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Simulation and Performance Optimization of an Amperometric Histamine Detection System †

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Abstract: One of the most widely known biogenic amines is histamine, which plays an important role in the human immune system. Some people suffer from allergic reactions after a histamine-rich diet; this is called histamine intolerance. The aim of this work is to develop a quick and reliable method for the detection and quantification of histamine in food, based on an electrochemical approach. In presence of biogenic amines, a reduction cascade induces a current at the working electrode. Prior to chronoamperometric measurements, Finite Elemente simulations were performed. The results are presented in this work.

Keywords: electrochemical simulation; histamine detection; chronoamperometry; biosensor

1. Introduction

The functions of biogenic amines in the human body are manifold. These include the regulation of body temperature, stomach pH and vasoactive effects [1]. The consumption of food containing excess of biogenic amines is suspected to induce several symptoms such as diarrhea, headaches, rhinoconjunctival symptoms, asthma, hypotension, arrhythmia, urticaria, pruritus and flushing. Currently, there is a lack of reliable methods for the detection of biogenic amines Therefore interpretation of results from clinical studies leading to conclusive correlations between histamine and the afore mentioned symptoms is difficult. In our study we aim to quantify biogenic amines indirectly by inducing a redox cascade. The oxidation of biogenic amines by diamine oxidase (DAO) to aldehydes produces hydrogen peroxide (H_2O_2) as a by-product. In a second reaction H_2O_2 is used to oxidize 3,3′,5,5′-tetramethylbenzidin (TMB), catalyzed by horseradish peroxidase (HRP), which is then detected by amperometry.

2. Materials and Methods

Chronoamperometric detection is based on the chemical oxidation or reduction of the requested analyte. A step-voltage is applied between two electrodes and the current is monitored over time. Oxidation or reduction of an analyte causes rapid current response to the step-voltage. This can be measured by a high sensitive potentiostat. In this case, the oxidized TMB is reduced at the working electrode (see Figure 1) and the amount of induced current directly correlates with the concentration of biogenic amine in the sample (see Figure 1) [2,3].

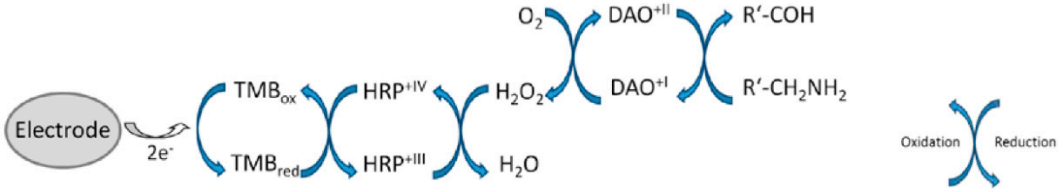


Figure 1. A redox cascade is used for the indirect detection of biogenic amines. The oxidation of biogenic amine by DAO reduces O_2 to H_2O_2. In a second reaction, the generated H_2O_2 is used to oxidize a co-substrate (TMB) catalyzed by horseradish peroxidase (HRP). Reduction of the co-substrate at a working electrode induces a current which directly correlates with the amount of biogenic amines in the sample.

In a simulation study, a 3D model was generated in COMSOL Multiphysics 5.0 (COMSOL, Inc., Burlington, MA, USA), which also accounted for diffusion of the individual species. To determine the concentration of the oxidized TMB (TMBox) generated by the reaction cascade, the rate of product formation v was calculated using Michaelis Menten kinetics (Equation (1)). The variable substrate concentration c_s, as well as the Michaelis constant K_M and the maximum achieved reaction rate V_{max} were taken from a previous experimental study [4].

$$v = \frac{V_{max} c_s}{K_M + c_s} \quad (1)$$

In addition, electrochemical measurements were carried out using a potentiostat (Reference 600™, Gamry Instruments, Warminster, PA, USA). HRP, H_2O_2 and phosphate-buffered saline (PBS) were purchased from Merck (Merck KGaA, Darmstadt, Germany). A ready-to-use TMB solution and stop solution (2M H_2SO_4) were purchased from R&D Systems (R&D Systems, Inc., Minneapolis, MN, USA). A 1:1 mixture of HRP stock solution (0.2 U/mL) and the TMB ready-to-use solution was used to generate Solution A. Different amounts of hydrogen peroxide were added to reach the final concentrations of 3.8, 15.3, 61.2, 244.7 and 1000 µM. The reaction was stopped by addition of 10 µL of the stop solution after 5 min. Hundred microliters of the sample was pipetted onto the screen printed electrodes (Gwent Group, Pontypool, UK, [4]).

3. Results and Discussion

3.1. Simulations

The concentration of the generated oxidized TMB after 5 min of incubation with HRP and variable initial concentrations of H_2O_2 were simulated using a 3D finite element model. The results of the simulations are shown in Figure 2.

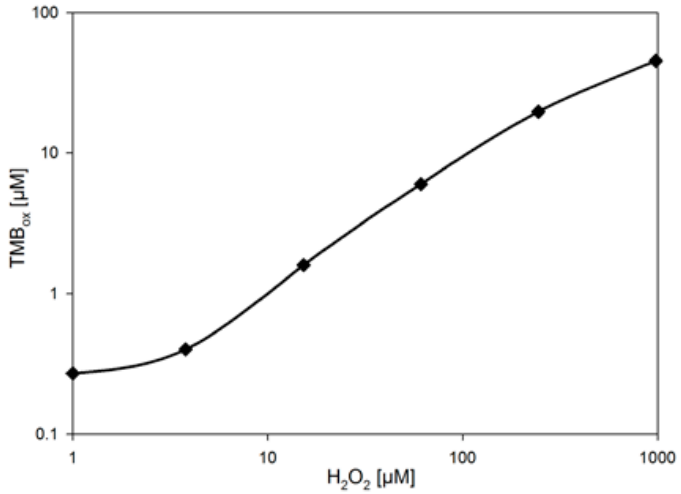


Figure 2. Generated TMBox after 5 min in dependency on the initial H_2O_2 concentration.

3.2. Experiments

Generated TMBox concentrations were calculated by simulation. Higher concentrations induced more negative current at the working electrode (Figure 3).

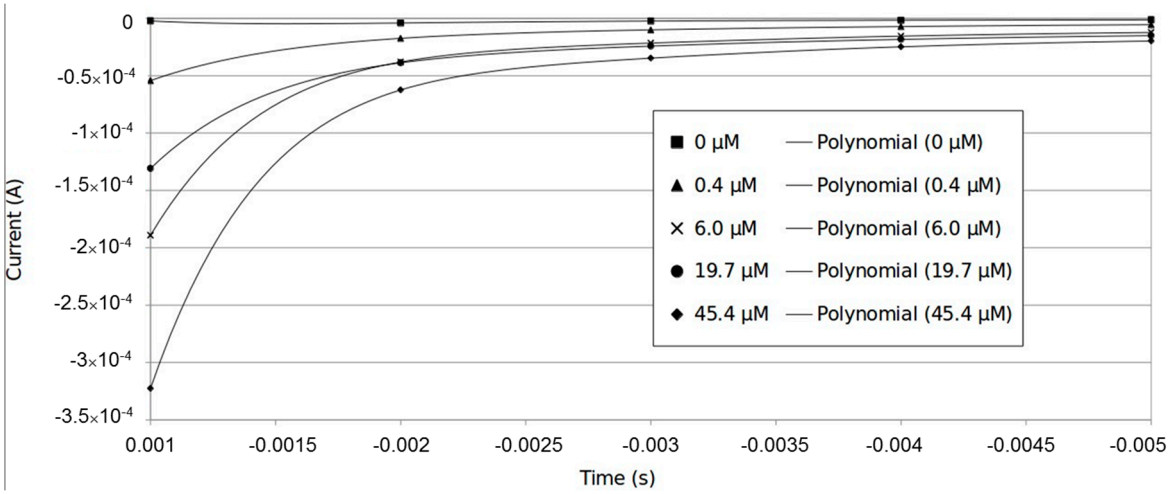


Figure 3. Experimental Results. The chronoamperometric plots show the time-dependency of the current during the reduction of TMBox at the working electrode.

According to the literature [5], current density is directly proportional to the inverse root of time, which is plotted in Figure 4. The slope (k) of the regression line depends on the initial concentration of TMBox.

In Figure 5, the slope of different TMBox concentrations (as shown in Figure 4) was plotted against the concentration of the generated TMBox after 5 min of incubation with H_2O_2. A logarithmic relationship is observed. Due to the bijectivity in the selected concentration range, one can conclude from the slope to the initial H_2O_2 concentration. The limit of detection is observed at about 0.1 µM of oxidized TMB. For comparison, a spectrophotometric measurement of the solutions after addition of the stop solution has been performed. This type of measurement offers a slightly higher limit of detection which accounts for about 0.15 µM of oxidized TMB (data not shown).

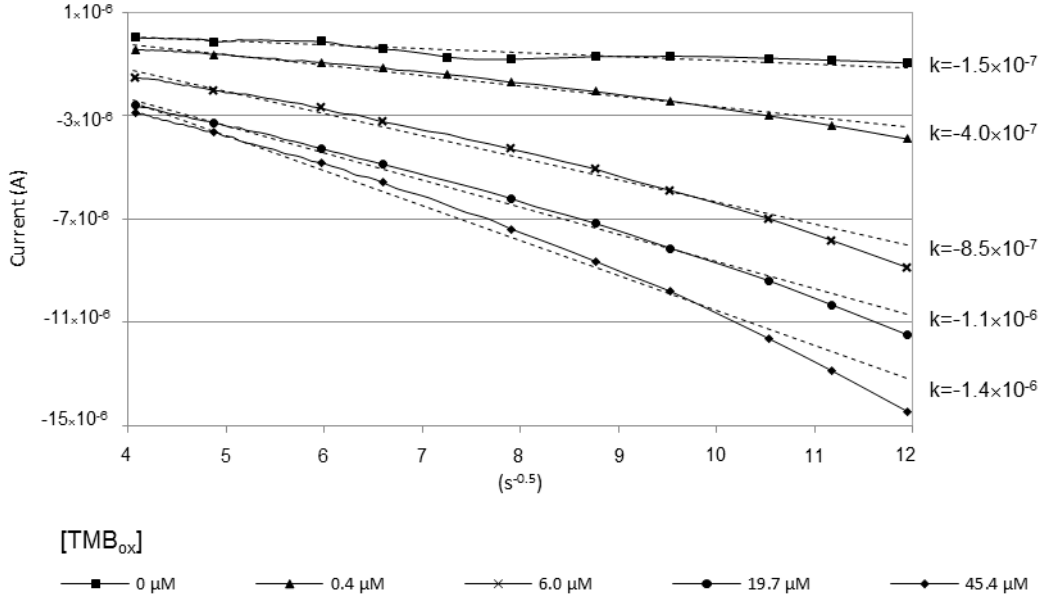


Figure 4. Experimental Results. The measured current is plotted against the inverse root of time and linear regression lines are calculated.

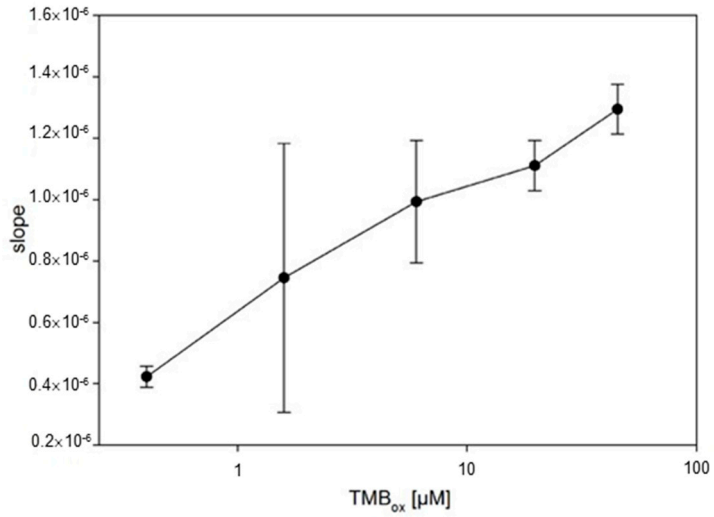


Figure 5. Concentration of oxidized TMB plotted against the slope of the regression lines (n = 2).

4. Conclusions

Chronoamperometry is a high promising instrument to detect small amounts of oxidized TMB, which can also be included into small hand-held devices for point of care application. The current setup is slightly more sensitive then photometry. Additionally, there are possibilities for further optimization such as different electrode material or sample volume. This will be regarded in the next steps of our study.

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Conflicts of Interest: The authors declare no conflict of interest. The funding agency had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Maintz, L.; Novak, N. Histamine and histamine intolerance. *Am. J. Clin. Nutr.* **2007**, *85*, 1185–1196.
2. Olivieri, A.; Tipton, K.F.; O'Sullivan, J. Characterization of the in vitro binding and inhibition kinetics of primary amine oxidase/vascular adhesion protein-1 by glucosamine. *Biochim. Biophys. Acta* **2012**, *1820*, 482–487.
3. Kergaravat, S.V.; Pividori, M.I.; Hernandez, S.R. Evaluation of seven cosubstrates in the quantification of horseradish peroxidase enzyme by square wave voltammetry. *Talanta* **2012**, *88*, 468–476.
4. Product Information Sheet. Available online: www.gwent.org/gem_data_sheets/biosensor_products/base_electrodes/be2050824d1.pdf (accessed on November 2013).
5. Bard, A.J.; Faulkner, L.R.; Leddy, J.; Zoski, C.G. *Electrochemical Methods: Fundamentals and Applications*; Wiley: New York, NY, USA, 1980.



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