Journal of The Electrochemical Society, **162** (14) B360-B362 (2015)
0013-4651/2015/162(14)/B360/3/$33.00 © The Electrochemical Society

Carbon Modified Paper Based Sensors

Raluca-Ioana Stefan-van Staden,[a,b,*,z] Iuliana Moldoveanu,[a,b] Carmen Cristina Surdu-Bob,[a,c] Marius Badulescu,[c] and Jacobus Frederick van Staden[a,*]

[a]*Laboratory of Electrochemistry and PATLAB Bucharest, National Institute of Research for Electrochemistry and Condensed Matter, Bucharest, Romania*
[b]*Faculty of Applied Chemistry and Material Science, Politehnica University of Bucharest, Bucharest, Romania*
[c]*Low Temperature Plasma Laboratory, National Institute for Lasers, Plasma and Radiation Physics (NILPRP), Magurele, Romania*

Disposable sensors are essential for biomedical analysis. Therefore, paper based sensors may became a good alternative to expensive tools and methods used to date in clinical laboratories. New stochastic sensors based on carbon thin films deposited on adsorbent and glossy papers modified with nanostructured material such as 5,10,15,20-tetraphenyl-21H,23H-porphyrin were proposed. Carcinoembryonic antigen – a biomarker very often used for cancer diagnosis as well as for follow up of cancer treatment, was selected as model analyte. Qualitative and quantitative assay was performed using the new proposed sensors. The sensors can be used as disposable sensors for biomedical analyses with high reliabilities.
© 2015 The Electrochemical Society. [DOI: 10.1149/2.0831514jes] All rights reserved.

Manuscript submitted June 16, 2015; revised manuscript received October 9, 2015. Published October 21, 2015.

Paper based disposable sensors are very promising for biomedical analysis where fast, cheap and easy to use tools are requested. To date cellulose-carbon nanotube-based paper transistors, cellulose-SnO – based biosensors and pH sensors, TiO_2 – cellulose composites based sensors, GaN-cellulose composites based sensors,[1–4] inkjet-printed polyaniline modified screen-printed carbon electrodes[5] as well as electrochemical sensing in paper-based microfluidic devices[6] were proposed.

Carcinoembryonic antigen (CEA) is a tumor marker found in many types of cells associated with tumors.[7,8] It is always a need to check the whole blood of suspected cancer patients for this biomarker; also this biomarker is used to verify if the treatment selected for cancer is efficient. Therefore, in this paper CEA was selected as model analyte for the new designed paper based disposable sensor. Until now, CEA was assayed by different methods including ELISA assay,[9–11] fluorescence immunoassay (FIA),[12] radioimmunoassay,[13,14] chemiluminescence[15] and electrochemical methods.[16,17]

The purpose of this work was to develop new disposable sensors based on carbon thin films obtained using plasma deposition on glassy and adsorbent paper. This are the first disposable stochastic sensors designed. Nanostructured material such as 5,10,15,20-tetraphenyl-21H,23H-porphyrin (P) was used as modifier. The advantages of developing such sensors versus the methods already described in the literature, as well as versus the standard method practiced in the clinical laboratories (ELISA) are: these sensors are the only ones that can perform both qualitative and quantitative reliable analysis directly from whole blood, no sampling being required for such sensors; the sensors are disposable, avoiding cross-contamination; the price of the analysis is far low than any other type of analysis; it was proven that the reaction Ag-Ab used in ELISA is not specific, more interferences occurring, while the proposed sensors are selective for the assay of CEA.

Experimental

Materials and reagents.— 5,10,15,20-tetraphenyl-21H,23H-porphyrin (P) and CEA (carcinoembryonic antigen), monosodium phosphate, disodium phosphate were purchased from Aldrich (Milwaukee, USA); paraffin oil and NaN_3 were purchased from Fluka (Buchs, Switzerland). Monosodium phosphate and disodium phosphate were used for preparation of phosphate buffer 0.1 mol/L, pH = 7.4. Deionized water obtained from a Millipore Direct-Q 3 System (Molsheim, France) was used for the preparation of all solutions. All standard solutions were prepared in buffer solution pH = 7.4, with NaN_3 0.1%.

Whole blood samples were obtained from five confirmed patients from the Universitary Hospital in Bucharest (ethics committee approval nr. 11/2013). The samples were used for screening tests using the proposed disposable stochastic sensor, without any sampling.

Instrumentation.— All diagrams were recorded using a PGSTAT 12 potentiostat/galvanostat connected to a three-electrode cell, and linked to a computer via an Eco Chemie (Utretch, The Netherlands) software version 4.9. A Pt electrode and an Ag/AgCl electrode served as the counter and reference electrodes in the cell. The pH measurements were performed using a CyberScan PCD 6500 Multiparameter.

Design of the sensors.— Two types of papers were used for plasma deposition: a glossy paper (Cglossy) and adsorbant paper (Cads).

Carbon films were deposited on paper using high voltage anodic plasma in high vacuum also called Thermionic Vacuum Arc (TVA).[18,19] A graphite rod was used as precursor. A stable plasma was ignited in Carbon vapors created using electron bombardment from a hot filament. The Carbon film deposited by TVA is hydrogen free and is made of atoms and energetic carbon ions only.[20] Film purity is ensured by the specificity of the TVA plasma of not needing a buffer gas. The plasma parameters used were: 1.1 Amperes discharge current and 800 V discharge voltage. The substrates were placed away from the plasma plume at 25 cm distance from the anode. Film topography depends on that of the substrate. Figure 1 shown SEM images of the films deposited onto glossy and adsorbent paper, respectively.

A drop of a 10^{-3} mol/L 5,10,15,20-tetraphenyl-21H,23H-porphyrin (P) solution was added on the active side of the sensor, and was let to dry in a dark place for 24 h.

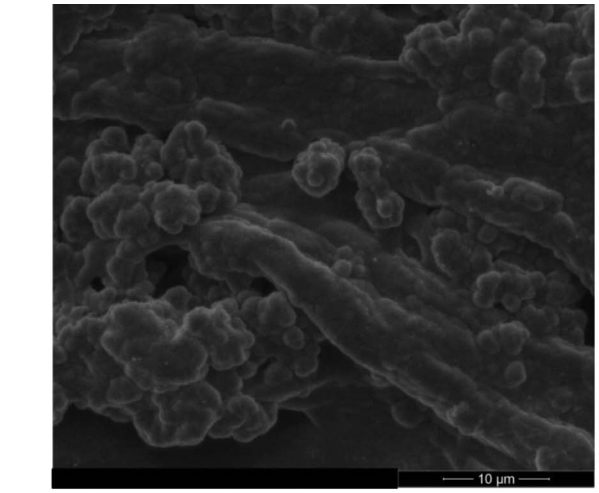
Stochastic method.— For the stochastic assay, a chronoamperometric technique was used for the measurement of t_{off} (the time when the channel/pore is losing the conductivity, and the current is dropping to zero, or very closed to zero value) and t_{on} (the time needed for interaction and redox processes inside the channel/pore) values at 125 mV. The electrodes were dipped into a cell containing solutions of antigen of different concentrations. The signature of CEA (t_{off} value) was determined in the diagrams recorded for the whole blood samples, followed by the determination of t_{on} value. Equations of calibration $1/t_{on}$ = f(Conc.) are determined using statistics. The unknown concentrations of the CEA in whole blood samples were determined from the equations of calibration.

Results and Discussion

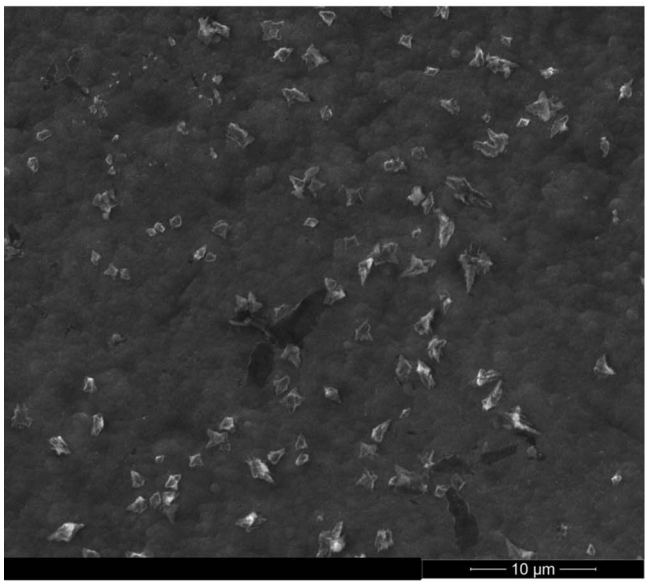
Response characteristics of the sensors used for screening of CEA in whole blood samples.— The response of the sensor is based on

*Electrochemical Society Active Member
[z]E-mail: ralucavanstaden@gmail.com

Downloaded on 2015-10-23 to IP 95.76.160.144 address. Redistribution subject to ECS terms of use (see ecsdl.org/site/terms_use) unless CC License in place (see abstract).



(a)



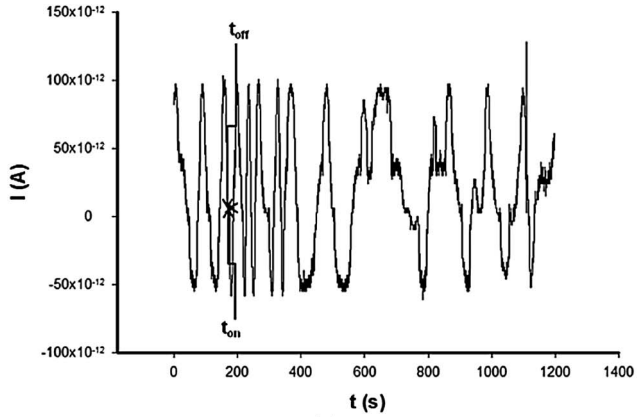
(b)

Figure 1. SEM images of (a) Cads and (b) Cglossy sensors.

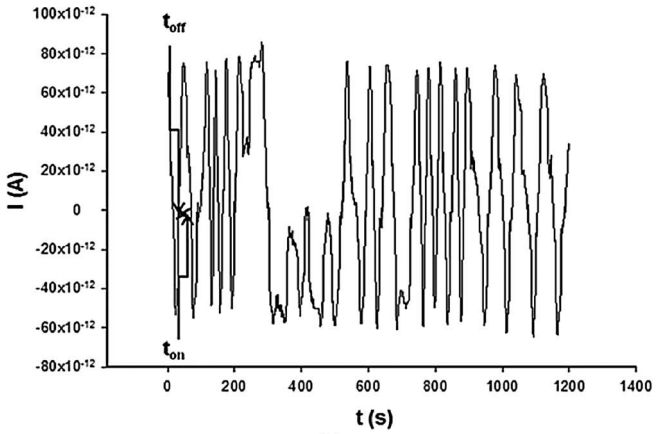
channel conductivity. Porphyrins are forming molecular aggregates with natural channels making them good candidates for stochastic sensing. Chronoamperometry is used to perform all measurements, at 125 mV vs Ag/AgCl. On the first stage, the CEA is blocking the channel, the intensity of current is dropping to 0 (the time when the current value is 0 is called t_{off} and is giving the signature of CEA). On the second stage, binding and electrochemical processes are taking place – this stage being characterized by the value of t_{off} (Figure 2).

The main advantage of stochastic sensors is that they can be used for the qualitative as well as quantitative analysis of CEA based on signature of the analyte (t_{off} values). The signature of the analyte is only depending on the size, geometry, and velocity of unfolding and migrating through channel of CEA, and not on the nature and complexity of the matrix (Table I). Therefore, a fast screening test can be performed using these sensors.

For quantification of CEA, t_{on} values are read and used. t_{on} values served also to assess the response characteristics of the sensor in stochastic sensing, as well as for the quantitative assessment of CEA in biological fluids. The best sensitivity, and limit of quantification (1.6 pg/mL) was given by the sensor based on Cads modified with P.



(a)



(b)

Figure 2. Specific diagrams obtained at screening of whole blood for CEA, using: (a) P/Cads; and (b) P/Cglossy.

A good reliability of the measurements was obtained when different disposable sensors were used for the measurements, RSD (%) values were lower than 0.1%.

Analytical applications - Screening of CEA in whole blood samples.— Qualitative and quantitative analysis of CEA was performed using the new disposable stochastic sensors. The identification of CEA in the whole blood samples was done in the diagrams obtained for whole blood samples (Fig. 2) accordingly with the signature of CEA (Table I).

Quantitative assay of CEA (Table II) was performed accordingly with the stochastic mode described above. A good correlation was obtained between the results given using both types of disposable sensors. This is also given by the pair-t test performed at 99.00%

Table I. Response characteristics of the disposable sensors for the assay of CEA.

Stochastic sensors based on	Signature, toff (s)	Linear concentration range[a]	Sensitivity[b]	Limit of quantification[c]
P/Cads	3.4	1.6×10^{-9}–1.6×10^{-7}	5.34×10^{5}	1.6
P/Cglossy	3	1.6×10^{-7}–1.6×10^{-4}	7.99×10^{1}	160

[a]mg/mL;
[b]s mg/mL;
[c]pg/mL

Downloaded on 2015-10-23 to IP 95.76.160.144 address. Redistribution subject to ECS terms of use (see ecsdl.org/site/terms_use) unless CC License in place (see abstract).

Table II. Determination of CEA in whole blood samples.

	CEA, ng/mL		
	Microsensor based on		
Sample no	P/Cads	P/Cglossy	Paired t-test
1	1.50 ± 0.04	2.15 ± 0.03	2.13
2	2.30 ± 0.03	2.20 ± 0.05	0.76
3	1.65 ± 0.03	1.25 ± 0.04	1.05
4	1.70 ± 0.02	1.57 ± 0.05	0.96
5	1.20 ± 0.02	1.70 ± 0.04	0.54

confidence level, all values being lower than the theoretical value 4.032.

Conclusions

New disposable stochastic sensors based on carbon thin films modified with 5,10,15,20-tetraphenyl-21H,23H-porphyrin (P) were made and used for assay of carcinoembryonic antigen in whole blood samples. The method is highly reliable, and very sensitive, being able to determine CEA in whole blood samples in concentration as small as 1.6 pg/mL.

Acknowledgments

This work was supported by PNII Program Partnership 2014–2016, MULTIMODESCREEN, Contract nr. 22/2014, PNII Ideas project number PN-II-ID-PCE-2011-3-0953 and by the Sectorial Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and the Romanian Government under the contract number POSDRU/159/1.5/S/137390/.

References

1. J. H. Kim, S. Mun, H. U. Ko, G. Y. Yun, and J. Kim, *Nanotechnol*, **25**, 7 (2014).
2. M. Kavruk, V. C. Ozalp, and H. A. Oktem, *J. Anal. Meth. Chem.*, 8 (2013).
3. K. F. Lei and S. I. Yang, *J. Nanosci. Nanotechnol.*, **13**, 6917 (2013).
4. D. D. Liana, B. Raguse, J. J. Gooding, and E. Chow, *Sensors*, **12**, 11505 (2012).
5. W. Kit-Anan, A. Olarnwanich, C. Sriprachuabwong, C. Karuwan, A. Tuantranont, A. Wisitsoraat, W. Srituravanich, and A. Pimpin, *J. Electroanal. Chem.*, **685**, 72 (2012).
6. Z. Nie, C. A. Nijhuis, J. Gong, X. Chen, A. Kumachev, A. W. Martinez, M. Narovlyansky, and G. M. Whitesides, *Lab Chip*, **10**, 477 (2010).
7. M. J. Duffy, A. van Dalen, C. Haglund, L. Hansson, R. Klapdor, R. Lamerz, O. Nilsson, C. Sturgeon, and O. Topolcan, *Eur. J. Cancer*, **39**, 718 (2003).
8. K. N. Sugahara, T. Teesalu, P. P. Karmali, V. R. Kotamraju, L. Agemy, D. R. Greenwald, and E. Ruoslahti, *Science*, **21**, 1031 (2010).
9. T. C. Pina, I. T. Zapata, F. C. Hernandez, J. B. Lopez, P. P. Paricio, and P. M. Hernandez, *Clinica Chimica Acta*, **305**, 27 (2001).
10. H. Kitagawa, E. Ohkouchi, A. Fukuda, K. Imai, and A. Yachi, *Jpn. J. Cancer Res.*, **77**, 922 (1986).
11. Y. Kumada, Y. Ohigashi, Y. Emori, K. Imamura, Y. Omura, and M. Kishimoto, *J Immunol Meth*, **385**, 15 (2012).
12. K. Barbee, A. Hsiao, E. Roller, and X. Huang, *Lab on a Chip*, **10**, 3084 (2010).
13. M. A. Virji, D. W. Mercer, and R. B. Herberman, *Cancer J. Clin.*, **38**, 104 (1988).
14. J. M. MacSween, N. L. Warner, A. D. Bankhurst, and I. R. Mackay, *Br. J. Cancer*, **26**, 356 (1972).
15. W. Dungchai, W. Siangproh, J. M. Lin, O. Chailapakul, S. Lin, and X. Ying, *Anal. Bioanal. Chem.*, **387**, 1965 (2007).
16. X. Sun and Z. Ma, *Anal. Chim. Acta.*, **780**, 95 (2013).
17. C. Chu, M. Li, S. Ge, L. Ge, J. Yu, M. Yan, X. Song, L. Li, B. Han, and J. Li, *Biosens. Bioelectron.*, **47**, 68 (2013).
18. C. Surdu-Bob, I. Mustata, and C. Iacob, *J. Optoelectron. Adv. Mat.*, **9**, 2932 (2007).
19. C. Surdu-Bob, G. Musa, V. Buck, and I. Mustata O. Filipov and A. Poukhovoi, *J. Optoelectron. Adv. Mat.*, **9**, 2657 (2007).
20. C. Surdu-Bob, R. Vladoiu, M. Badulescu, and G. Musa, *Diamond Rel. Mat.*, **17**, 1625 (2008).

Downloaded on 2015-10-23 to IP 95.76.160.144 address. Redistribution subject to ECS terms of use (see ecsdl.org/site/terms_use) unless CC License in place (see abstract).