anodic peak current increased linearly with clozapine concentration over a wide concentration range (from $0.05 \times 10^{-6}$ to $10 \times 10^{-6}$ M). Control measurements were made with an uncoated (i.e., bare) gold electrode and these results are also plotted in Figure 6b. While the DPV peak currents increased linearly with clozapine concentration, the slope of the curve (i.e., the sensitivity) for the bare gold electrode was 30-fold lower compared to the electrode coated with the composite film. From the information in Figure 6b it was possible to calculate the limit of detection (LOD; 3 times the standard deviation of background).[38] The LOD for the CNT-chitosan coated electrode was 4-fold better than for the bare gold electrode—0.050 $\times 10^{-6}$ M (16.3 ng mL$^{-1}$) versus 0.2 $\times 10^{-6}$ M—and of an appropriate magnitude relative to the therapeutic range (from $1.0 \times 10^{-6}$ to $3.0 \times 10^{-6}$ M; 0.350–1 µg mL$^{-1}$).[8c]

2.3. Clozapine Analysis in Spiked Serum

Next, we assessed the detection from commercially available serum spiked with varying amounts of clozapine. Initial DPV measurements were made with an uncoated (i.e., bare) gold electrode and these results are shown in Figure 7a. The
first observation with the bare gold electrode is that an oxidation peak is observed for serum at +0.44 V (vs Ag/AgCl) even when no clozapine was added to the serum. This peak was not observed in buffer and thus is presumably due to redox-active metabolites in serum (e.g., uric acid, ascorbic acid, dopamine). The second observation with the bare gold electrode is that the addition of clozapine to the serum (5 × 10⁻⁶ to 20 × 10⁻⁶ M) did not result in substantial changes in the DPV response. This result is somewhat surprising but suggests that the gold electrode cannot discriminate between the electrochemical oxidation of clozapine and the oxidation of other redox-active interferents in the serum.

DPV measurements were also made using electrodes coated with the CNT-chitosan composite film in clozapine-spiked commercial serum. When serum was tested without clozapine addition Figure 7b shows an oxidation peak was observed at +0.15 V (vs Ag/AgCl) which is negatively shifted compared to the serum peak observed with the bare gold electrode (Figure 7a). Presumably, this shift in peak position is due to the electrocatalytic activities of the CNTs in the composite film. Importantly, this potential shift removes the serum peak from the voltage range where clozapine oxidation peaks are expected. When clozapine was added to the commercial serum, an oxidation peak is observed at 0.35 V (vs Ag/AgCl) and the peak current increased with the clozapine concentration.

We next performed DPV measurements of clozapine-spiked serum using triplicates of CNT-chitosan-coated electrodes to generate the standard curve in Figure 7c. As shown, the background-subtracted anodic peak currents increased linearly with clozapine concentration in the therapeutically relevant concentration range (1.0 × 10⁻⁶ – 3.0 × 10⁻⁶ M). From these results, the limit of detection (LOD) was calculated to be 0.5 × 10⁻⁶ M. Thus, compared to the bare-gold electrode the CNT-chitosan composite film confers selectivity by shifting the serum peak potential from the clozapine region and this enables the sensitive detection of clozapine.

A second set of serum samples were obtained from healthy volunteers. These samples were obtained by drawing blood, centrifuging, and then adding clozapine to the supernatant (i.e., the serum). Using triplicate CNT-chitosan coated electrodes, a standard curve was generated for these serum samples. Figure 7c shows considerable deviation between the standard curve generated from commercial serum and that generated from serum obtained from healthy volunteers. The deviation between the two serums may be different due to different sampling protocols with differing dilutions and additives (e.g., anticoagulants) between the commercial serum and the serum from healthy volunteers. In addition, Figure 7c compares the standard curves in serum against that obtained for buffered clozapine solutions (e.g., from Figure 6b). As indicated by the slopes of these curves the sensitivity for clozapine detection from serum is 10-fold less than from buffer.

The reasons for these differences between the serums and buffer are not clear but it illustrates the difficulty in performing sensitive analysis from such a complex and potentially time-varying background. In some cases, sample pretreatment steps (e.g., dilution with buffer, protein precipitation or filtering) can be performed to mitigate problems encountered.
with serum.\textsuperscript{[40]} Obviously, it would be desirable if analysis could be performed without the need for sample pretreatment. Nevertheless, the difficulty in understanding and compensating for the effects of serum on detection sensitivity illustrate the challenge of using a one-sensor one-analyte approach for detecting analytes from blood—especially when the signal to noise is low.

2.4. Analysis of Serum Samples from Schizophrenia Patients

Finally, we analyzed serum samples from two schizophrenia patients. One was an out-patient who was not receiving clozapine (CLZ-untreated). The other was an in-patient who was being treated with clozapine (CLZ-treated; 500 mg per day) along with several additional medications including glycopyrrolate (1 mg per day), simvastatin (20 mg per day), docusate sodium (200 mg per day), magnesium hydroxide (30 mL per day) (see Figure S1, Supporting Information, for further details of patient’s medication). Blood was drawn from these patients into a tube, centrifuged to remove clots and the resulting supernatant was used as serum. The serum samples from these patients were analyzed directly without performing any pretreatment steps.

We performed initial analysis on the patients’ serum samples using a bare gold electrode and Figure 8a shows the DPVs for these samples. The DPVs with the bare gold electrode show a broad peak +0.4 V with little discrimination between the serum from the clozapine-treated and untreated patient. These observations are similar to those in Figure 7a. These serum samples were also analyzed using the CNT-chitosan coated electrode and these DPV results are shown in Figure 8b. The DPV for the serum from the untreated patient shows that a strong peak appears at +0.2 V which is similar to the serum background peak observed in Figure 7b. Importantly, the serum from the untreated patient shows no peaks in the range of 0.3–0.4 V which is also consistent with results in Figure 7b. Presumably, the shift in the peak oxidation potential away from 0.4 V (bare gold electrode) is due to the electrocatalytic activity of the CNT.

Figure 8b shows that for serum from the clozapine-treated patient, a small DPV oxidation peak is observed at 0.35 V, which is consistent with clozapine peak position observed in Figure 7b. This clozapine electrochemical signal was verified by testing this serum in triplicate using three CNT-chitosan coated electrodes. All three electrodes consistently showed the oxidation peak at 0.35 V. Also, when the same electrode was used to test serum from the treated and untreated patient, we only observed the oxidation peak at 0.35 V in serum from the clozapine-treated patient. Figure S2 (Supporting Information) shows the individual DPV from CNT-chitosan coated electrodes.

To estimate the clozapine concentration in these patient serum samples, we used the standard curve for serum from healthy volunteers as shown in Figure 7c because the same protocol was used for sampling. The oxidation peak of CLZ-treated-patient’s serum in DPV of Figure 8b was estimated to have a serum clozapine concentration of 320 ng mL$^{-1}$. This value compares to the value 368 ng mL$^{-1}$ reported by a commercial laboratory as indicated in Figure 8c. This consistency indicates that our CNT-chitosan coated electrode could be a useful sensor for the therapeutic drug monitoring of clozapine for schizophrenia patients.\textsuperscript{[41]}

3. Conclusion

Sensor technology offers the potential to contribute important tools to understand and manage mental health disorders. For
instance, physical sensors that can be implanted, ingested or worn could provide valuable measurements (e.g., of speech, gait, and sleep) indicative of a patient’s status, the disease’s progression, or a treatment’s effectiveness. Our long term goal is to create the capabilities to access complementary chemical information (e.g., from blood, urine or saliva). To maximize the extraction of chemical information from a sample, we envision the use of a small array of semimart sensors in which each sensor-type offers appropriate sensitivities and overlapping selectivities. Toward this goal, we report the development of a sensor with electrocatalytic activities that can directly detect low levels of clozapine in the serum of patients undergoing treatment with this antipsychotic drug. Detection from serum is a critical step in the development of sensors for clinical analyses. Importantly, this sensor measurement required minimal sample pretreatment (only centrifugation to generate the serum), which can be contrasted to other approaches that employ additional steps to remove protein, dilute interferents or adjust pH to facilitate analysis. This sensor relies on the fabrication of a composite film composed of chitosan (allows programmable thin film assembly by electrodeposition) and multiwalled carbon nanotubes (confers electrocatalytic activity). Potentially, information obtained from this electrocatalytic sensor can be coupled with information obtained from other sensor types (e.g., a redox-cycling sensor) to provide a more complete understanding of a patient’s status and enable the better management of her/his affliction.

4. Experimental Section

Chemicals: The following were purchased from Sigma-Aldrich: chitosan, carbon nanotubes (carbon >95%, multiwalled), clozapine, and serum from human male AB plasma. The water (>18 MΩ) used in this study was obtained from a Super Q water system (Millipore). Chitosan solutions (0.9%, pH 5.5) were prepared by dissolving chitosan flakes (confers electrocatalytic activity). Potentially, information obtained from this electrocatalytic sensor can be coupled with information obtained from other sensor types (e.g., a redox-cycling sensor) to provide a more complete understanding of a patient’s status and enable the better management of her/his affliction. Clozapine Analysis in Serum: Commercial serum was purchased from Sigma-Aldrich. Also, serum samples from healthy volunteers or schizophrenia patients were collected from the Maryland Psychiatric Research Center, University of Maryland School of Medicine. The blood of patient was centrifuged and the resulting supernatant was taken using BD Vacutainer tube. It was apportioned into 1 mL aliquots and stored at –80 °C freezer before assay. The study to collect human serum from patients with schizophrenia and healthy controls was approved by the University of Maryland School of Medicine IRB and informed consent was obtained from all study participants prior to research procedure. The clozapine levels in serum from schizophrenia patients were analyzed independently by a commercial laboratory (LabCorp).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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