Chemical and biological sensors

Advancing Electrochemical Screening of Neurotransmitters Using a Customizable Machine Learning-Based Multimodal System

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Abstract—High throughput and automated optical readout systems are already an industry standard in life sciences for screening several reactions at once. However, such high throughput systems are in an inceptive stage for studying electrochemical interactions. This limitation, for example, slows down the process of establishing property-performance relation of novel materials for biochemical sensing. Herein, building on our prior work, we fabricate a low-cost customizable platform to screen response of acetic acid-treated laser induced graphene to identify and quantify four biogenic amine neurotransmitters in artificial saliva, namely dopamine, serotonin, epinephrine, and norepinephrine, which due to similar molecular structures are difficult to differentiate using conventional electrochemical methods. Our analytical platform analyzes multiple sensors at once and processes the data using machine learning to rapidly screen the material–molecule interactions by combining several electrochemical spectral components (fingerprints). Combining multiple spectral features, both within one electrochemical module and across different modules, significantly improves the sensor performance and allows identification of the biomolecules using the same material system. The proposed automated electroanalytical system can be used to screen material–molecule interactions as well as high throughput point-of-care testing for rapid, multiplexed, and low-cost molecular detection.

Index Terms—Chemical and biological sensors, electrochemical sensors, automated, machine learning, multimodal, multiplexed.

I. INTRODUCTION

The ever-increasing need for molecular sensing for various applications ranging from disease diagnosis [1] to hazardous chemical detection [2] has allowed electrochemical sensors to emerge as a popular choice due to their high sensitivity, selectivity, low cost, stability, and simple and real-time readout [3]. Traditionally, the electrochemical sensor community has heavily relied on material engineering to enhance the sensor performance [4]. However, the task of benchmarking the process-property relation of various materials for optimizing electrochemical sensors is often tedious due to the nature of the measurements and the added complexity of handling liquids with electronics. High throughput, automated screening is already an industrial standard in optical methods, especially in life sciences where several samples can be analyzed at once [5], [6]. However, automated and high throughput testing is in its inceptive stages for studying electrochemical interactions [7]. Previous studies have explored methods to enable high throughput testing, such as the use of an array of electrodes in the same liquid cell to perform measurements for several sensors or the use of a single sensor that is inserted into several independent liquid cells [8]. However, the problem with these approaches for benchmarking and optimizing sensor performance through material engineering is the crosstalk and cross contamination between the sensors and the media. Hence, to obtain reliable results, an independent sensor and liquid medium are preferred for each test to avoid any chance of cross contamination. Due to the slow nature of this process (i.e., enhancing sensitivity through material engineering), several other avenues have emerged in the form of device design [9], multiplexed sensing [10], multimodal sensing, waveform engineering [11], etc.

Multiplexing is a powerful method to enhance the sensor sensitivity and the diagnosis accuracy. Multiplexing improves sensor performance by detecting multiple targets. Conventional approaches for multiplexing include spatial separation [12], independent capture molecules [13], temporal separation [14], mass measurement [15], high-performance liquid chromatography [16], spectroscopic separation [17], among others [10]. Spectroscopic approaches are attractive in the sense that the sensor design and the material are fixed, and the spectroscopy data is used to extract a large amount of information. Among electrochemical methods, potentiometric methods, such as cyclic voltammetry (CV) [18], square wave voltammetry (SWV) [19], and differential pulse voltammetry (DPV) [20], are common examples of spectroscopic (i.e., peak-based) measurements [21], in the sense that various molecules and reactions add different spectral components to the final output waveform. Further, novel methods have been developed, such as semicircular voltammetry, with added benefit of custom-tailored waveforms to enhance detection sensitivity [11]. Conventionally, various voltammetry methods have been used independently for sensing and usually the spectral strength of only one component/peak is chosen to extract the concentration of a target analyte. However, several peaks could be attributed to the presence of a target molecule due to consequent reactions [22], which could be used for extracting information and enhancing the diagnostic accuracy. However, since the reaction mechanisms for many molecules are unknown, it is challenging to manually assign and classify the components to particular molecules.

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Previously, we developed a multimodal machine learning-based system for multiplexed detection of two analytes (uric acid and tyrosine in sweat and saliva) with electrodeposited molybdenum polysulfide on laser induced graphene (LIG) [23]. In the present letter, we benchmark interaction of acetic acid-treated laser induced graphene (AA-LIG) with four biogenic amine neurotransmitters: dopamine (DA), serotonin (SER), epinephrine (EP), and norepinephrine (NEP). These molecules are difficult to differentiate using conventional electrochemical methods [24], as they have similar molecular structures and physiochemical properties. Our machine learning-based automated analytical system combines features from high throughput/automated sensing with spectroscopic multiplexing in order to solve their mutual problems, i.e., the difficulty to manually process high throughput data and the need for a large dataset for spectroscopic multiplexing. Machine learning has already made long strides in material discovery and omics, however in electrochemical community, due to the lack of large datasets, this process has been slow. Machine learning can be used to efficiently process and classify the electrochemical data and is an emerging area in electrochemical sensing [25]. In this letter, we have made improvements to our previous hardware setup in the form of a better sensor holder and contact mechanism along with custom-designed multiplexer and potentiostats. The developed multiplexer reads several sensors at once while efficiently assigning a commercial or custom-made potentiostat with custom electrochemical modules to collect a wide variety of spectroscopic data. Data processing approaches are then used to automatically process the signals and identify each target analyte and predict their concentration. The system is able to collect the spectroscopic data and the software pipeline processes the data to accurately predict the target neurotransmitter concentration with a limit of detection (LOD) lower than conventional single peak analysis.

II. MATERIALS AND METHODS

A. Electrochemical Modules

To study the sensitivity and selectivity of various electrochemical modules, the choice was restricted to SWV, DPV, and large amplitude ac voltammetry (LAACV) with the first two being popular choices for analysis, whereas the latter is a less explored method that applies a complex sinusoid superimposed ramp waveform eliciting a complex response which can be decomposed into several harmonics [26]. All module parameters are optimized to obtain the maximum primary oxidation peak of DA (50 μM) in Dulbecco's phosphate buffered saline.

B. Sensor and Sensor Holder

The LIG sensor is fabricated and used as the testbed to demonstrate the proof of concept of the proposed analytical method. Direct laser scribing of polyimide (with a CO_2 laser, 10.6 μM) leads to formation of a three-dimensional porous graphene film [27]. A standard three-electrode configuration of working electrode (WE), counter electrode, and reference electrode (RE) are designed using LIG. After fabrication, the LIG sensor is treated with acetic acid for ~12 h to improve its electrical conductivity (AA-LIG). It is shown that acetic acid treatment increases the ratio of carbon–carbon bonds [28]. In order to achieve a reliable contact to the sensors, robust and nondestructive mechanisms are needed as AA-LIG is fragile toward shearing forces. Zero insertion force connectors are used to make the contact in the form of custom-designed spring-loaded gold probes [Fig. 1(a)]. A custom sensor holder is designed to house a set of four sensors along with the capability to make the contact as well as to attach an external RE (such as glass Ag/AgCl RE) to each individual sensor when needed [Fig. 1(b)]. The electrical measurements are automated as described in the next sections.

C. Potentiostats

A 4-channel commercial potentiostat [PA, MultiPalmsens4, Palmsens, Inc.; marked in Fig. 2(a)] is used to perform CV, SWV, and DPV

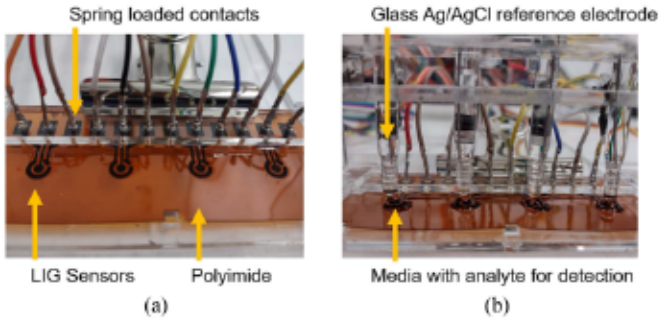

Fig. 1. Sensor and sensor holder setup. (a) Spring-loaded contacts to make reliable contact to acetic acid-treated laser induced graphene (AA-LIG) sensors on flexible polyimide. (b) Sensors in the sensor holder with glass Ag/AgCl reference electrodes and liquid media.

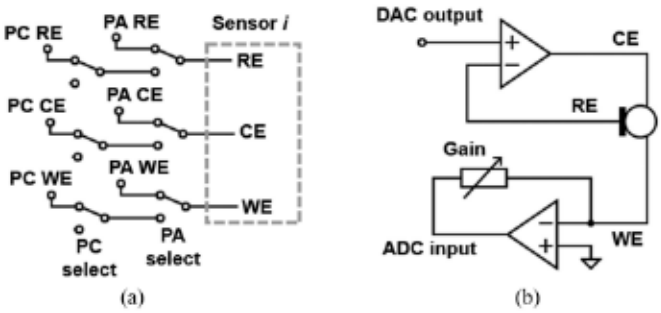

Fig. 2. Block circuit diagram of the readout system: (a) Multiplexer routing diagram from eight sensors to different potentiostats, PA and PC, for multiplexed switching. (b) Custom potentiostat for large amplitude ac voltammetry measurements.

measurements. Since LAACV is not usually available on commercial platforms, a custom potentiostat [PC, as indicated in Fig. 2(a)] is built to perform LAACV. The block circuit diagram of the single channel custom potentiostat is shown in Fig. 2(b). In brief, a Teensy 3.5 microcontroller is used to generate the potential waveform, a sinusoid (100 Hz; 100 mV V_{pk}) superimposed upon a ramp (50 mVs^{-1} scan rate). A low input bias operational amplifier (TLC2264, Texas instruments) is used to create the basic potentiostat that ensured the potential between the WE and RE is as indicated by the microcontroller.

D. Multiplexer

The chosen electrochemical methods, SWV, DPV, and LAACV, are all needed to be performed on the same sensor to collect maximal spectral data. In order to do this, a single sensor is dynamically connected to two potentiostats: PA and PC as mentioned in Section II-C. PA has four channels, whereas PC only has one channel. This imbalance is compensated by the time each potentiostat needs to collect data from a sensor. Each SWV measurement takes about 0.75 min to complete and each DPV measurement takes 2 min. We performed three scans of each measurement mode; hence, PA needed a total of ~8–9 min with a sensor to complete a measurement set of SWV and DPV. LAACV from PC on the other hand takes ~2 min to complete three scans. Hence, in this letter, the optimal design is chosen so a total of eight sensors can be measured at once: four of them assigned to PA for parallel measurements (total time: ~8–9 min) and four of them to PC for sequential measurement (total time: ~8 min). This design with matching measurement time maximizes the potentiostat usage. In order to achieve multiplexing, an eight-sensor to two-potentiostat multiplexer is designed with the block circuit diagram shown in Fig. 2(a). Commercially available relays are used to build the prototype multiplexer and an Arduino mega board controls the relay operation. A python-based interface is written and used to communicate and control

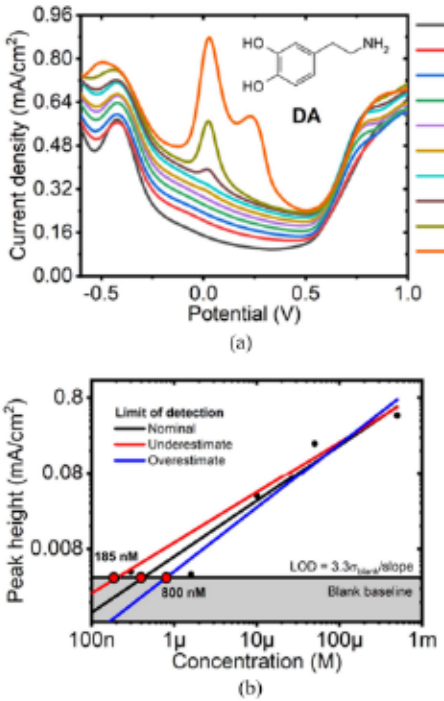

Fig. 3. Differential pulse voltammetry (DPV) results for dopamine (DA) in artificial saliva. (a) Raw data showing the baseline shift and peaks. (b) Limit of detection (LOD) calculated for DPV data shown in (a) based on the major peak amplitude. Nominal LOD along with underestimated and overestimated values are calculated to show the error range.

the multiplexer automatically, switching between measurements and monitoring the data collection. This customizable nature of the potentiostat allows for mixing and matching between available resources and the needed modules.

E. Cost of the Multimodal Readout System

The custom potentiostat, mainly made of Teensy 3.5 (~$30 USD) and the amplifier (~$3), costs roughly ~$40 to make per channel and is suitable for current ranges μA and above. The customizable nature allows for the user to tune the system performance as needed. The multiplexer capable of routing several potentiostats to sensors is mainly comprised of commercially available 16 channel relay boards (~$16) and a microcontroller (~$35). Overall, in this implementation, the cost for a two-potentiostat to eight-sensor multiplexer is estimated to be around $130. It should be noted that a typical cost of a commercial multichannel potentiostat can be upwards of $10k [29].

III. RESULTS

AA-LIG sensors were fabricated and tested with DA, SER, EP, and NEP in artificial saliva. Concentrations from pM to uM were added to each sensor sequentially with four replicates for each case. An example DPV data for DA is shown in Fig. 3(a) along with the corresponding conventional peak analysis to extract the LOD of the sensor in Fig. 3(b). LOD based on manual analysis is found to be 400 nM for the AA-LIG sensor.

A. Feature Extraction and Machine Learning for Analyte Quantification

In contrast to the manual method, using a python script, the raw data is automatically decomposed into constituent Gaussian peaks, minimizing the root mean square error. The peak fitting parameters, such as

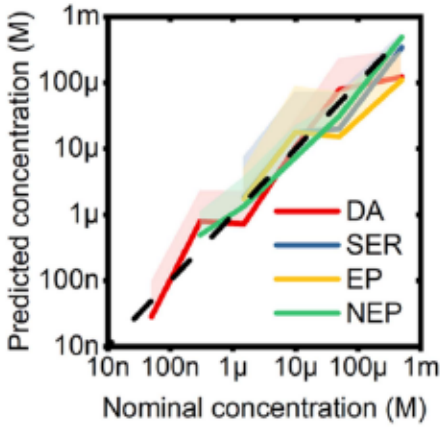

Fig. 4. Machine learning-based concentration estimation of neurotransmitters. Ideal prediction is denoted by the dashed black line (each error bar has >100 data points, total 16 independent devices in this figure).

TABLE 1. Multimodal Electrochemical Combination

Modules	Analyte threshold concentration (*nM*)			
	DA	*SER*	*EP*	*NEP*
DPV	50	1500	1500	300
SWV	300	1500	1500	300
LAACV	300	1500	1500	1500
DPV + SWV	50	300	300	1
DPV + SWV + LAACV	10	300	1000	300

peak height, width, and location, are distilled from the raw data. These features are then used to train a regressor to predict the concentration of a target molecule. The data are split into 90% for training and 10% for testing, and a leave-one-out style of repeated analysis is used to assess the results. Among various regressor classifiers that are compared, it is found that k-nearest neighbors and decision tree regressor (DT) perform the best. The prediction results using DT are plotted in Fig. 4, showing a linear response. Below a concentration termed as the threshold concentration (TC), the performance degrades; we term this TC as the sensor's LOD. With the proposed model, it is found that using features from just the DPV method results in a TC of 50 nM for DA, i.e., eight times lower than the LOD obtained using the conventional single-peak method (discussed in Fig. 3), which is attributed to the inclusion of multiple peaks in analysis. Furthermore, using the same preprocessing algorithm as well as the same trained machine learning model, the system could differentiate between the four analytes and selectively detect when they are independently present in the media.

B. Effect of the Multimodal Readout

Next, we studied the effect of multimodal sensing, i.e., instead of just using one electrochemical method (e.g., DPV), the data from two or more electrochemical methods are combined (e.g., DPV+SWV and DPV+SWV+LAACV) to predict the analyte concentration. In the proposed method, this is achieved by collating features from different measurements for the same sample condition to generate a super-feature vector, which is used to train and test the classifier in a similar method mentioned in Section III-A. It is found that the combination of multiple methods resulted in a lower TC, and hence a lower LOD than conventional single peak and single mode analysis as shown in Table 1. As highlighted in the table, combination of modules leading to lowest LOD varies for different molecules. The

improved LOD is attributed to the utilization of more spectral data than what is available in a single mode dataset. The machine learning model is able to deconvolute and learn these miniscule patterns that is hard to do manually, highlighting the strength of the proposed method.

IV. CONCLUSION

A multiplexed and multimodal electrochemical screening setup is designed to efficiently screen and test several sensors for detecting multiple target molecules. With a set of chosen constraints for the electrochemical modules, an optimized design with the capability of simultaneously measuring eight sensors is implemented and analyzed to detect four neurotransmitters using AA-LIG sensors. A multiplexer is designed and built for simultaneous data collection. The customizable feature of this design allows for mixing and matching of various measurement modules to optimize the analytical output. It also allows collection of a large amount of data needed for machine learning-based analysis to efficiently screen several materials and molecules combinations. Further, by combining multimodal electrochemical data and the use of a wider range of spectral components, our method improves the sensor's LOD for the studied analytes up to eight times compared to conventional single-peak methods. The proposed system can lead to more efficient and rapid sensor/material benchmarking, reliable multiplexed sensing, and high throughput point of care testing to measure several sensors at once, allowing for improved diagnostic outcomes as well as reducing the analytical cost and time.

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