ANALYSIS OF CARCINOEMBRYONIC ANTIGEN USING POTENTIOMETRIC SENSORS

Ionela Raluca COMNEA-STANCU[1], Raluca-Ioana STEFAN-VAN STADEN[2], Jacobus Frederick VAN STADEN[3], Camelia STANCIU-GAVAN[4]

Potentiometric sensors based on graphite and graphene pastes modified with 5,10,15,20-tetraphenyl-21H,23H porphyryn (P) were proposed for the assay of carcinoembryonic antigen (CEA). The limits of quantification for carcynoembrionic antigen were 1,6x10-11 mg/mL using the sensor based on P/Graphite and 1,6x10-6 mg/mL using the sensor based on P/Graphene. The sensors were applied for the analysis of carcinoembryonic antigen in whole blood samples.

Keywords: potentiometric sensors, carcinoembryonic antigen, graphite, graphene

1. Introduction

Carcinoembryonic antigen (**CEA**) is an oncofetal protein attached to epithelial-cell apical membrane via its c-terminal glycosylphosphatidylinositol anchor, a member of the immunoglobulin superfamily of cell adhesion molecules (IgCAMs) [1]. It weights approximately 200 kDa [2], is belonging to a group of substances known as the tumor-associated antigens (TAA), and is one of the most specific carcinoembryonic protein found in many types of cells associated with tumors [3,4].

According with the literature the level of carcinoembryonic antigen (**CEA**) is highly elevated in neoplastic diseases of the colon, breast, lung, prostate,

[1] PhD student, Department of Analytical Chemistry and Environmental Engineering, Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest, Laboratory of Electrochemistry and PATLAB Bucharest, National Institute of Research for Electrochemistry and Condensed Matter, Bucharest, Romania, e-mail: comnea.ionela@yahoo.com

[2] Prof. dr. habil, Department of Analytical Chemistry and Environmental Engineering, Faculty of Applied Chemistry and Materials Science, University POLITEHNICA of Bucharest; researcher, Laboratory of Electrochemistry and PATLAB Bucharest, National Institute of Research for Electrochemistry and Condensed Matter, Bucharest, Romania e-mail: ralucavanstaden@gmail.com

[3] Researcher, PhD, Laboratory of Electrochemistry and PATLAB Bucharest, National Institute of Research for Electrochemistry and Condensed Matter, Splaiul Independentei No.202, Bucharest, Romania e-mail: koosvanstaden2012@yahoo.com

[4] MD, Department of Surgery 4, University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania, e-mail: camelia_gavan@yahoo.com

bladder, pancreatic, stomach and gynecological malignancies, in all cases exceeding 5ng/mL [5,6]. Because the level of **CEA** in human serum and other biofluids has a balanced relationship with the progress of cancer, it has been extensively studied as a tumor marker for clinical monitoring, valuing clinical therapeutic effect, and predicting cancer recurrence and metastasis [4].

Until now, **CEA** has been widely investigated with different methods, but the most commonly used for the detection of this biomarker is ELISA (enzyme-linked immunosorbent assay). This method is based on the principle of immunoassay with enhanced detection of enzymes for biomolecules in the field of life science. It is a sensitive and useful assay for serum samples, at the same time very laborious and time consuming [7-10].

Tsai et al, tried to improve the efficiency of ELISA by integrating the sandwich immunoassay with functional magnetic and fluorescent nanoparticles in magnetic separators. They achieved a lower determination limit and wider linear range than ELISA, and also they reduced the time of analysis to one third of ELISA [11].

Quantitative real-time polymerase chain reaction (qRT-PCR) is being utilized for detecting tumor markers which are supposed to be expressed only in tumor cells and not in cells from surrounding tissue. qRT-PCR assay showed high sensitivity and suitability for the detection of **CEA** in gastric cancer patients, and it may offer a promising tool for the early detection of micrometastatic tumor cells in gastric cancer patients [12].

Another recent molecular biological protocol using the realtime quantitative reverse transcription polymerase chain reaction (RQ-PCR) targeting CEA appears to be an attractive method with high objectivity [13,14].

In the recent years, researchers have focused on the development, preparation and application of nanocomposites materials, like horseradish peroxidase-anti-CEA-4-aminothiophenol-thionine-gold nanoparticles [15], PTCA/Au/GS (3, 4, 9, 10-perylenete-tracarboxylic dianhydride (PTCA)/Au/graphene sheets) [16], CdS/DNA and PbS/DNA nanochains [17], bimetallic AuPt nanochains [18], $[Ag–Ag_2O]/SiO_2$ nanocomposite material [19], etc. for the construction of sensitive immunosensors for **CEA** detection.

Also, sensors based on AlN-film bulk acoustic resonator (FBAR) has become one of the most promising candidates for biosensors, because the sensor can obtain a much higher acoustic velocity of 12,350 m/s than the previous quartz crystal microbalance (QCM), and surface acoustic wave (SAW) sensors. AlN-based FBAR sensors can be manufactured with low cost, small size, and the ability to be integrated with monolithic microwave integrated circuits (MMICs) [20,21].

Han et al, succeded to fabricate a simple, label, free, and ultrasensitive amperometric immunosensor based on GO–Thi–Au (graphene oxide-thionine–

gold) nanocomposites for carcinoembryonic antigen (**CEA**) detection. The immunosensor exhibited good selectivity and stability with an ultra low detection limit of 0.05 fg/mL, and a linear concentration range from 0.1 fg/mL to $1x10^9$ fg /mL, and was used to detect the **CEA** concentration in human serum samples [22].

The aim of this work was to perform the analysis of carcinoembryonic antigen using a potentiometric sensor. We proposed two sensors based on carbon matrices modified with 5,10,15,20-tetraphenyl-21H,23H-porphyrin.

2. Experimental

2.1. Reagents and materials

Carcinoembryonic antigen (**CEA**), graphene powder, graphite powder and the 5,10,15,20-tetraphenyl-21H,23H-porphyrin (P), monosodium phosphate and disodium phosphate were purchased from Sigma Aldrich (Milwaukee, USA), paraffin oil and NaN_3 was 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% in a ratio water:buffer solution 1:1 (v/v). Serial dilution technique was used for the preparation of standard solutions of different concentrations. All solutions were fresh prepared before measurements.

2.2. Apparatus

An Ivium potentiostat/galvanostat was used for all potentiometric measurements. Ag/AgCl (0.1 mol/L KCl) electrode served as reference electrode in the cell.

2.3. Sensors design

Modified graphene and graphite pastes were prepared as follows: the powder of each material was mixed with paraffin oil to form a paste. 50 µL from the 10^{-3} mol/L solution of the electrochemical active compound (5,10,15,20-tetraphenyl-21H,23H-porphyrin, dissolved in tetrahydrofuran-THF) were added to each 100 mg of paste. The modified paste was placed into a plastic tube with a diameter of approximately 250 µm. Electric contact was obtained by inserting an Ag wire into the modified paste. The surface of the sensor was wetted with deionized water and polished with alumina paper (polishing strips 30144-001, Orion) before using. When not in use, the sensors were stored in a dry state at room temperature.

2.4. Recommended procedure

Direct potentiometry was used for the measurements of the potential of each standard solution ($1.6x10^{-11}$ – $1.6x10^{-2}$ mg/mL). The range of concentration was selected to cover both early detected patients as well as patients in late stages. The electrodes were placed in stirred standard solution while the potential was recorded, and graphs of E(mV) versus –log conc **CEA** were plotted. The unknown concentrations were determined from the calibration graphs (Fig. 1).

2.5. Sample preparation for whole blood

Whole blood samples were obtained from the Universitary Hospital in Bucharest (Ethics committee approval nr 11/2013) from 5 different patients diagnosed with different tumors, with concentrations between 2 and 10ng/mL. These samples were used for the assay of **CEA** without any preparation. The apparatus cell was filled with the whole blood and the potential developed was measured. The unknown concentration was determined from the calibration graphs as described in the direct potentiometry method.

3. Results and discussion

3.1. The response characteristics of potentiometric sensors

The sensors based on graphite and graphene pastes modified with 5,10,15,20-tetraphenyl-21H,23H-porphyrin (P) were tested using direct potentiometric method for the analysis of **CEA**. The mechanism of potential development was based on the interaction between CEA and porphyrin at the membrane-solution interface. Both sensors showed linear and near-Nernstian response, so that they can be used for the analysis of **CEA**.

Table 1 shows the response characteristics of the sensors used for the assay of **CEA**. The sensor based on graphite paste modified with 5,10,15,20-tetraphenyl-21H,23H-porphyrin (P) can be used for the assay of **CEA** in the linear concentration range $1.6x10^{-11}$–$1.6x10^{-8}$ mg/mL and the sensor based on graphene paste modified with 5,10,15,20-tetraphenyl-21H,23H-porphyrin (P) in the linear concentration range $1.6x10^{-6}$–$1.6x10^{-3}$ mg/mL. The differences between the linear concehtration ranges are connectec with the type of matrix used for the sensor designed, as well as with the conductivity of the matrix. The time of analysis was 5 minutes, because the response time for both sensors on the linear range of concentration was 4.5min.

The lowest limit of quantification was exhibited by the sensor based on graphite paste modified with 5,10,15,20-tetraphenyl-21H,23H-porphyrin (P) (Table 1). While the sensor based on P/graphite cover the **CEA** values for early detection of cancer, the sensor based on P/graphene cover the **CEA** values for patients on later stages.

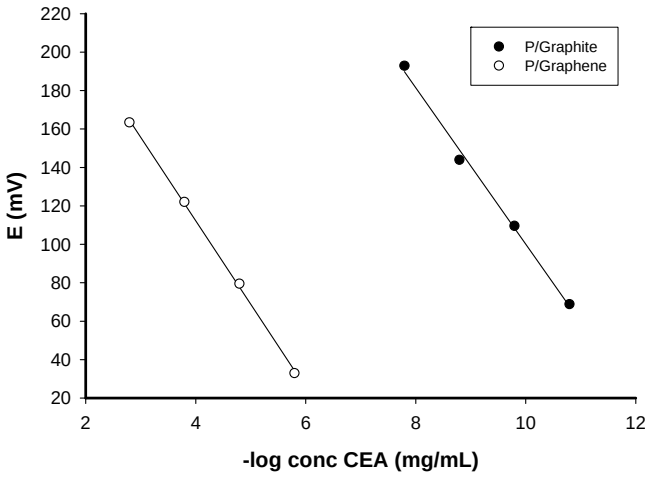

Fig. 1. Calibration graphs obtained for CEA using the sensors based on P/Graphite and P/Graphene

(Table 1)

Response characteristics of potentiometric sensors for the assay of CEA

Potentiometric sensors based on	E^0 (mV)	Slope (mV/decade of concentration	Working concentration range (mg/mL)	Limit of quantification (mg/mL)	Limit of detection (mg/mL)
P/Graphite	475.31	-37.55	$1.6x10^{-11}$-$1.6x10^{-8}$	$1.6x10^{-11}$	$2.77\ x10^{-12}$
P/Graphene	285.88	-43.39	$1.6x10^{-6}$-$1.6x10^{-3}$	$1.6x10^{-6}$	$1.68\ x10^{-7}$

3.2. Analytical applications

The response characteristics (linear concentration rangem limits of determination, sensitivity, selectivity) show that the sensors can be used for the assay of **CEA** in biological fluids. Therefore, the proposed sensors, were used for the analysis of **CEA** in whole blood samples.

Five blood samples were provided from the hospital, and used as collected, for the assay of **CEA** using the designed sensors. Recovery tests of **CEA** in whole blood samples were performed by spiking the whole blood samples with known concentrations of **CEA**. Recordings of potentials were done for the original and spiked solution. The final concentrations were selected to fit into the

linear concentration range of each sensor. The results of the recovery tests are shown in Table 2.

(Table 2)

Recovery of CEA in whole blood samples

Sample no.	***% Recovery***	
	P-Graphite	***P-Graphene***
1	*89.10*	*90.73*
2	*101.20*	*92.03*
3	*100.09*	*94.63*
4	*101.03*	*92.40*
5	*100.10*	*93.72*

*N=3

Accordingly with the values shown by the recovery test the sensor based on P/Graphene is the best for the assay of **CEA** in whole blood samples. The sensor based on P/graphite detected **CEA** at a lower concentration, and therefore the recovery values are not enough satisfactory.

4. Conclusions

Two potentiometric sensors based on graphite paste modified with 5,10,15,20-tetraphenyl-21H,23H-porphyrin (P), and graphene paste modified with 5,10,15,20-tetraphenyl-21H,23H-porphyrin (P) were used for the assay of **CEA** from whole blood samples.

The proposed sensors presented good and reliable response characteristics for the assay of carcinoembryonic antigen. Only the microsensor based on P-Graphene was able to assay carcinoembryonic antigen in whole blood samples, with acceptable accuracy.

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R E F E R E N C E S

[1] *O. Arrieta, D. Saavedra-Perez, R. Kuri, A. Aviles-Salas, L.Martinez, D. Mendoza-Posada, P. Castillo, A. Astorga, E. Guzman, J. De la Garza,* "Brain metastasis development and poor survival associated with carcinoembryonic antigen (CEA) level in advanced non-small cell lung cancer: a prospective analysis", BMC Cancer, **vol. 9**, 2009, pp. 119-127.

[2] *T. Špringer, J. Homola,* "Biofunctionalized gold nanoparticles for SPR-biosensor-based detection of CEA in blood plasma", Anal Bioanal Chem **vol. 404**, 2012, pp. 2869–2875.

[3] *W.Limbut, P. Kanatharana, B.Mattiasson, P. Asawatreratanakul, P. Thavarungkul,* "A reusable capacitive immunosensor for carcinoembryonic antigen (CEA) detection using thiourea modified gold electrode", Analytica Chimica Acta, **vol. 561**, 2006, pp. 55–61.

[4] *Z. Chen, Y. Lei, Z. Liang, F. Li, L. Liu, C.Li, F. Chen,* "A highly sensitive label-free resonance light scattering assay of carcinoembryonic antigen based on immune complexes", Analytica Chimica Acta, **vol. 747**, 2012, pp. 99– 105.

[5] *F. Naghibalhossaini, P. Ebadi,* "Evidence for CEA release from human colon cancer cells by an endogenous GPI-PLD enzyme" Cancer Letters, **vol. 234**, 2006, pp. 158–167.

[6] *C. Xu, Y. Sun, Y. Wanga, Y. Yan, Z. Shi, L. Chen, H.Lin, S. Lu, M. Zhu, C. Su, Z. Li,* "CEA promoter-regulated oncolytic adenovirus-mediated Hsp70 expression in immune gene therapy for pancreatic cancer", Cancer Letters, **vol. 319**, 2012, pp. 154–163.

[7] *X. Yang, D. Wang, Z. Yang, Y.Qing, Z. Zhang, G.Wang, Z. Yang, Z. Wang,* "CEA is an Independent Prognostic Indicator that is Associated with Reduced Survival and Liver Metastases in SCLC", Cell Biochem Biophys, **vol. 59**, 2011, pp. 113–119.

[8] *J. M. Flores-Fernández, E. J. Herrera-López, F. Sánchez-Llamas, A. Rojas-Calvillo, P. A.Cabrera-Galeana, G. Leal-Pacheco, M. G. González-Palomar, R. Femat, M. Martínez Velázquez,* "Development of an optimized multi-biomarker panel for the detection of lung cancer based on principal component analysis and artificial neural network modeling", Expert Systems with Applications, **vol. 39**, 2012, pp. 10851–10856.

[9] *I. Ghosh, D.Bhattacharjee, A. K. Das, G. Chakrabarti, A. Dasgupta, S. K. Dey,* "Diagnostic Role of Tumour Markers CEA, CA15-3, CA19-9 and CA125 in Lung Cancer", Ind J Clin Biochem, **vol. 28**, 2013, pp. 24–29.

[10] *H. He, G. Chen, L. Zhou, Y. Liu,* "A joint detection of CEA and CA-50 levels in saliva and serum of patients with tumors in oral region and salivary gland", J Cancer Res Clin Oncol, **vol 135**, 2009, pp. 1315–1321.

[11] *H. Y. Tsai,C. Y. Chang, Y. C. Li, W. C. Chu, K. Viswanathan, C. Bor Fuh,* "Detection of carcinoembryonic antigen using functional magnetic and fluorescent nanoparticles in magnetic separators", J Nanopart Res, **vol. 13,**2011, pp. 2461–2467.

[12] *L. Dardaei, R. Shahsavani, A. Ghavamzadeh, M. Behmanesh, E. Aslankoohi, K. Alimoghaddam, S. H. Ghaffari,* "The detection of disseminated tumor cells in bone marrow and peripheral blood of gastric cancer patients by multimarker (CEA, CK20, TFF1 and MUC2) quantitative real-time PCR", Clinical Biochemistry, **vol. 44**, 2011, pp. 325–330.

[13] *Y. Fujita, M. Terashima, Y. Hoshino, S. Ohtani, S. Kashimura, N. Kanzaki, F. Osuka, M. Kogure, M. Gotoh,* "Detection of cancer cells disseminated in bone marrow using real-time quantitative RT-PCR of CEA, CK19, and CK20 mRNA in patients with gastric cancer", Gastric Cancer, **vol. 9**, 2006, pp. 308–314.

[14] *G. Wang, Z. Wang, X. Liu, Z. Tang, Y. Liu, X. Li, X. Zhou, H. Gong,* "Detection of CEA mRNA in patients with non-small cell lung cancer and it's significance", Chinese-German Journal of Clinical Oncology, **vol. 7**, 2008, pp. 620–622.

[15] *W. Jiang, R. Yuan, Y. Chai, L. Mao, H. Su,* "A novel electrochemical immunoassay based on diazotization-coupled functionalized bioconjugates as trace labels for ultrasensitive detection of carcinoembryonic antigen", Biosensors and Bioelectronics, **vol. 26**, 2011, pp. 2786–2790.

[16] *Q. Zhu, Y. Chai, R.Yuan, Y. Zhuo, J. Han, Y. Li, N. Liao,* "Amperometric immunosensor for simultaneous detection of three analytes in one interface using dual functionalized graphene

sheets integrated with redox-probes as tracer matrixes", Biosensors and Bioelectronics, **vol. 43**, 2013, pp. 440–445.

[17] *F. Y. Kong, B. Y. Xu, J. J. Xu, H. Y. Chen*, "Simultaneous electrochemical immunoassay using CdS/DNA and PbS/DNA nanochains as labels", Biosensors and Bioelectronics, **vol. 39**, 2013, pp. 177–182.

[18] *X. Cao, N. Wang, S. Jia, L. Guo, K. Li*, "Bimetallic AuPt nanochains: Synthesis and their application in electrochemical immunosensor for the detection of carcinoembryonicantigen" Biosensors and Bioelectronics, **vol. 39**, 2013, pp. 226–230.

[19] *Y. Yuan, R. Yuan, Y. Chai, Y. Zhuo, L. Mao, S. Yuan*, "A novel label-free electrochemical immunosensor for carcinoembryonic antigen detection based on the [Ag–Ag2O]/SiO2 nanocomposite material as a redox probe", Journal of Electroanalytical Chemistry, **vol. 643**, 2010, pp. 15–19.

[20] *T. Y. Lee, J. T. Song*, "Detection of carcinoembryonic antigen using AlN FBAR", Thin Solid Films, **vol. 518**, 2010, pp. 6630–6633.

[21] *E. Kim, Y. K. Choi, J. Song, J. Lee*, "Detection of various selfassembled monolayers by AlN-based film bulk acoustic resonator", Materials Research Bulletin, **vol. 48**, 2013, pp. 5076-5079.

[22] *J. Han, J. Ma, Z. Ma*, "One-step synthesis of grapheneoxide–thionine–Au nanocomposites and its application for electrochemical immunosensing", Biosensors and Bioelectronics, **vol. 47**, 2013, pp. 243–247.