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# Amperometric Bioelectronic Tongue for glucose determination

Yazan Al-Issa [a], John Njagi [b], Stephanie C. Schuckers [a], Ian I. Suni [c,*]

[a] Department of Electrical and Computer Engineering, Clarkson University, Potsdam, NY 13699, USA
[b] Department of Chemistry and Biomolecular Scinece, Clarkson University, Potsdam, NY 13699, USA
[c] Materials Technology Center, Department of Chemistry & Biochemistry, and Department of Mechanical Engineering & Energy Processes, Southern Illinois University, Carbondale, IL 62901, USA

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ABSTRACT

An amperometric Bioelectronic Tongue is reported for glucose determination that contains eight sensor electrodes constructed using different metal electrodes (Pt, Au), oxidoreductase enzymes (glucose oxidase, ascorbate oxidase, uricase), and membrane coatings (Nafion, chitosan). The response to varying concentrations of glucose, ascorbic acid, uric acid, and acetaminophen was tested for two models, concentration determination by current density measurements at individual electrodes and concentration determination by a linear regression model for the entire electrode array. The reduced chi-squared for the full array model was found to be about one order of magnitude lower than that for the individual-electrode model. Discrimination of glucose from chemical interference by the other three species is accomplished through a combination of enzyme catalysis, metal electrocatalysis, and membrane surface charge. The benefit of incorporating enzyme electrodes into the sensor array is illustrated by the lower correlation coefficients between different enzyme electrodes relative to non-enzyme coated electrodes. This approach can be more generally applied to detection of other substrates of oxidoreductase enzymes.


## 1. Introduction

Accurate monitoring of blood glucose levels is critical for health management of diabetes and hypoglycemia, allowing patients to monitor the effects of diet and exercise, and to make decisions regarding insulin dosage and timing. Glucose monitoring with handheld devices is often based on amperometric detection of either $O_2$ depletion or $H_2O_2$ formation during enzyme-catalyzed glucose oxidation at enzyme-coated Pt electrode [1,2]. However, the accuracy of glucose determination is limited by the possible presence of other electrochemically active species such as ascorbic acid, uric acid, and acetaminophen [1,2].

Chemical interference can be compensated for by using perm-selective membranes such as Nafion [3,4] and co-immobilization of glucose oxidase together with ascorbic oxidase [5], which pre-oxidizes ascorbate. However, the use of perm-selective membranes creates a diffusion barrier that increases response time and reduces sensitivity. In addition, since perm -elective membranes are often deposited by spin coating, the membrane pore size and thickness are difficult to control [6]. Chemical interference by other species that can be oxidized or reduced is also problematic during oxidoreductase-based biosensing of alcohols, phenols, sugars, and metabolic intermediates by amperometric monitoring of either $O_2$ depletion or $H_2O_2$ formation [7–12].

This problem is addressed here by creation of a Bioeletronic Tongue for glucose determination within a mixture of chemically interfering species. The concept of a Bioelectronic Tongue was recently reported, combining hardware, an array of amperometric or potentiometric sensor electrodes, and software, pattern recognition algorithms [13–19]. While the Electronic Nose and Tongue are well-established, the limited sensitivity and selectivity of the individual sensor elements limit accuracy of the overall device [20–22], so sensor elements that incorporate biomolecules has emerged as a major research thrust [22]. Here we report an amperometric Bioelectronic Tongue that contains enzyme electrodes for determination of glucose ascorbic acid, uric acid, and acetaminophen in controlled mixtures, and compare the response of individual sensors with the pattern response of the entire array. While others have reported electrode arrays that include multiple oxidoreductase enzymes, they have analyzed only the individual electrode responses, not the collective array response [7,8,11,12].

## 2. Experimental

### 2.1. Materials and reagents

Three different oxidoreductase enzymes, glucose oxidase (type X-S, from *Aspergillus niger*), ascorbate oxidase (from *Cucurbita*

* Corresponding author. Tel.: +1 (618) 453 7822.
*E-mail address:* isuni@siu.edu (I.I. Suni).

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species), and uricase (from *Anthrobacter globulin*), were all purchased from Sigma Aldrich. Chitosan, acetaminophen, $AuCl_3$ (30 wt% weight in dilute HCl), $H_2PtCl_6$ hydrate, 5 wt% Nafion, 25 wt% glutaraldehyde, uric acid, glucose, potassium phosphate, and sodium nitrate were also purchased from Sigma Aldrich. L-Ascorbic acid was purchased from Fisher Scientific, while acetic acid was purchased from Alfa Aesar.

### 2.2. Biosensor array

The biosensor array contains eight one-mm diameter Cu wires embedded into a virgin Teflon holder and arranged concentrically, as illustrated in Fig. 1. An array of six Pt and two Au working electrodes was created by electrodeposition of first Au and then Pt onto the exposed ends of the Cu wires. Direct electrodeposition of Pt onto Cu yielded unstable amperometric signals, probably due to poor Pt nucleation [23,24], which exposes the underlying and not biocompatible Cu wire. Au electrodeposition provides enhanced nucleation, preventing any Cu surface exposure.

The eight working electrodes were coated with different metals, different membranes, and different enzymes as indicated in Table 1. For sensor element #1, two units of glucose oxidase were mixed in 2 μl of chitosan, cast onto the electrode, and allowed to dry at room temperature for 1 h. For sensor element #2, two units of glucose oxidase and 10 units of ascorbate oxidase were mixed in 2 μl of chitosan, cast onto the electrode, and allowed to dry at room temperature for 1 h. For sensor element #3, the uricase/chitosan coating was formed by a five-step procedure: (a) deposit 2 μl chitosan, (b) deposit two unit of uricase, (c) cross-link with 25 wt% glutaraldehyde for 20 min, (d) deposit 2 μl of chitosan, and (e) dry for ½ h, then incubate in 0.1 M PBS. Uricase immobilization was more challenging than glucose oxidase or ascorbate oxidase. In order to obtain a stable amperometric signal from the uricase-coated electrode, the more robust procedure of glutaraldehyde cross-linking was needed, rather than simply casting the mixture of chitosan and enzyme onto the electrode surface. After steps a, b, and e, the sensor elements were allowed to dry at room temperature for 1 h. For sensor element #4, ten units of ascorbate oxidase were mixed in 2 μl of chitosan, cast onto the electrode, and allowed to dry at room temperature for 1 h. Sensor elements #5–8 were similarly coated with either chitosan alone, or first chitosan and then Nafion. Nafion coatings alone were unstable, typically delaminating during the course of testing.

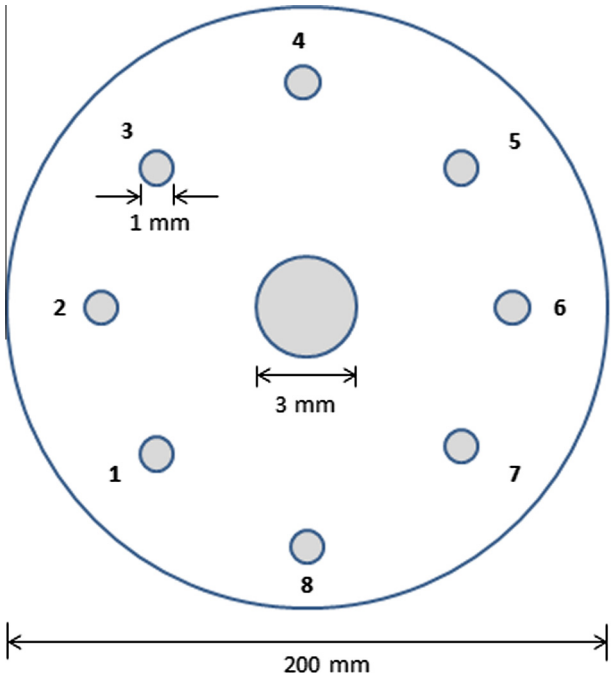


**Fig. 1.** Schematic of the eight-electrode sensor array.

### 2.3. Electrochemical measurements

The virgin Teflon holder with embedded Pt and Au working electrodes was placed inside a virgin Teflon electrochemical cell, which also contains an Ag/AgCl reference electrode and Pt wire counter electrode. Amperometric measurements were performed on the electrode array using a CHI-1030B multi-potentiostat from CH Instruments. The biosensor array was sequentially exposed to different concentrations of glucose (0–0.75 mM), uric acid (0–0.15 mM), ascorbic acid (0–0.15 mM), and acetaminophen (0–0.15 mM). Sensor electrodes #1, 2, and 5–8 measure anodic currents associated with oxidation of $H_2O_2$, ascorbic acid, uric acid, acetaminophen, and possibly other species that can be oxidized. Sensor electrodes #3 and 4 measure cathodic currents, predominantly associated with $O_2$ reduction. The amperometric response of the entire biosensor array to the different solution concentrations was obtained three times, twice for training, and once for testing. The test solutions were stirred continuously during all measurements.

## 3. Data analysis

### 3.1. Multivariate regression model

A multivariate regression model is created using the measured current density from the eight electrodes as the inputs. Both the dependent and the independent variables are scaled, although this only has a minor effect on the results. The model output is the estimated concentration of the four analytes in a mixture. First, a coefficient matrix is created based on the measured electrode readings and the known concentrations for the training data set. This coefficient matrix is then used to estimate analyte concentrations based on the measured current readings for the test data set.

Both multi-electrode and individual electrode models are computed and then compared. For the individual-electrode model, regression is computed for each combination of analyte and individual electrode current density. For the full array model, regression is computed for the four analytes using all eight electrode readings simultaneously. Eq. (1) describes the complete electrode array model, while Eq. (2) describes the individual electrode models. The multivariate regression model based on the eight electrodes is:

$$\boldsymbol{C} = \boldsymbol{jB} + \boldsymbol{A} \tag{1}$$

where $\boldsymbol{C}$ is a $1 \times 4$ array representing the four analytes: $C_1$ represents the glucose concentration, $C_2$ the ascorbic acid concentration, $C_3$ the uric acid concentration, and $C_4$ the acetaminophen concentration; $\boldsymbol{j}$ is a $1 \times 8$ current density matrix for the eight electrodes; $\boldsymbol{B}$ is an $8 \times 4$ coefficient matrix, and $\boldsymbol{A}$ is a $1 \times 4$ error or bias in the corresponding concentration. The analyte concentrations studied are chosen to be reasonably low so that a linear fit is expected. The regression equation for the single electrode model is:

$$C_i = j_k B_{ik} + A_{ik} \tag{2}$$

where $C_i$ represents the concentration for a single analyte, $B_{ik}$ is the associated coefficient for the model, $j_k$ is the specific electrode current density, and $A_{ik}$ the bias.

**Table 1**
Electrode preparation for biosensor array.

| Sensor element | Electrode material | Membrane coating | Enzyme(s) | Applied potential (vs. Ag/AgCl) (mV) |
|---|---|---|---|---|
| #1 | Pt | Chitosan | Glucose oxidase | +700 |
| #2 | Pt | Chitosan | Glucose oxidase + ascorbate oxidase | +700 |
| #3 | Pt | Chitosan | Uricase | −600 |
| #4 | Pt | Chitosan | Ascorbate oxidase | −300 |
| #5 | Pt | Chitosan | None | +650 |
| #6 | Pt | Chitosan, then Nafion | None | +650 |
| #7 | Au | Chitosan | None | +650 |
| #8 | Au | Chitosan, then Nafion | None | +650 |

### 3.2. Leave-One-Out Cross-Validation

All data from one experiment are collected, and the resulting dataset contains 45 samples. The Leave-One-Out Cross-Validation (LOOCV) methodology is used to train and validate the model. The advantage of this method is that there is no data lost [25]. The disadvantage is that this method is computing intensive and can be time consuming.

For a dataset of $N$ observations, the Leave-One-Out Cross-Validation (LOOCV) method performs $N$ training iterations. In each iteration, the LOOCV takes $(N-1)$ samples for model training and the remaining sample for validation and the obtained model is stored. In other words, it is repeated $N$ times so that every time one different sample is used as a validation sample. Finally, the model coefficients are computed by averaging all the models obtained during the iterative training process [26,27].

### 3.3. Performance metrics

Goodness-of-fit is quantified by the reduced chi-squared ($\chi^2_{red}$), which is normalized to the number of degrees of freedom ($\upsilon$):

$$\chi^2_{red} = \frac{\chi^2}{\upsilon} = \frac{1}{\upsilon}\sum_i \frac{(y_i - \bar{y})^2}{\sigma^2} \quad (3)$$

## 4. Results and discussion

### 4.1. Individual-electrode model

A regression model is computed for each analyte to predict its concentration from the current density measurements at each of the eight electrodes individually. Table 2 provides correlation coefficients between the actual and predicted value for each of the four concentrations at all eight electrodes. Table 2 illustrates that the glucose concentration ($C_1$) is most highly correlated with $j_1$ and $j_2$, the ascorbic acid concentration ($C_2$) with $j_4$, the uric acid concentration ($C_3$) with $j_3$ and $j_5$, and the acetaminophen concentration with $j_7$ and $j_8$. Fig. 2A–D provide a graphical comparison between the actual and estimated concentrations of glucose, ascorbic acid, uric acid, and acetaminophen for the individual electrode (#1, 4, 3 and 8) that best predicts these concentrations (glucose, ascorbic acid, uric acid, and acetaminophen).

**Table 2**
Correlation coefficients ($\rho$) between actual concentrations ($C_i$) and electrode current densities ($j_i$).

| $\rho(j_i, C_j)$ | $C_1$ | $C_2$ | $C_3$ | $C_4$ |
|---|---|---|---|---|
| $j_1$ | 0.904 | 0.196 | 0.392 | 0.348 |
| $j_2$ | 0.876 | 0.118 | 0.450 | 0.380 |
| $j_3$ | 0.067 | 0.487 | 0.895 | 0.055 |
| $j_4$ | 0.008 | 0.938 | 0.284 | −0.020 |
| $j_5$ | 0.080 | 0.498 | 0.798 | 0.476 |
| $j_6$ | 0.255 | 0.382 | 0.599 | 0.650 |
| $j_7$ | 0.146 | 0.509 | 0.591 | 0.727 |
| $j_8$ | 0.177 | 0.258 | 0.497 | 0.860 |

These indicate the electrode most sensitive to each of the four analytes.

Table 2 shows that electrodes #1 and 2 are much more highly correlated with the glucose concentration that the rest of the electrode array. Table 2 also illustrates that the correlation coefficients are similar (0.86–0.94) between each of the four analytes and the electrode that best predicts its concentration. Thus Table 2 alone would appear to indicate that the concentration of all four analytes can be equally well determined by current density measurements at individual electrodes. However, comparison of the root-mean-squared error (RMSE) for predicting the concentration of all four analytes illustrates that this is not true, and that predicting the glucose concentration is more difficult. Predicting glucose has an RMSE of 0.427, whereas predicting the other three concentrations has an RMSE ranging from 0.076 to 0.089. This reflects the fact that ascorbic acid, uric acid, and acetaminophen can all be oxidized at electrodes #1 or 2, thus obscuring the correct glucose concentration, which is measured indirectly as oxidation of a reaction product, $H_2O_2$. On the other hand, since glucose is not electrochemically active, the presence of glucose does not interfere with concentration measurement of the other three analytes.

Table 3 provides the correlation coefficients between the current densities measured at the eight electrodes. This illustrates that electrodes #1 and 2 are highly correlated (0.989), since glucose oxidase is immobilized at both of these sensor electrodes. Electrodes #3 and 4 are also correlated (0.667), since both electrodes measure $O_2$ depletion. However, all of the other correlation coefficients are low (<0.4) between the four enzyme electrodes (#1–4). On the other hand, the current densities at electrodes without an immobilized enzyme (#5–8) are highly correlated (>0.78). This

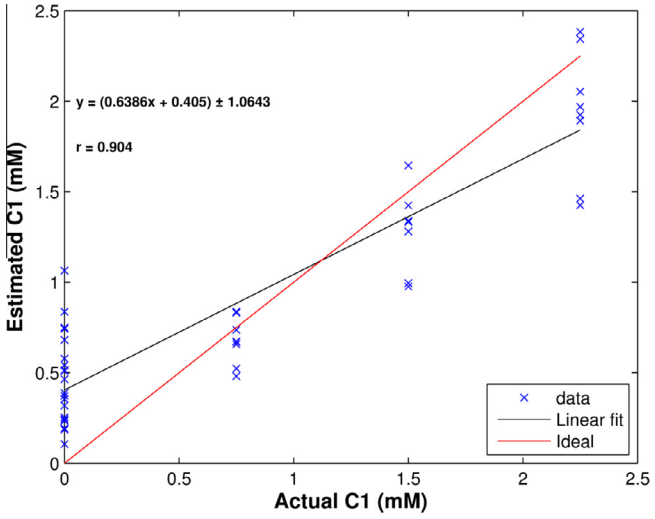


**Fig. 2A.** Performance of the individual-electrode model for predicting the concentrations of glucose ($C_1$) based on electrode 1.

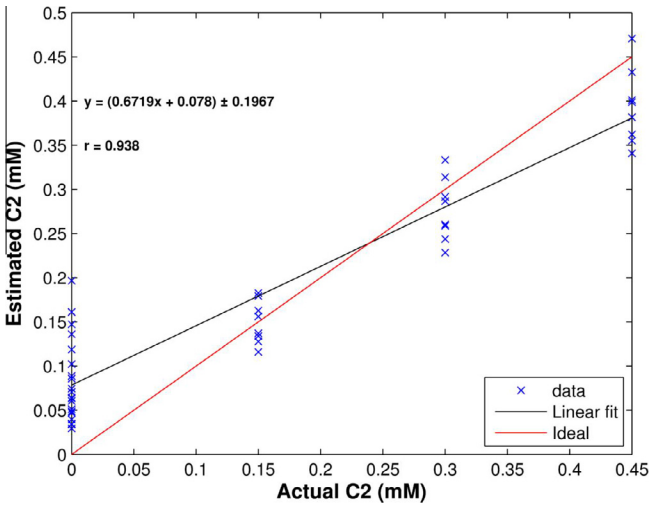


**Fig. 2B.** Performance of the individual-electrode model for predicting the concentrations of ascorbic acid ($C_2$) based on electrode 4.

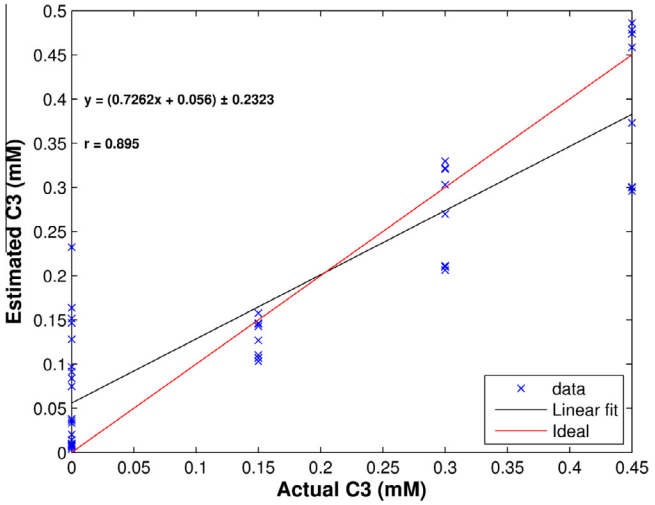


**Fig. 2C.** Performance of the individual-electrode model for predicting the concentrations of uric acid ($C_3$) based on electrode 3.

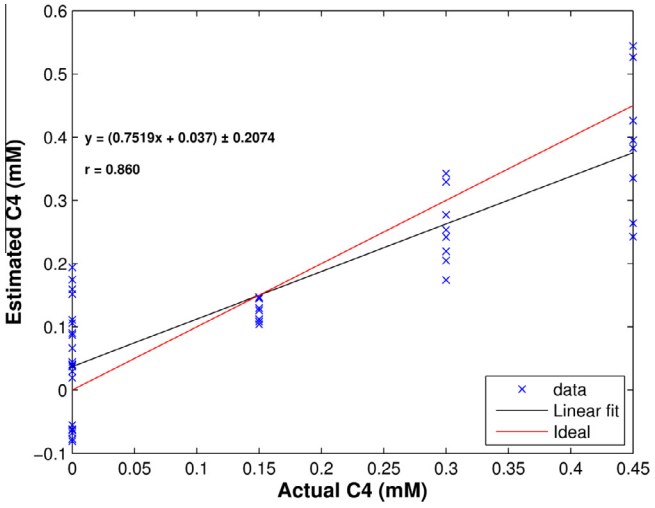


**Fig. 2D.** Performance of the individual-electrode model for predicting the concentrations of acetaminophen ($C_4$) based on electrode 8.

illustrates the value of having enzyme electrodes in the sensor array, since this yields current density measurements that are much less correlated.

The goodness-of-fit for the individual-electrode model can be estimated from the reduced chi-squared, which is given in Table 4. These are presented separately for each of the four analytes to illustrate the differing levels of predictive accuracy, and for easier comparison to the full-array model discussed below. For the individual-electrode model, the acetaminophen concentration is most poorly determined.

### 4.2. Full-array model

A regression model is also computed for each analyte to predict its concentration from the current measurements at all eight electrodes simultaneously, referred to here as the full array model. Fig. 3A–D provide a graphical comparison between the actual and estimated concentrations of glucose, ascorbic acid, uric acid, and acetaminophen for the full-array model. Table 4 presents a comparison between the individual-electrode and full array models, summarizing the slopes, intercepts and correlation coefficients for the graphical results of Figs. 2 and 3. In all cases, the full array model provides a much more accurate measurement of all four analyte concentrations, as evidenced by correlation coefficients quite close to unity, y-intercepts almost equal to zero, and a nearly unity linear relationship between the actual and model-predicted analyte concentrations.

Table 4 also presents and compares the reduced chi-squared for both models. Table 4 illustrates that the reduced chi-squared for the full array model is about 10× lower than for the individual electrode model. The acetaminophen concentration in particular is determined much more accurately by the full array than the individual-electrode model. However, acetaminophen is less well determined than the other three analytes because no enzyme electrode is included in the array with specificity to acetaminophen.

### 4.3. Choice of sensor materials

The best way to understand the materials' choice at the different sensor electrodes is to compare the response of each electrode

**Table 3**
Correlation coefficients between measurements at different sensor electrodes.

| Sensor | $j_1$ | $j_2$ | $j_3$ | $j_4$ | $j_5$ | $j_6$ | $j_7$ | $j_8$ |
|---|---|---|---|---|---|---|---|---|
| $j_1$ | 1 | | | | | | | |
| $j_2$ | 0.989 | 1 | | | | | | |
| $j_3$ | 0.382 | 0.397 | 1 | | | | | |
| $j_4$ | 0.173 | 0.112 | 0.667 | 1 | | | | |
| $j_5$ | 0.475 | 0.500 | 0.890 | 0.615 | 1 | | | |
| $j_6$ | 0.579 | 0.615 | 0.603 | 0.353 | 0.826 | 1 | | |
| $j_7$ | 0.525 | 0.548 | 0.694 | 0.526 | 0.925 | 0.892 | 1 | |
| $j_8$ | 0.528 | 0.576 | 0.485 | 0.226 | 0.787 | 0.889 | 0.938 | 1 |

**Table 4**
Comparison of fitting parameters ($r$, slope, and intercept) and good-ness-of-fit ($\chi^2_{red}$) for the both full array model and the individual electrode model for all four analytes.

| | | $r$ | Slope | Intercept (mM) | $\chi^2_{red}$ |
|---|---|---|---|---|---|
| Individual-electrode | $C_1$ | 0.904 | 0.6386 | 0.405 | 0.240 |
| | $C_2$ | 0.938 | 0.6719 | 0.078 | 0.191 |
| | $C_3$ | 0.895 | 0.7262 | 0.056 | 0.210 |
| | $C_4$ | 0.650 | 0.7519 | 0.037 | 0.262 |
| Full array | $C_1$ | 0.999 | 0.9981 | 0.001 | 0.002 |
| | $C_2$ | 0.984 | 0.9373 | 0.016 | 0.040 |
| | $C_3$ | 0.992 | 0.9944 | −0.001 | 0.018 |
| | $C_4$ | 0.988 | 0.9788 | 0.003 | 0.028 |

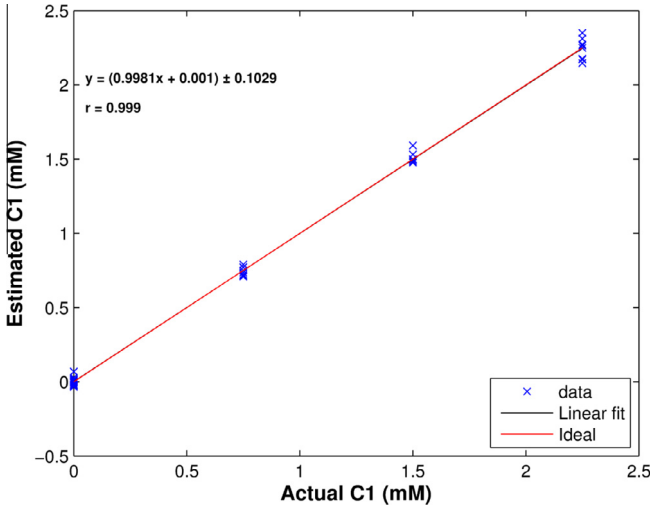


**Fig. 3A.** Performance of the full-array array model for predicting the concentrations of glucose ($C_1$).

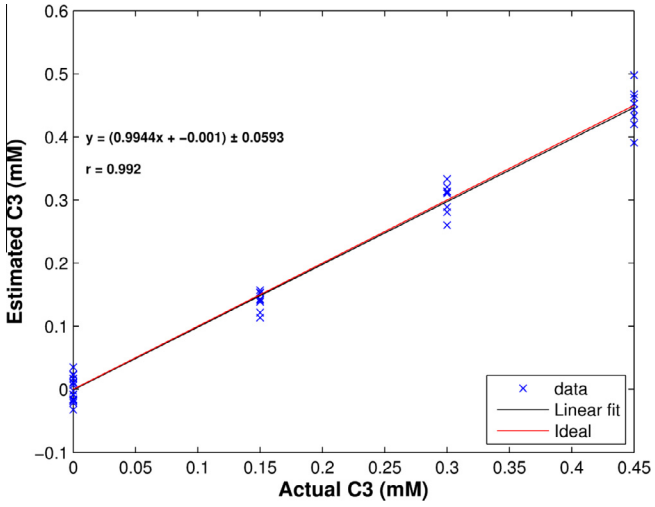


**Fig. 3C.** Performance of the full-array array model for predicting the concentrations of uric acid ($C_3$).

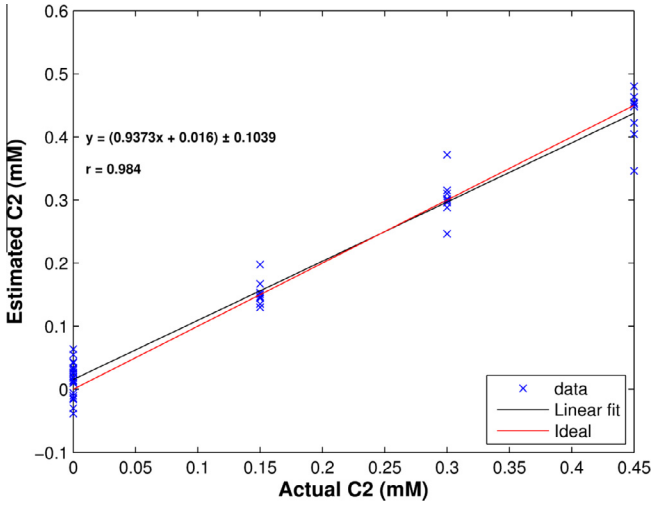


**Fig. 3B.** Performance of the full-array array model for predicting the concentrations of ascorbic acid ($C_2$).

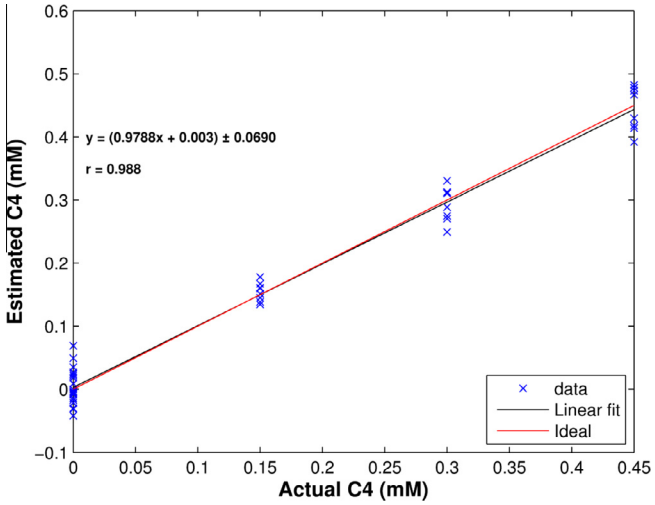


**Fig. 3D.** Performance of the full-array array model for predicting the concentrations of acetaminophen ($C_4$).

to glucose, ascorbic acid, uric acid, and acetaminophen, as summarized in Table 2. The sensor array contains four enzyme electrodes (#1–4) at which the following reactions are catalyzed by glucose oxidase, ascorbate oxidase, and uricase, respectively:

$$C_6H_{12}N_6O_3(\text{glucose}) + O_2 + H_2O \leftrightarrow C_6H_{12}O_7 + H_2O_2 \quad (4)$$

$$C_6H_8O_6(\text{L-ascorbic acid}) + \frac{1}{2}O_2 \leftrightarrow C_6H_6O_6 + H_2O \quad (5)$$

$$C_5H_4N_4O_3(\text{uric acid}) + O_2 + H_2O \leftrightarrow C_4H_4N_4O_2 + CO_2 + H_2O_2 \quad (6)$$

Amperometric biosensors based on $O_2$ reduction or $H_2O_2$ oxidation have been reported for glucose, ascorbic acid, and uric acid, based on the similarity of these oxidoreductase enzyme reactions [1,2,28–32].

The concentration of glucose is monitored at sensor elements #1 and #2 by the oxidation of $H_2O_2$ at +700 mV vs. Ag/AgCl, as shown in Table 1. At this potential, uric acid, ascorbic acid, and acetaminophen can all be readily oxidized, so they may interfere with accurate glucose determination. This motivated the co-immobilization of ascorbate oxidase with glucose oxidase at sensor element #2 in order to pre-oxidize ascorbate, therefore reducing its interference with glucose detection. However, the results in Table 2 for the individual-electrode model illustrate that electrode #1 ($r = 0.904$) is slightly better than electrode #2 ($r = 0.876$) for detecting glucose in these four-analyte mixtures.

According to the results in Table 2, ascorbic acid is best determined at sensor electrode #4, at which ascorbate oxidase is immobilized, and uric acid is best-determined at sensor electrode #3, at which uricase is immobilized. Both sensor electrodes are operated at cathodic potentials and are employed to detect the current density from oxygen ($O_2$) reduction. In Eqs. (4)–(6) above, all three oxidoreductase enzymes consume $O_2$, so all three reactants (glucose, ascorbic acid, uric acid) can be detected by measuring the extent of oxygen depletion at the corresponding electrodes. This method is used here for detection of ascorbic acid and uric acid, but not for detection of glucose. However, this method was employed for early glucose biosensors utilizing the Clark electrode [33,34].

The use of two different electrode materials, Au and Pt, was originally motivated by the observation of electrocatalytic effects in sensors that detect $H_2O_2$ [35–37]. Inspection of Table 2 suggested that Au electrodes might be more capable of discriminating acetaminophen from the other three analytes than Pt electrodes.

The correlation coefficients between acetaminophen and the two Au electrodes ($j_7$ and $j_8$) are 0.73 and 0.86, respectively. These values are almost as high as those for electrodes at which the oxidoreductase enzyme corresponding to the analyte of interest is immobilized (0.904 for $j_1$, 0.938 for $j_4$, and 0.895 for $j_3$). This suggests that Au electrodes are electrocatalytic towards acetaminophen oxidation, and this effect has indeed been recently reported [38–41].

The use of different membrane materials is motivated by the different electrostatic behavior of Nafion and chitosan. Nafion is a sulfonated tetrafluoroethylene that is highly anionic in neutral buffer solutions due to its numerous F atoms and $SO_3^-$ groups. For this reason, Nafion and its composite membranes have been proposed for enzymatic glucose biosensors in order to repel negatively charged species such as urate and ascorbate, while allowing permeation of neutral glucose molecules [42]. However, Nafion films are difficult to incorporate into enzymatic glucose biosensors due to a tendency to crack, and inadequate reproducibility [6,43]. In addition, the negatively charged sulfonic groups on Nafion can also concentrate cationic species present in the physiological system, resulting in interference with the measured glucose signal [6].

Chitosan was introduced into the glucose Bioelectronic Tongue because this polymer is widely known as a polycationic polymer that is positively charged in neutral buffer solutions. Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). The sensor array contains two electrodes (#5 and #7) with no immobilized enzyme that are coated with chitosan (cationic), and two electrodes (#6 and #8) with no immobilized enzyme that are coated Nafion (anionic). The situation is complicated somewhat by the inability to make pure Nafion coatings that are stable for the duration of these experiments. As described in the Experimental Section, the Nafion coatings atop electrodes #6 and #8 are deposited atop an underlying chitosan coating to improve the adhesion and durability of Nafion.

The correlation coefficients in Table 3 illustrate that the electrodes with an anionic outer Nafion layer are less sensitive to the presence of anionic species (ascorbic acid and uric acid) than those with a cationic outer chitosan layer. In other words, for both $C_2$ and $C_3$, the current densities $j_6$ are less than $j_5$ and the current densities $j_8$ are less than $j_7$. The simplest explanation is that the anionic membrane coatings on electrodes #6 and #8 repel urate and ascorbate, while the cationic membrane coatings on electrodes #5 and #7 do not.

## 5. Conclusions

An amperometric Bioelectronic Tongue was tested for determination of glucose in mixtures that also contain ascorbic acid, uric acid, and acetaminophen. This device contains eight sensor electrodes constructed using different metal electrodes (Pt, Au), oxidoreductase enzymes (glucose oxidase, ascorbate oxidase, uricase), and membrane coatings (Nafion, chitosan). The system response was tested using two models, concentration determination by individual electrodes and concentration determination by a linear regression model for the entire electrode array. The reduced chi-squared for the full array model was found to be about one order of magnitude lower than that for the individual-electrode model. Discrimination of glucose from chemical interference by the other three species is accomplished through a combination of enzyme catalysis, metal electrocatalysis, and membrane charge. Interference from ascorbic acid and uric acid is eliminated by using sensor electrodes coated with ascorbate oxidase and uricase, respectively. Interference from acetaminophen is eliminated partly by the use of an Au electrode, which is electrocatalytic towards acetaminophen oxidation, and partly through use of membrane materials Nafion and chitosan, which are negatively and positively charged, respectively, at physiological pH. The benefit of incorporating enzyme electrodes into the sensor array is illustrated by the lower correlation coefficients between different enzyme electrodes relative to non-enzyme coated electrodes. This approach may be generalized to other detection of other substrates of oxidoreductase enzymes.

## Conflict of interest

The authors declare that there is no conflict of interest.

## Acknowledgements

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