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SELF-ASSEMBLED MONOLAYERS FOR URICASE ENZYME ABSORPTION IMMOBILIZATION ON SCREEN-PRINTED GOLD ELECTRODES MODIFIED.

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CONTENT

INTRODUCTION

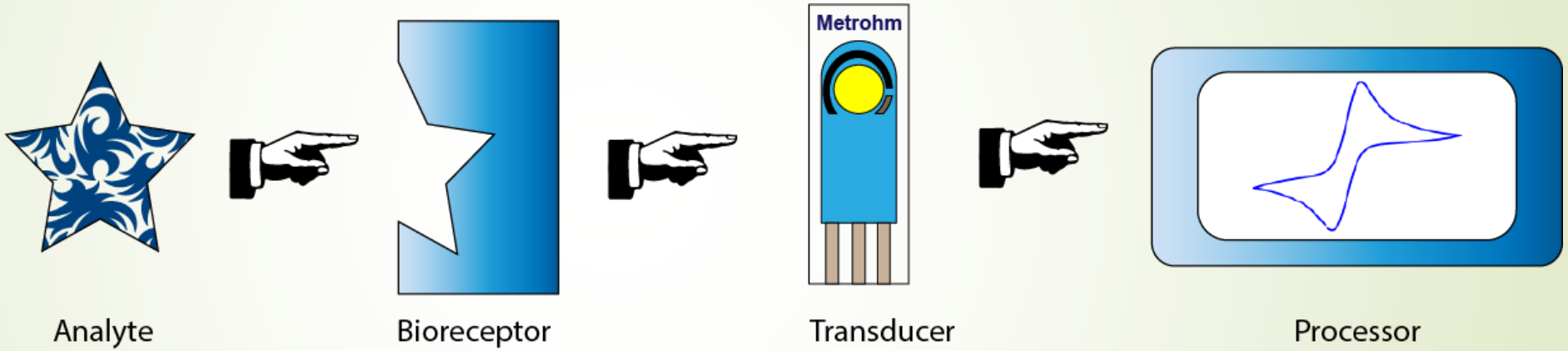


Figure 1. General operating principle of a biosensor

[1]. A. Singh et al, Biosensors, 2021
[2]. I.S. Kucherenko et al, Nanoscale Advances, 2019

UA = Uric Acid
W = Women
M = Men

INTRODUCTION

- UA is a relevant biomarker to immune system [3], due to your relationship with multiple diseases, such as diabetes mellitus, kidneys stones and arthritis [4].

- The normal UA levels in human body are:

1. **Blood serum**: 1 - 6 mg/dL (W), 1.5 – 7 mg/dL (M) [5].
2. **Saliva**: 3.35 ± 0.45 mg/dL [6].
3. **Urine:** 23.54 - 73.97 mg/dL [7].

Figure 2. Swollen and deformed fingers due to gout [8].

[3]. A. Vernerová et al, Clinical Chemistry and Laboratory Medicine, 2020.
[4]. S.H. Han et al, Scientific Reports, 2022.
[5]. J. Maiuolo et al, International Journal of Cardiology, 2016.
[6]. A. Jaiswal et al, Cureus, 2021.
[7]. S.K. Ponnaiah et al, Journal of Physical Chemistry B, 2018.
[8]. Istock web site: https://www.istockphoto.com/es

INTRODUCTION

- The self-assembled monolayers (SAM) are spontaneously formed molecular assemblies over a solid substrate [8].

- The main SAM advantages in the enzyme immobilization processes are [9]:

  - Favor a correct enzyme orientation.
  - Avoid enzyme denaturation by conductive effects.
  - Avoid the agglomeration of protein elements on the surface.

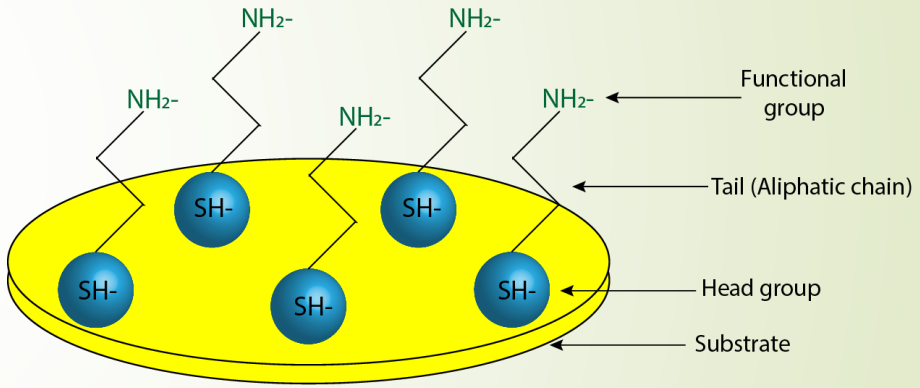


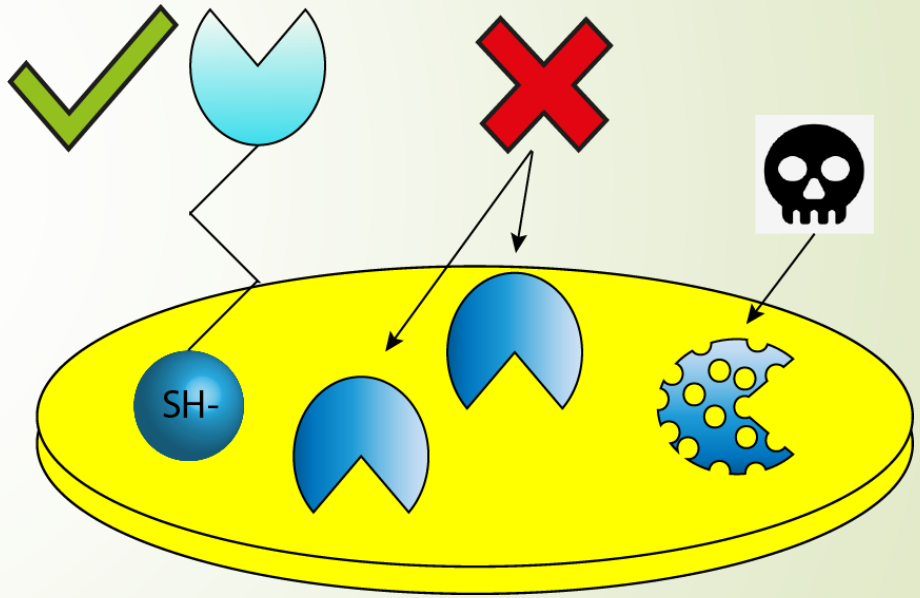
Figure 3. SAM's general structure

[9]. M. Singh et al, Journal of Materials Chemistry C, 2020.
[10]. S.R. Chinnadayyala et al, Sensors (Switzerland), 2019.

JUSTIFICATION

- The main disadvantage of enzymatic immobilization is that it itself reduces biological and catalytic activity [9,10].

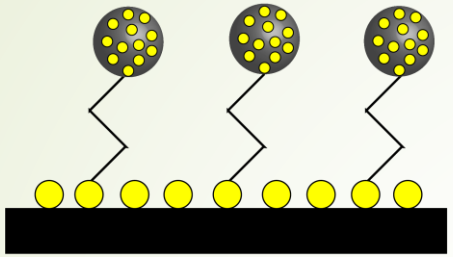
- The use of thiols for the formation of SAM, on a working surface is an attractive alternative for a enzyme safeguarding process.



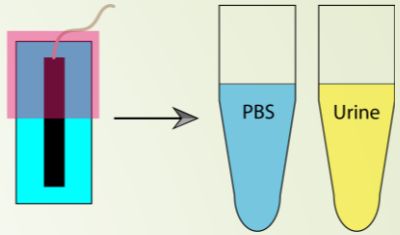
[10]. S.R. Chinnadayyala et al, Sensors (Switzerland), 2019.
[11]. H. Yang et al, Sensors and Actuators B: Chemical, 2016.

[12]. A. Arroquia et al, Materials Sciencr and Engineering C ,2020
[13]. H. wenzheng et al, IEEE Sensors Journal, 2021.
[14]. B. Kulyc et al, Carbon, 2022

STATE OF THE ART

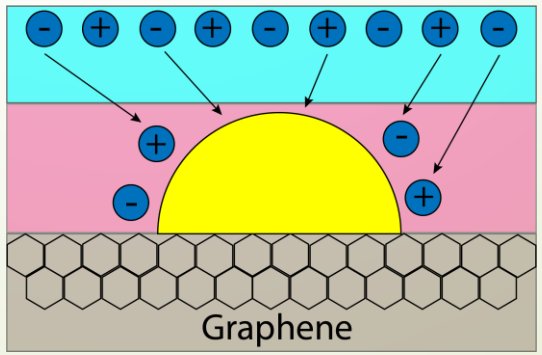


H. Wenzheng et al: Simultaneous electrochemical detection of UA and DA based on mass transfer with gold-doped graphene electrodes [13].



2020 2021 2022

A. Arroquia et al: Electrochemical detection of UA, with decorated-polydopamine nanospheres, anchored by SAM on AuNPs [12].



B. Kulyc et al: Non-enzymatic electrochemical detection of UA with LIG electrodes in PBS and dilute urine human samples [14].

AuNPs = Gold nanoparticles
DA = Dopamine
PBS =Phosphate saline buffer
LIG = Laser induced graphene

AuSPE = Gold screen-printed electrode
WE = Working electrode.
CE = Counter electrode.
RE = Reference electrode.

METODOLOGY

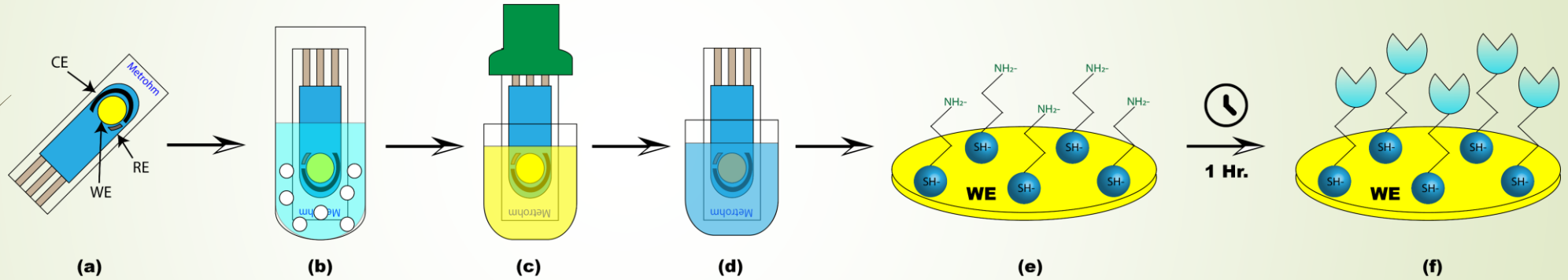
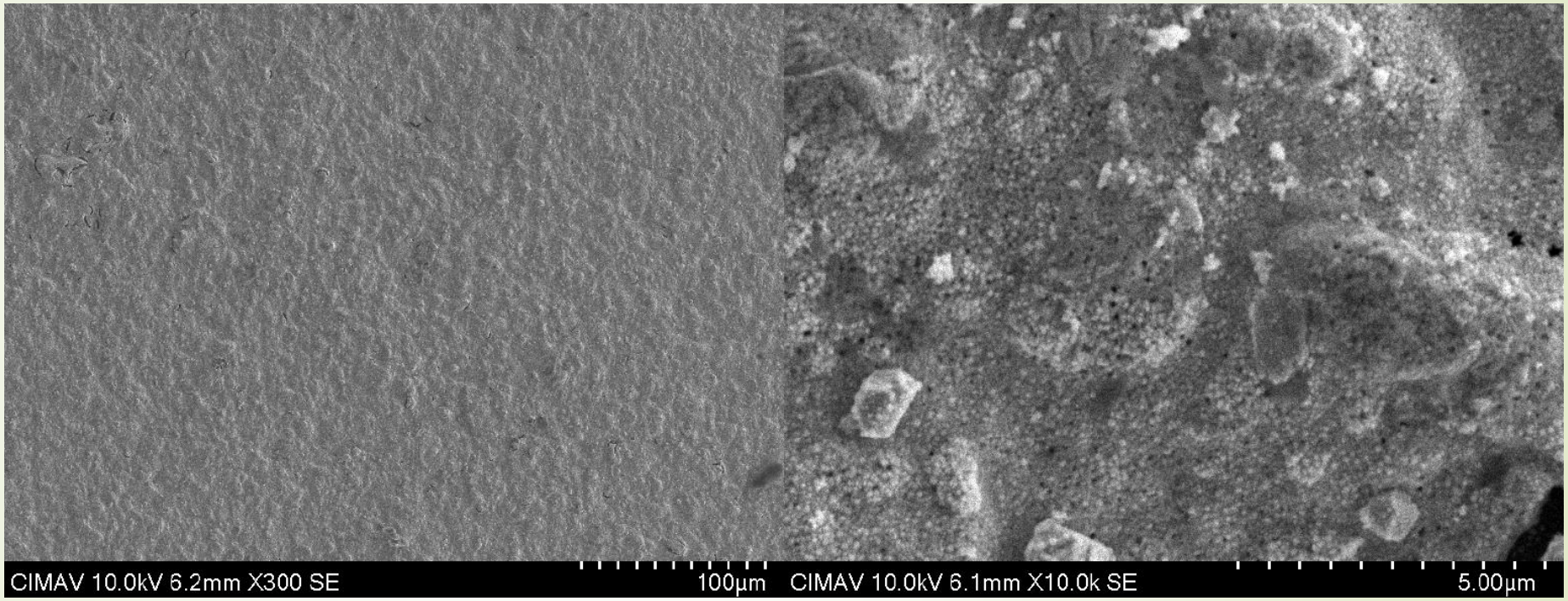


Figure 4. General methodology of the working surface modifications: (A) Bare AuSPE (B) Gold working surface activation with KOH/H_2O_2, (C) AuNPs electro-deposition by Cyclic voltammetry (CV) with $HAuCl_4$, (D) SAM formation by CYS solution for 24 hours of incubation, (E) SAM structure on working surface, and (F) Complete assembly: Au/KOH/AuNPs/SAM/Uox biosensor.

KOH = Potassium hydroxide
H_2O_2 = Hydrogen peroxide
$HAuCl_4$ = Chloroauric acid
CYS = Cysteamine
Uox = Uricase

[15]. H.D. Hernández Moreno, Centro de Investigación y Estudios Avanzados del IPN, 2021.
[16]. L.M. Fischer et al, Microelectronic Engineering, 2009.
[17]. M.S. El-Deab et al, Journal of The Electrochemical Society, 2003.
[18]. C. Leitao et al, IEEE Sensors Journal, 2021.
[19]. J, Kim et al, Biosensors & Bioelectronics, 2015.

RESULTS



(A) **(B)**

Figure 5. SEM of the AuSPE working surface: (A) View at x300 of gold working electrode morphology, and (B) View at x10000 of the gold working electrode with AuNPs electrodeposited. (Data obtained from The Center for Research in Advanced Materials (CIMAV S.C.), Chihuahua, México.)

[20]. C. Gabellini et al, ACS Nano, 2022.

$K_3[FeCN_6]$ = Potassium ferricyanide
KCl = Potassium chloride

RESULTS

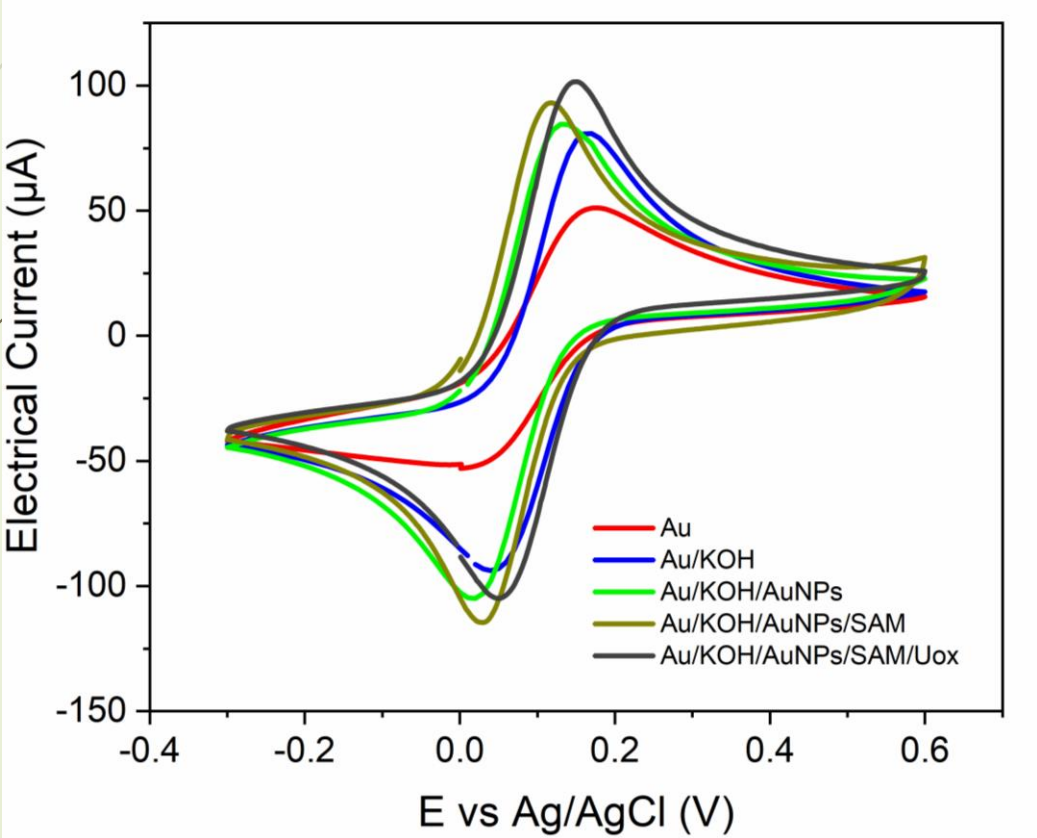


Figure. 6. Electrochemical characterization by CV in redox probe $K_3[FeCN_6]$/KCl at 5 mM/100 mM for each working surface modification stage,

Table I. Characterization results (n=6)

Surface	Oxidation electric current (µA)	ΔV (V)
Au	43.2536 ± 10.8665	0.1844 ± 0.0028
Au/KOH	79.7000 ± 4.4248	0.1366 ± 0.0024
Au/KOH/AuNPs	83.9967 ± 0.5202	0.1133 ± 0.0047
Au/KOH/AuNPs/SAM	93.8700 ± 0.9435	0.0940 ± 0.0093
Au/KOH/AuNPs/SAM/Uox	101.6000 ± 2.9561	0.0896 ± 0.0024

LOD = Limit of detection
PBS = Phosphate buffer saline

RESULTS

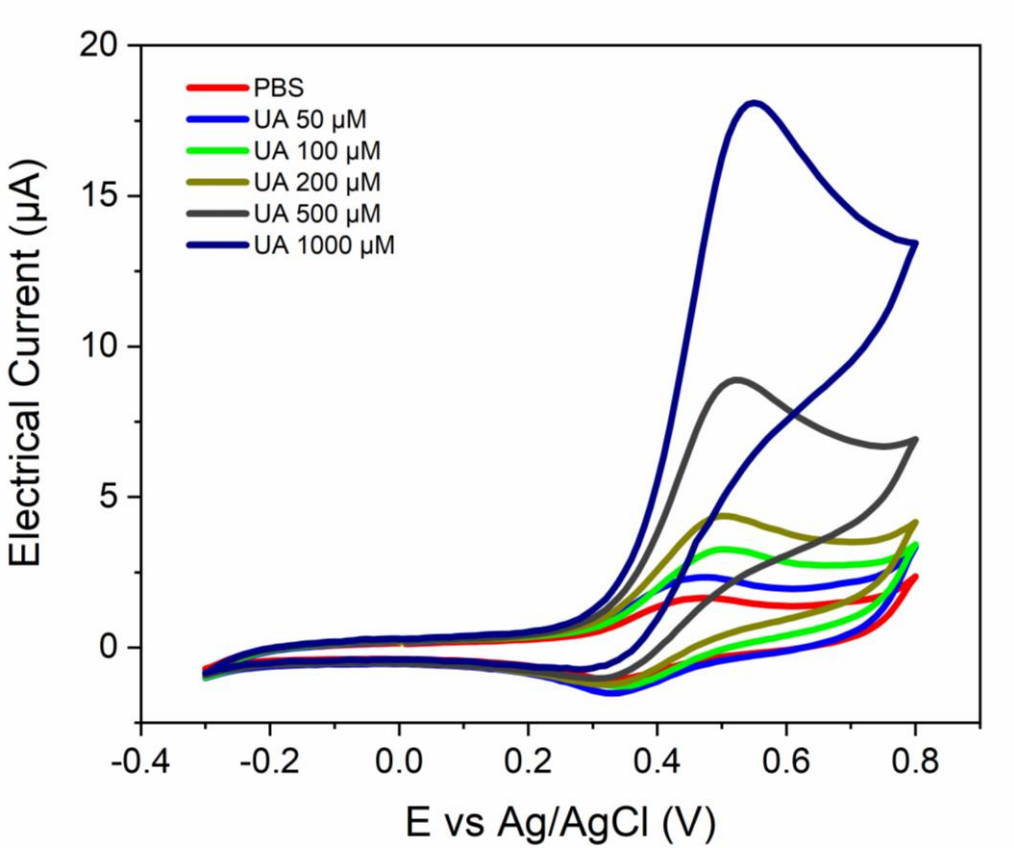


Figure 7. UA detection by CV at different concentrations.

LOD = Limit of detection
PBS = Phosphate buffer saline

RESULTS

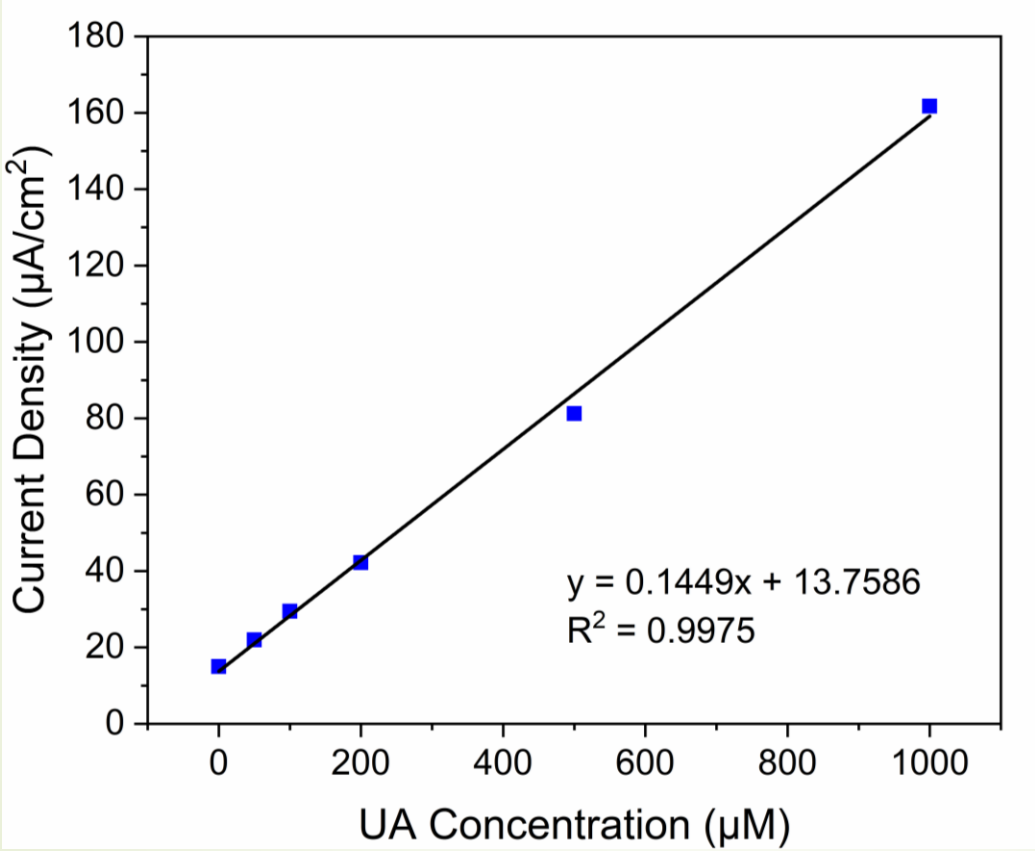


Figure 8. Linear regression of the current density depending to the UA concentration

Table II. Sensing parameters

Kinetic constant μA/(μM)cm²	0.1449
Linear range (μM)	50 – 1000
LOD (μM)	4.4969
Oxidation Potential (V)	0.5 V

RESULTS

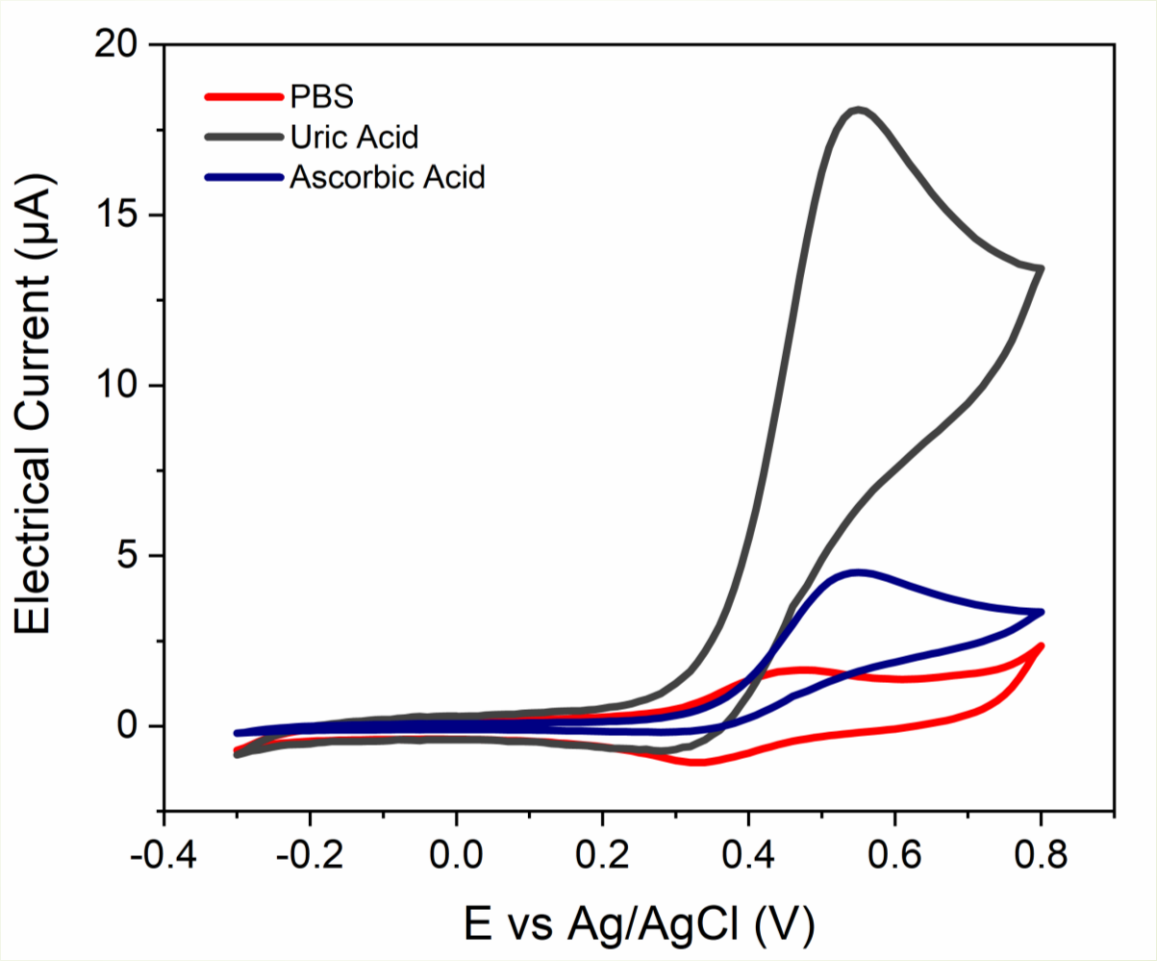


Figure. 9. Selectivity assay by CV in UA and AA solutions at 1 mM.

CONCLUSIONS

- The thiol-based SAM on AuSPE as working surface was used for physical immobilization of Uox and subsequently in the detection of UA.
- Surface modification was corroborated by SEM and CV, while UA detection was performed using CV in a range from 50 µM to 1000 µM.
- The device presented a great selectivity to UA molecules against AA molecules oxidation as an interfering analyte.
- The reported analytical results, showed our device as attractive an alternative for easy and fast UA monitoring.

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REFERENCES

1. Singh, A.; Sharma, A.; Ahmed, A.; Sundramoorthy, A.K.; Furukawa, H.; Arya, S.; Khosla, A. Recent Advances in Electrochemical Biosensors: Applications, Challenges, and Future Scope. Biosensors 2021, 11, 1–31, https://doi:10.3390/bios11090336.
2. Kucherenko, I.S.; Soldatkin, O.O.; Kucherenko, D.Y.; Soldatkina, O. V.; Dzyadevych, S. V. Advances in Nanomaterial Appli-cation in Enzyme-Based Electrochemical Biosensors: A Review. Nanoscale Adv. 2019, 1, 4560–4577, https://doi:10.1039/c9na00491b.
3. Vernerová, A., Kujovská Krčmová, L., Melichar, B., Švec, F.: Non-invasive determination of uric acid in human saliva in the diagnosis of serious disorders. Clin. Chem. Lab. Med. 59, 797–812 (2021). https://doi.org/10.1515/cclm-2020-1533.
4. Han, S.H.; Ha, Y.J.; Kang, E.H.; Shin, K.; Lee, Y.J.; Lee, G.J. Electrochemical Detection of Uric Acid in Undiluted Human Saliva Using Uricase Paper Integrated Electrodes. Sci. Rep. 2022, 12, 1–10, https://doi:10.1038/s41598-022-16176-5.

REFERENCES

5. Maiuolo, J.; Oppedisano, F.; Gratteri, S.; Muscoli, C.; Mollace, V. Regulation of Uric Acid Metabolism and Excretion. Int. J. Cardiol. 2016, 213, 8–14, https://doi:10.1016/j.ijcard.2015.08.109.
6. Jaiswal, A.; Madaan, S.; Acharya, N.; Kumar, S.; Talwar, D.; Dewani, D. Salivary Uric Acid: A Noninvasive Wonder for Clinicians? Cureus 2021, 13, https://doi:10.7759/cureus.19649.
7. Ponnaiah, S.K.; Periakaruppan, P.; Vellaichamy, B. New Electrochemical Sensor Based on a Silver-Doped Iron Oxide Nanocomposite Coupled with Polyaniline and Its Sensing Application for Picomolar-Level Detection of Uric Acid in Human Blood and Urine Samples. J. Phys. Chem. B 2018, 122, 3037–3046, https://doi:10.1021/acs.jpcb.7b11504.
8. Istock web site: https://www.istockphoto.com/es/search/2/image?phrase=Uric%20acid%20gout

REFERENCES

9. Singh, M.; Kaur, N.; Comini, E. The Role of Self-Assembled Monolayers in Electronic Devices. J. Mater. Chem. C 2020, 8, 3938–3955, https://doi:10.1039/d0tc00388c.
10. Chinnadayyala, S.R.; Park, J.; Kim, Y.H.; Choi, S.H.; Lee, S.M.; Cho, W.W.; Lee, G.Y.; Pyun, J.C.; Cho, S. Electrochemical Detection of C-reactive Protein in Human Serum Based on Self-assembled Monolayer- Modified Interdigitated Wave-shaped Electrode. Sensors (Switzerland) 2019, 19, 1–15, https://doi:10.3390/s19245560.
11. Yang H.; Zhou H.; Hao H.; Gong Q.; Nie K. Detection of Escherichia coli with a label-free impedimetric biosensor based on lectin functionalized mixed self-assembled monolayer. Sensors and Actuators B: Chemical 2016, 229, 297-304, https://doi.org/10.1016/j.snb.2015.08.034.
12. Arroquia, A.; Acosta, I.; Armada, M.P.G. Self-Assembled Gold Decorated Polydopamine Nanospheres as Electrochemical Sensor for Simultaneous Determination of Ascorbic Acid, Dopamine, Uric Acid and Tryptophan. Mater. Sci. Eng. C 2020, 109, 110602, https://doi:10.1016/j.msec.2019.110602.

REFERENCES

13. Wenzheng H.; Xiongying Y.; Tianhong C. Flexible Electrochemical Sensor With Graphene and Gold Nanoparticles to Detect Dopamine and Uric Acid. IEEE Sensors Journal 2021, 21(23), 26556-26565, https://doi.org/10.1109/JSEN.2021.3122326.
14. Kulyk, B.; Pereira, S.O.; Fernandes, A.J.S.; Fortunato, E.; Costa, F.M.; Santos, N.F. Laser-Induced Graphene from Paper for Non-Enzymatic Uric Acid Electrochemical Sensing in Urine. Carbon N. Y. 2022, 197, 253–263, https://doi:10.1016/j.carbon.2022.06.013.
15. Hernandez, H.D. Diseño y desarrollo de un biosensor para la detección de ácido úrico basado en monocapas autoorganizadas (SAMs) fabricadas sobre electrodos serigrafiados de oro, CINVESTAV-IPN 2021, Ciudad de México, México.
16. Fischer, L.M.; Tenje, M.; Heiskanen, A.R.; Masuda, N.; Castillo, J.; Bentien, A.; Émneus, J.; Jakobsen, M.H.; Boisen, A. Gold Cleaning Methods for Electrochemical Detection Applications. Microelectron. Eng. 2009, 86, 1282–1285, https://doi:10.1016/j.mee.2008.11.045.

REFERENCES

17. El-Deab, M.S.; Okajima, T.; Ohsaka, T. Electrochemical Reduction of Oxygen on Gold Nanoparticle-Electrodeposited Glassy Carbon Electrodes. J. Electrochem. Soc. 2003, 150, A851, https://doi:10.1149/1.1574806.
18. Leitao, C.; Pereira, S.O.; Alberto, N.; Lobry, M.; Loyez, M.; Costa, F.M.; Pinto, J.L.; Caucheteur, C.; Marques, C. Cortisol In-Fiber Ultrasensitive Plasmonic Immunosensing. IEEE Sens. J. 2021, 21, 3028–3034, https://doi:10.1109/JSEN.2020.3025456.
19. Kim, J.; Imani, S.; de Araujo, W.R.; Warchall, J.; Valdés-Ramírez, G.; Paixão, T.R.L.C.; Mercier, P.P.; Wang, J. Wearable Salivary Uric Acid Mouthguard Biosensor with Integrated Wireless Electronics. Biosens. Bioelectron. 2015, 74, 1061–1068, https://doi:10.1016/j.bios.2015.07.039.
20. Gabellini, C.; Şologan, M.; Pellizzoni, E.; Marson, D.; Daka, M.; Franchi, P.; Bignardi, L.; Franchi, S.; Posel, Z.; Baraldi, A.; et al. Spotting Local Environments in Self-Assembled Monolayer-Protected Gold Nanoparticles. ACS Nano 2022, 16, 20902–20914, https://doi:10.1021/acsnano.2c08467.