

## Amperometric Xanthine Biosensor Based on Electrodeposition of Pt Nanoparticles on Polycyclotetrasiloxane Modified Electrode

Álvaro Boluda<sup>1</sup>, M. P. García Armada<sup>1</sup>, J. Losada<sup>1</sup>, B. Alonso<sup>2</sup>, C. M. Casado<sup>2</sup>

<sup>1</sup> Dpto. Ingeniería Química Industrial y M. A., Universidad Politécnica de Madrid, José Gutiérrez Abascal, 2, 28006 Madrid, Spain

<sup>2</sup> Dpto. Química Inorgánica, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain  
[pilar.garcia.armada@upm.es](mailto:pilar.garcia.armada@upm.es)

### Abstract.

Xanthine (3,7-dihydro-purine-2,6-dione) is present in most body tissues and fluids. It is generated from guanine by guanine deaminase and from hypoxanthine by xanthine oxidase (XOD). The determination of xanthine level in blood and tissue is essential for diagnosis and medical management of various diseases like hyperuricemia, gout, xanthinuria and renal failure. The determination of xanthine is also used in the food industries for the quality control of fish products. After the death of a fish, ATP is degraded into xanthine, which increases with storage. Thus xanthine attracts much attention as an indicator for fish freshness [1]. Xanthine oxidase is a molybdenum, iron and labile sulfur containing flavoprotein that catalyzes the oxidation of hypoxanthine to xanthine and xanthine to uric acid and plays an important role in controlling purine metabolism. In the literature, several analytical methods such as electrophoresis, high-performance liquid chromatography (HPLC), amperometric and voltammetric methods have been reported for quantitative determination of xanthine and various electrochemical xanthine biosensors based on the immobilized xanthine oxidase have been reported [2]. However these XOD based biosensors have some common drawbacks such as poor stability, reusability, slow electron transfer, and complexity of immobilization.

Several metal nanoparticles have been used as electrode modified materials in the electrochemical biosensors of xanthine [3-5] and it is demonstrated that gold and platinum nanoparticles could increase the surface area and conducive to electron transfer with strong catalytic properties [6]. In accordance to these studies, Pt nanoparticles (PtNPs) show a little better catalytic behavior in the electrochemical oxidation of the enzymatically generated H<sub>2</sub>O<sub>2</sub> than AuNPs, consequently we have used Pt nanoparticles in our biosensor.

In this work we present a novel and selective xanthine electrochemical biosensor, based on xanthine oxidase immobilized in a nanostructured electrode surface prepared with an electrodeposited cyclotetrasiloxane polymer functionalized with ferrocene units [7] (Fig. 1) used as bed for the deposition of Pt nanoparticles [8]. Figure 2 shows the SEM micrographs of both Pt wires modified with the polymer and the polymer with PtNPs in which a uniform distribution of PtNPs is showed.

Once the modified electrodes were characterized, the kinetics was studied by application of Laviron model. The  $\Delta E_p$  values remained invariable with the increasing scan rate indicating that there are no kinetic limitations [9]. The polymer and PtNPs-polymer electrodes showed excellent electrocatalytic activity toward the oxidation and reduction of hydrogen peroxide in the phosphate buffer solution (pH 7.0). The Koutecky-Levich studies with rotary electrode let us to know the kinetics of the electrooxidation of H<sub>2</sub>O<sub>2</sub> and determine the rate constants,  $k_{obs} = 43450 \text{ M}^{-1}\text{s}^{-1}$  and  $80617 \text{ M}^{-1}\text{s}^{-1}$  for the polymer and PtNPs-polymer modified electrodes respectively. These results show a good electrocatalytic behavior.

Xanthine oxidase was immobilized by covalent cross-linking [10] with BSA and glutaraldehyde, and its good electrocatalytic behavior has allowed us to develop an efficient sensor capable of measuring xanthine at potentials from 0.4 V and -0.1 V (vs. SCE). The linear relationship between the current response and the concentration of XA ranging from 0 to 3,8 mM was obtained with a detection limit of 0.24  $\mu\text{M}$  at pH 7.0, sensitivity of  $0.146 \text{ A M}^{-1} \text{ cm}^{-2}$  at 0.4 V applied potential (Vs. SCE) and higher values for increasing applied potentials. The kinetics of biosensor was also studied and an apparent Michaelis-Menten of 0.7 mM was obtained.

The effect of the interferences caused by ascorbic acid (AA), glucose and uric acid (UA) was also studied.

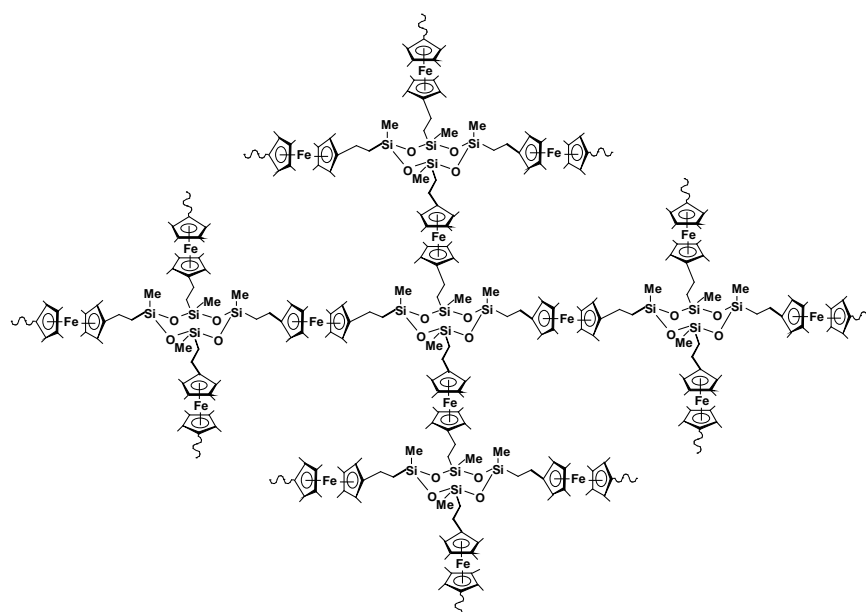
This new biosensor has been applied successfully to monitoring the meat freshness in fishes and to determine the xanthine derivative teophylline, present in antiasthmatic drugs.

### References

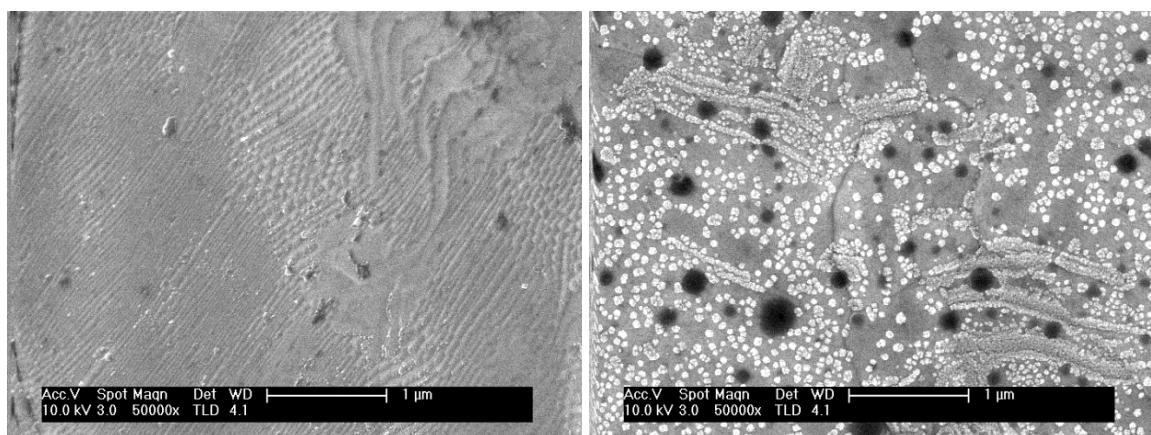
[1] Rooma Devi, Manish Thakur, C.S. Pundir, *Biosensors and Bioelectronics* **26** (2011) 3420–3426.

- [2] Rooma Devi, Sandeep Yadav, C.S. Pundir, *Colloids and Surfaces A: Physicochem. Eng. Aspects*, **394** (2012) 38–45.
- [3] Dan Shan, Yan-Na Wang, Huai-Guo Xue, Serge Cosnier, Shou-Nian Ding, *Biosensors and Bioelectronics*, **24** (2009) 3556–3561.
- [4] Rooma Devi, Sandeep Yadav, Renuka Nehra, Sujata Yadav, C.S. Pundir, *Journal of Food Engineering*, **115** (2013) 207–214.
- [5] Chandra Shekhar Pundir, Rooma Devi, *Enzyme and Microbial Technology*, **57** (2014) 55–62.
- [6] Salih Zeki Bas, Handan Gulce, Salih Yıldız, Ahmet Gulce, *Talanta*, **87** (2011) 189–196.
- [7] J. Losada, M.P. García Armada, I. Cuadrado, B. Alonso, B. González, C.M. Casado, J. Zhang, *Journal of Organometallic Chemistry*, **689** (2004) 2799–2807.
- [8] Salih Zeki Bas, Handan Gulce, Salih Yıldız, *Journal of Molecular Catalysis B: Enzymatic*, **72** (2011) 282–288.
- [9] H. D. Abruña, *Electrochemical Interfaces: Modern Techniques for in-situ Interface Characterization*. VCH Pub. (1991) Florida, U.S.A.
- [10] M. Zhou, L. Shang, B. Li, L. Huang, S. Dong, *Biosensors and Bioelectronics*, **24** (2008) 442–447.

## Figures



**Figure 1.** Cyclotetrasiloxane backbone



**Figure 2.** UHSEM micrographs of Pt wires modified with the cyclotetrasiloxane polymer (left) and PtNPs - cyclotetrasiloxane polymer (right)