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*Publication date:*
2021

*Document Version*
Publisher's PDF, also known as Version of record

# Enzymatic Histamine Biosensor Based On Prussian Blue-Modified 3D Pyrolytic Carbon Microelectrodes

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**Abstract:** In allergic reactions, mast cells and basophils release 25-65 ng/ml histamine, which is about 200-600 nM [1,2]. Here, we propose an electrochemical enzymatic biosensor, with a novel method of measurement, which can enable detection of histamine in this range. Pyrolytic carbon working electrode (WE) with 3D microstructures is fabricated by photolithography with SU-8 photoresist followed by pyrolysis[3]. Thereafter, it is modified with Prussian Blue (PB) film by electrodeposition[4]. Next, diamine oxidase (DAO) in a solution containing BSA and glutaraldehyde is cross-linked on the WE. The mechanism of histamine detection is analogous to charge/ discharge cycles of a capacitor. First, the PB on WE is electrochemically reduced to form Prussian White (PW) resulting in charge accumulation in the PW/PB layer (charging). Second, the electrode is exposed to the solution containing histamine at an open circuit while the open circuit potential (OCP) is recorded. Histamine is oxidized to imidazole acetaldehyde and hydrogen peroxide ($H_2O_2$) is generated at the electrode by DAO. Subsequently, $H_2O_2$ oxidizes PW producing PB (discharging) imposing a positive shift in OCP. The amount of the produced PB is then assessed by recording chronoamperometric current by applying a step of reducing potential (recharging). The charge is obtained by integrating the area under the current. The obtained results shows that the combination of 3D pyrolytic carbon with the described two-step electrochemical method- i) open circuit potentiometry followed by ii) chronoamperometry- can practically provide the same sensitivity that is conventionally acquired from platinum microelectrodes in flow injection systems. Currently, we are optimizing the biosensor aiming for histamine detection in human LAD2 mast cells medium.

**References**

[1] Iwaki, S., Ogasawara, M., Kurita, R., Niwa, O., Tanizawa, K., Ohashi, Y., & Maeyama, K. (2002), *Analytical Biochemistry*, 304(2), 236–243.

[2] Jackson, J. A., Riordan, H. D., Neathery, S., & Revard, C. (1998). *Journal of Orthomolecular Medicine*, 13(4), 236–240.

[3] Hemanth, S., Caviglia, C., & Keller, S. S. (2017), *Carbon*, 121, 226–234.

[4] De Mattos, I. L., Gorton, L., & Ruzgas, T. (2003), *Biosensors and Bioelectronics*, 18(2-3), 193–200.