September 19, 2023

To
Scientific Editors
Sensors

Re: Response to reviewers' comments on research article entitled, "A Facile Graphene Conductive Polymer Paper Based Biosensor for Dopamine, TNF-α, and IL-6 Detection"

Dear Editor,

The authors would like to appreciate the constructive comments received from you and your expert reviewers for improving the quality of our manuscript. Based on your valuable comments, the manuscript has been revised and our detailed responses along with related changes are explained after reviewers' comments, point by point.

Enclosed for your reconsideration is a revised version of our research article entitled "A Facile Graphene Conductive Polymer Paper Based Biosensor for Dopamine, TNF-α, and IL-6 Detection", for publication in the Sensors Journal. The revised manuscript includes all the changes/modifications that were suggested by the reviewers.

We greatly appreciate this opportunity and thank you for reconsidering our submission.

Sincerely yours,

Ali Ashraf

Ali Ashraf
Assistant Professor
Mechanical Engineering
University of Texas Rio Grande Valley,
Edinburg, TX-78539.

Authors' Responses to reviewers' comments on "A Facile Graphene Conductive Polymer Paper Based Biosensor for Dopamine, TNF-α, and IL-6 Detection"

Please note that the authors' responses to reviewers' comments are printed in blue, immediately following the comments and the corresponding revisions/changes are highlighted in yellow within the revised manuscript.

Reviewer #2

Here in this work, the authors have developed paper-based portable (point-of-care), affordable (laser cut) microfluidic biosensor for the detection of biomarkers in healthcare applications. The device is essentially a paper which is dip-coated (single/double layer) with conductive polymers functionalized graphene acting as an electrode.

The authors demonstrated electrochemical detection of biomolecules of interest such as dopamine, cytokine (TNF-a, IL-6) using CV and EIS and measured limit of detection (LOD) experimentally.

The authors showed proof of attachment between graphene conductive polymer with cellulose fiber using SEM and Raman spectroscopy. The authors have performed adequate morphological characterization for hierarchical structure of the sensing substrate, drying temperature dependent film stability, and ink conductivity, viscosity/ink thickness.

The methodology of analyte detection relies on increasing analyte concentration dependent increase in charge-transfer resistance, owing to increased biomolecule adsorption on the surface. Selective detection of antibody/antigen pair has been demonstrated. The dopamine, TNF-a detection performance for different electrochemical biosensors have been compare nicely. The ability of detection of IL-6 in human serum, and potential opportunity for early cancer detection is highly promising.

- The authors would like to thank the reviewer #2 for kindly reviewing our manuscript and providing us expert opinions and valuable comments. The following addresses the reviewer's comments.

Suggestions for revision of specific statements/issues

1. It would be better to use the same term to designate a given item. Such as 'conductive polymers functionalized graphene' and 'graphene and conductive polymer' have been used in different lines of the text.

- Thanks to the reviewer for the valuable suggestions.
- We fully agree with the reviewer, and we have designated a short form "GCPPS" for Graphene Conductive Polymer Paper based Sensor. Therefore, we have changed the following sentences in different sections:

Abstract

"In this paper, we present decorated paper with graphene and conductive polymer (herein referred to as Graphene Conductive Polymer Paper based Sensor or GCPPS) for sensitive detection of biomolecules."

1. Introduction

"In this paper, we fabricated a miniaturized graphene-PEDOT:PSS coated pa-per-based biosensor (GCPPS) with laser engraving to detect dopamine, TNF-α, and IL-6."

2.7. Electrochemical Analysis of Dopamine/TNF-α/IL-6

"This paper-based sensor was connected with Autolab PGSTAT302N (from Metrohm) via an adapter to accommodate GCPPS sensors."

4. Conclusions

In this study, a laser-cut paper-based electrochemical sensor coated with G-PEDOT:PSS (GCPPS) ink prepared using planetary mixer has been developed, and it demonstrated excellent performance in detecting dopamine, TNF-α, and IL-6 after modification by mild dry oxidation.

2. Line 180: The authors utilized a three-electrode setup instead of two-electrode for better quantification. It is often confusing to understand what are the electrodes. One can still identify, G-PEDOT:PSS as working electrode, the other two electrode in this three-electrode setup is not clearly mentioned in the text or figures. This should be done.

- Thanks to the reviewer for his/her valuable comment.
- We agree with the reviewer, and we adopted the three-electrode configuration instead of two electrodes. In section 2.3. Laser Cutting of Filter Paper and Electrode Dip Coating: we mentioned that the working electrode (middle one in Fig. 1b: Fabricated Sensor) and counter electrode (left one depicted in Fig. 1b: Fabricated Sensor) was dip coated with G-PEDOT:PSS ink whereas the reference electrode was dip coated with Ag ink (right one depicted in Fig. 1b: Fabricated Sensor).
- We have added the following sentences to make a clear description about the electrode configurations of our sensor.

2.3. Laser Cutting of Filter Paper and Electrode Dip Coating.

"Three electrode method was used in this experimental design which consists of counter, working and reference electrode. The counter electrode (C.E., left one in Fig. 1b: Fab-ricated Sensor) and the working electrode (W.E., middle one in Fig. 1b: Fabricated Sensor) were dip-coated into the G-PEDOT:PSS ink, and Ag ink was used for the reference electrode (right one depicted in Fig. 1b: Fabricated Sensor)."

2.7. Electrochemical Analysis of Dopamine/TNF-α/IL-6.

"Three electrode method consists of working electrode, counter electrode, and reference electrode in which the potential is kept constant in working electrode with respect to reference electrode and the potentiostat measures the current accurately between counter and working electrodes."

3. In Figure 1b, the process of converting dip coated electrodes into the fabricated sensor is not clearly explained. Should be described elaborately.

- Thanks to the reviewer for pointing this to our attention.
- We fully agree with the reviewer, and we added some additional information mentioned in comment #2 that describes Figure 1b more elaborately.

4. In Figure S1, the difference between graphene attached to the conductive polymer (S1b) and graphene with conductive polymer coating structure (S1c,d) is not clearly understood.

- Thanks to the reviewer for bringing this to our attention.
- Figure S1 c-d are both describes the same g-PEDOT:PSS ink on the filter paper. To avoid confusion, we are removing the Figure S1c,d from supplementary.

5. The figures are presented very poorly. Particularly the data points. This can be vastly improved, which further enhances readability. Such as in Fig 3 or any other EIS measurement related data instead of just connecting the data points, a guide to eye line matching semicircle or semicircle fits would have been better. In fact, the authors argued that, the Nyquist plot obtained in Fig. 3a-b was fitted with Randles equivalent circuit 4 components: solution resistance (Rs), charge transfer resistance (Rct), double layer capacitance (Cdl), and Warburg diffusion (Zw)). So, the data and fit overlay should have been shown instead of connecting the data points only.

- Thanks to the reviewer for his/her valuable comments.
- We agree with the reviewer that the data points with connecting lines make the figure a bit confusing. However, the data presented here with connecting lines in Figure 3b are directly obtained from the Autolab Potentiostat. We used the Randles equivalent circuit (with 4 components) to make an equivalent semi-circle to obtain the charge transfer resistance. Although the fit circuit data makes the figure more readable but at the cost of distorting the original data.
- To keep the original data, we plotted the data points with connecting lines. To make it more readable, we used an enlarged view of each Nyquist plot at a low-frequency range (at the tail end of the Nyquist plot).
- To enhance the quality of the Figure set, we are planning to send the Figures separately.

6. In Fig. S5, the increase of negative peak current with increasing Dopamine concentration is evident. However, the linearity (if it is!!) is not shown. Should be plotted separately.

- We thank the reviewer for the comment.
- We fully agree to the reviewer that in Fig. S5, the increase of negative peak current with increasing Dopamine concentration is evident. We used cyclic voltammetry to get the oxidation-reduction peak behavior of our sensor.
- The peak current vs concentration plot is added in Figure S5. However, the relation between peak current with concentration is almost linear. So, we are removing linear from the sentence mentioned in 3.2. Dopamine Detection to avoid confusion.

"The negative peak current (reduction) for dopamine showed an increase with the concentration (25 µM to 400 µM) (Figure S5c)."

New Plot:

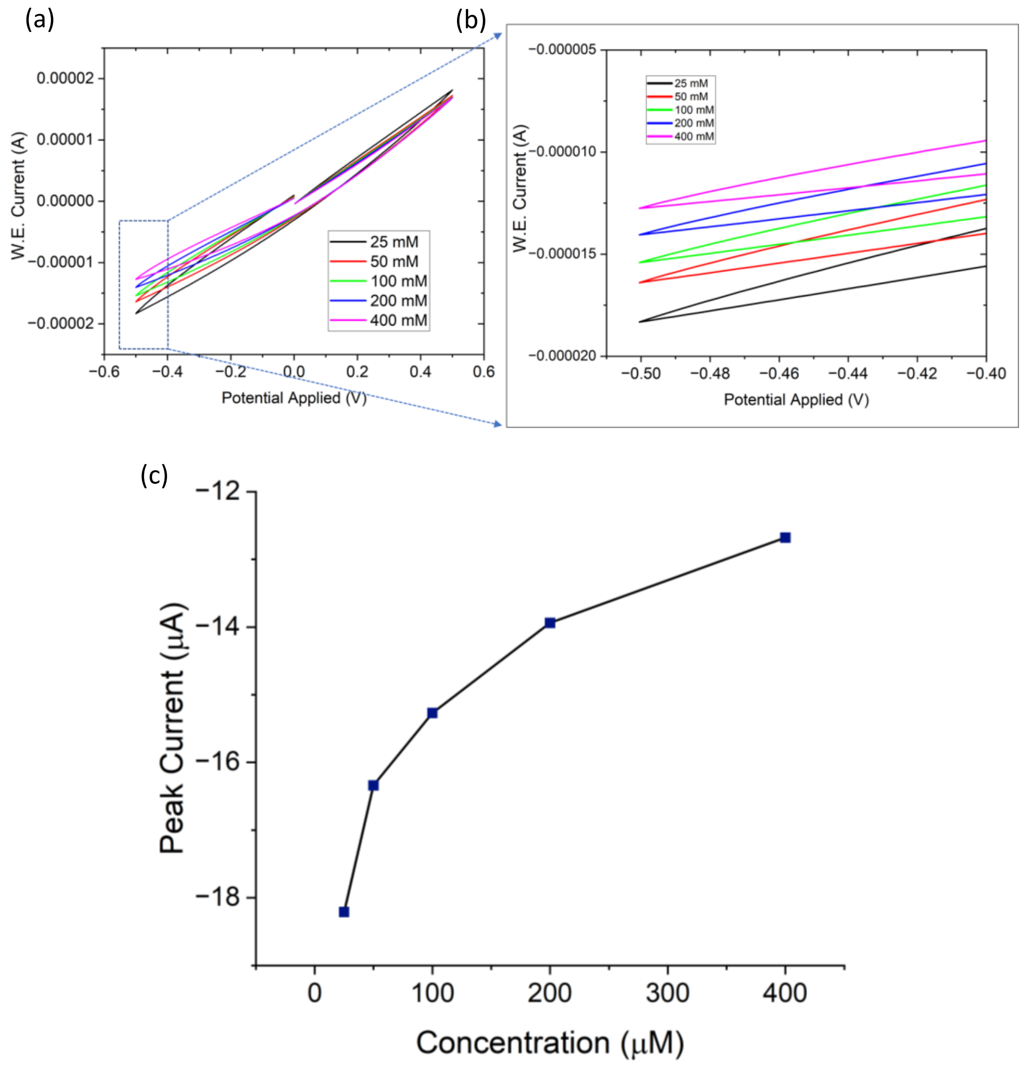

Figure S5. Cyclic Voltammetry performance on single-layer paper-based biosensor. (a) Cyclic Voltammetry (CV) performance for single layer coated paper-based electrodes during dopamine detection, (b) enlarged view of Figure S5 (a), (c) Peak current-concentration plot of CV performed on paper-based biosensor.

7. For TNF-a detection, the biosensor was single or double-layer dip coated?

- We thank the reviewer for bringing this to our attention.
- For TNF-a detection, the biosensor was double-layer dip coated. We have added the following senstences in section 3.3. TNF-α Detection

  "Similar to dopamine detection, EIS was performed to quantify the TNF-α detection using a Nyquist plot in a double layer dip coated sensor."

8. The experiments lack demonstration of reproducibility of the measurements line duplication or triplication of same experiments. As a result, concentration dependent measurements missing error limit with respect to the measurements.

- Thanks to the reviewer for the valuable comment.
- We have triplicated experiments for IL-6 detection in human serum (Figure 7) since this was the most challenging biosensing test in physiological media. At first, we tried to determine the capability of our sensor to detect various biomolecules of interest (dopamine, TNF-α, IL-6) in phosphate buffer saline, and later focused on the reproducibility of the sensor during IL-6 detection in human serum. We have added the following lines in the introduction to clarify this.

  " In this paper, we fabricated a miniaturized graphene-PEDOT:PSS coated pa-per-based biosensor (GCPPS) with laser engraving to detect dopamine, TNF-α, and IL-6. Initially we focused on detectability of the biomolecules using the sensor. For the final test of IL-6 detection in human serum, we triplicated the experiments to verify reproducibility."