ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2015, *60*(5-6), 447-451

PAPERS

PLATFORM BASED ON MICROSENSORS USED FOR THE SCREENING OF HER-1 IN PERITONEAL FLUID

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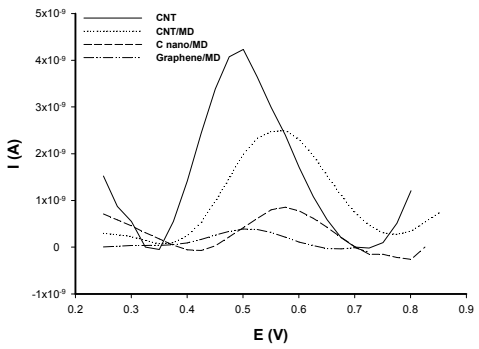
Received November 10, 2014

Four platforms based on amperometric microsensors designed with different carbon matrices: graphene, carbon nanotubes and carbon nanopowder were proposed for the detection of HER-1 in peritoneal fluid. Maltodextrins (dextrose equivalent, DE 11-13) was used as electrocatalyst. The amperometric microsensors proposed covered the linear concentration range between $1.94x10^{-7}$ and $3.04x10^{-3}$mg/mL. The best sensitivity ($5.09x 10^{-6}$ mg/mL) and the lowest limit of detection ($1.77x10^{-10}$mg/mL) were achieved with the sensor based on carbon nanopowder modified with maltodextrin. The modified sensors showed a good reproducibility and high selectivity in the determination of HER-1 in peritoneal fluid.



INTRODUCTION*

Cancer is a major public health concern world-wide. An increase in its incidence is expected every year.[1] HER-1 plays an important role in the human epithelial cancers, e.g., NSCLC. HER-1 plays an important role in tumor growth and progression, by stimulating cancer cell proliferation.[2] Overexpression and activating mutations of the HER-1 are frequently observed and associated with poor prognosis.[3-5] HER-1 – the first member of the EGFR tyrosine kinase family,[6] is expressed in epithelial cells.[7]

Different techniques including, enzyme linked immunosorbent assay,[8,9] fluorescence,[10] electro-chemical methods,[11, 12] were used until now for detection of HER-1.

The purpose of this work was to evaluate and develop a method that can determine HER-1 in peritoneal fluid. Platforms based on amperometric sensors based on different carbon matrices such as graphene, carbon nanotubes and carbon nanopowder, modified with maltodextrin were designed, developed and evaluated. The modified sensors showed a good reproducibility and high selectivity in the determination of HER-1 in biological fluid such as peritoneal fluid.

EXPERIMENTAL

Materials and Reagents

HER-1, maltodextrin (dextrose equivalent 11-13) (MD), graphene, carbon nanopowder (Cnano), carbon nanotube

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(CNT), were obtained from Aldrich (Milwaukee, USA); paraffin oil was obtained from Fluka (Buchs, Switzerland). Titrisol buffer solution (pH=7.4) were obtained from Merck. Deionised water for the solutions' preparation was obtained from a Millipore Direct-Q 3 System (Molsheim, France). All standard solutions were buffered at pH=7.73 with a solution containing: Na^+ 146.57mmol/L; K^+ 4.80 mmol/L; Ca^{2+} 0.89 mmol/L; HCO_3^- 30.54 mmol/L, the ratio between deionised water and buffer being 1:1 ratio (v:v). The same solutions with pHs between 7.46 and 8.10 were prepared to determine the influence of pH on the response of the electrodes.

Design of the active materials for the platform

The matrices used for the construction of the electroactive materials were: graphene, carbon nanopowder and carbon nanotube. Paraffin oil was added over carbon material's powder (graphene, carbon nanopowder, or carbon nanotubes) until a paste was formed. 25µL solution of MD (10^{-3} mol/L MD (DE 11-13) aqueous solution) was added to 100mg of paste to obtained the modified paste. The modified paste was filled into a plastic tube with a diameter of 30µm. An Ag wire inserted in the modified paste was used as electrical contact. The active side of the working sensors (modified paste) was wetted with deionised water and polished with alumina paper (polishing strips 30144-001, Orion) before starting the measurements. When not in use, the platforms were stored in a dry state at room temperature. Surface analysis of the pastes was performed using AFM (Fig. 1).

Apparatus

A PGSTAT 12 coupled with a computer, and driven by software Ecochemie (version 4.9) were used for all amperometric measurements. Pt and Ag/AgCl electrodes were used as counter and reference electrodes in the cell. pH measurements were performed using Cyberscan PCD 6500 pH/mV-meter from Eutech Instruments.

Recommended procedures

Differential pulse voltammetry (DPV)

All *differential pulse voltammetry* measurements were performed at a constant temperature of 25°C. The scan rate was 25mV/s. The platform was dipped into a cell containing standard solutions of different concentrations, from $4.48x10^{-13}$ to $3.04x10^{-3}$ mg/mL. The heights of the peaks were measured (see Table 1 for the potentials) and were plotted versus concentrations of standard solutions of HER-1. The unknown concentrations were determined from the calibration graphs.

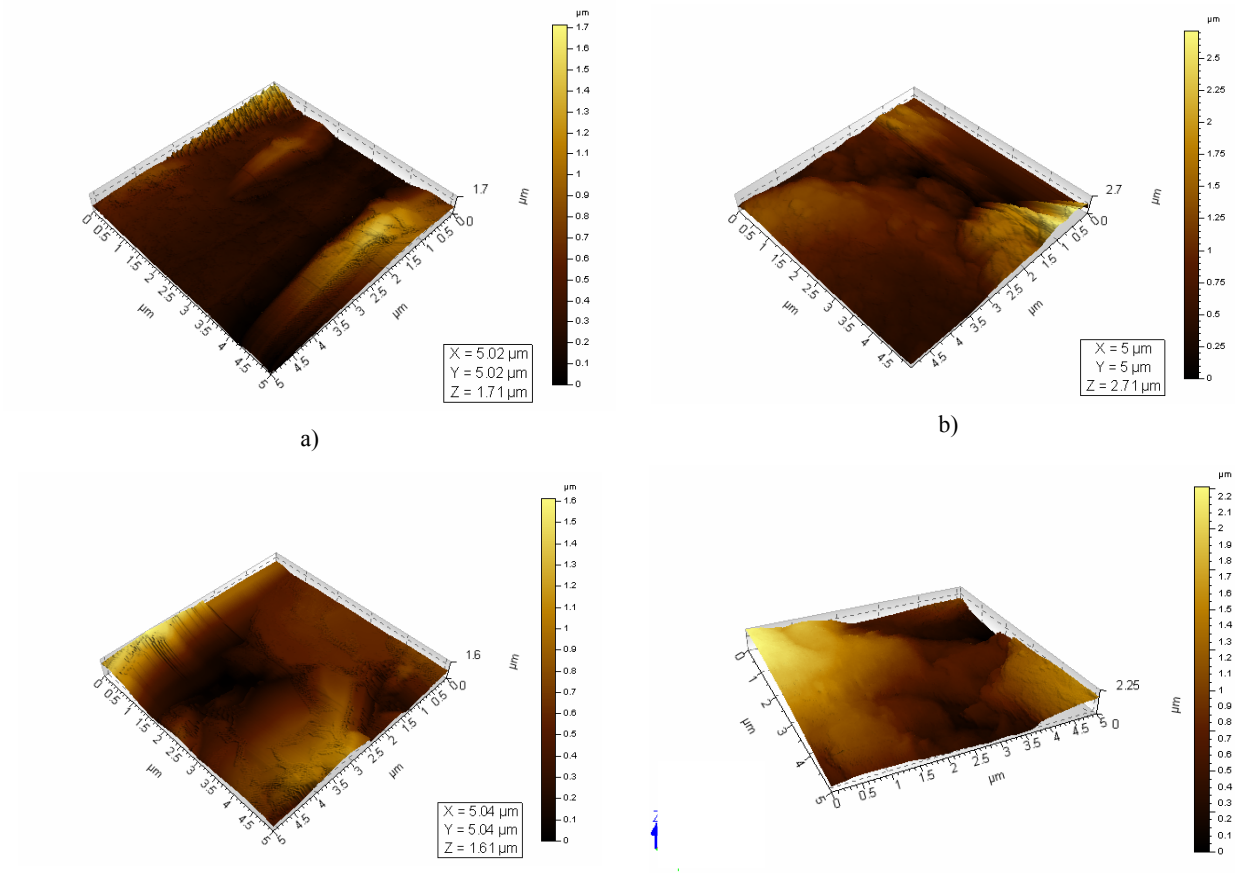

Fig. 1 – 3D of the AFM image for the pastes based on a) MD/graphene; b) MD/Cnano; c) MD/CNT; d) CNT.

Table 1

Response characteristics of amperometric microsensors

Amperometric sensors based on	**Calibration equation[a]**	**Limit of quantification (mg/mL)**	**Sensitivity (A/mg/mL)**	**Linear concentration (mg/mL)**	**LOD (mg/mL)**	**E (mV)**
MD/Graphene	H=0.09(±0.02)+4.55(±0.12)x10^4xC r=0.9982	1.94x10^{-7}	4.55x10^4	1.94x10^{-7} – 2.43x10^{-5}	2.68x10^{-8}	576±12
MD/C nano	H=0.51(±0.15)+5.09(±0.09) x10^6xC r=0.9999	1.94x10^{-7}	5.09x10^6	1.94x10^{-7} – 4.86x10^{-6}	1.77x10^{-9}	576±14
MD/CNT	H=0.83(±0.13)+8.41(±0.10) x10^4xC r=0.9971	1.94x10^{-7}	8.41x10^4	1.94x10^{-7} – 1.21x10^{-4}	1.70x10^{-7}	500±14
CNT	H=0.42(±0.15)+3.74(±0.08)x10^3xC r=0.9996	9.27x10^{-7}	3.74x10^3	9.27x10^{-7} – 3.04 x10^{-3}	5.30x10^{-7}	500±15

[a] <H> = nA (peak height); <C> =mg/mL (concentration of HER-1). All results are the average of ten determinations.

RESULTS AND DISCUSSION

Response characteristics of the amperometric sensors

The response characteristics were determined at pH 7.73, the average of the pH values characteristics for the peritoneal fluid; the range of pH determined by Noh[13] for peritoneal fluid being 7.46-8.10, the solution containing the following ions in certain concentrations: Na^+ 146.57mmol/L; K^+ 4.80 mmol/L; Ca^{2+} 0.89 mmol/L; HCO_3^- 30.54 mmol/L. No variation of peak height was recorded when the pH was varied between 7.46 and 8.10.

The response characteristics of the amperometric sensors were determined using DPV, and are shown in Table 1. The proposed amperometric microsensors covered the linear concentration range $1.94x10^{-7}$-$3.04x10^{-3}$mg/mL. Equation of Otto was used for calculation of limits of detection:[14]

$$DL \quad \frac{I_B \quad 3 \quad _S \quad a}{S}$$

where I_B is the background current recorded, σ_S is the standard deviation for the measurement of the background current, a is the slope of the calibration equation. All statistics for sensor validation were done accordingly with Otto.[14]

The best sensitivity and lowest limit of detection were obtained using the platform based on MD/C nano (Table 1). The lowest sensitivity was obtained for non-modified CNT paste based sensor platform; accordingly, the MD was proving to be a good electrocatalyst for the analysis of HER-1 using DPV technique. The amperometric microsensors were used reliable for more than 3 months of continuous utilization for the assay of HER-1 in peritoneal fluid (RSD of the slopes were less than 1.00%).

Analytical applications

Peritoneal fluid was obtained from a confirmed patient presenting gastric cancer (ethics committee approval nr. 11/2013) and was analyzed without any sampling. DPV was used for the analysis of HER-1 in peritoneal fluid. The results obtained in our laboratory (Table 2) were compared with those provided by the clinical laboratories (immunopathology, and ELISA technique ($9.50x10^{-7}$mg/mL)).

Table 2

Recovery of HER-1 in peritoneal fluid using amperometric microsensors

Amperometric sensors based on	**Peritoneal fluid**
	mg/mL, HER-1
Graphene /MD	$1.19 (\pm 0.21) \times 10^{-6}$
C nano/MD	$9.62(\pm 0.09) \times 10^{-7}$
CNT/MD	$9.70(\pm 0.23) \times 10^{-7}$
CNT	$1.03(\pm 0.42) \times 10^{-6}$
ELISA method	1.05×10^{-6}
Bias, %	2.39

CONCLUSIONS

The four platforms based on amperometric microsensors designed with different matrices of carbon (graphene, carbon nanotubes and carbon nanopowder) modified with maltodextrin proposed for the determination of HER-1 in peritoneal fluid revealed good response characteristics when DPV method was used. The microsensor of choice (based on its high sensitivity, and lower limit of detection achieved) was the one based on MD and C nanopowder. The results obtained for the assay of HER-1 in peritoneal fluid using the microsensors based platforms are in agreement with the results obtained using ELISA (standard method), proving that the platforms can be reliable used for the analysis of HER-1 in such biological fluid. The main feature of the microsensors is their utilization for the assay of HER-1 in serum or whole blood samples, as well as their utilization for other biomarkers specific to gastric cancer.

Acknowledgements: This work was supported by PNII Program Capacity, 2012-2014, Contract nr. 3ERC-like/2012. Iuliana Moldoveanu acknowledges the support of the Sectorial Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and the Roumanian Government under the contract number POSDRU/159/1.5/S/137390/.

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