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–SnO2 – based biosensors and pH sensors, TiO2 – cellulose composites based sensors, GaN-cellulose composites based sensors,11–12 inkjet-printed polyaniline modified screen-printed carbon electrodes5 as well as electrochemical sensing in paper-based microfluidic devices6 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 chemiluminescence15 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. These are the first disposable stochastic sensors designed. Nanostructured material such as 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 NaN3 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 NaN3, 0.1%.

Whole blood samples were obtained from five confirmed patients from the University 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 (Utrecht, 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 adsorbent 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 toff (the time when the channel/poro is losing the conductivity, and the current is dropping to zero, or very closed to zero value) and tton (the time needed for interaction and redox processes inside the channel/poro) 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

\[ t_{\text{off}} = \frac{1}{v_{\text{on}}} \] 

were determined in the diagrams recorded for the whole blood samples, followed by the determination of t off value. Equations of calibration for 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

Carbon Modified Paper Based Sensors

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Disposable sensors are essential for biomedical analysis. Therefore, paper based sensors may become 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.
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 $t_{\text{off}}$ 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 $t_{\text{off}}$ (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 ($t_{\text{off}}$ 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, $t_{\text{off}}$ values are read and used. $t_{\text{off}}$ 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 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>
<thead>
<tr>
<th>Stochastic sensors based on</th>
<th>Signature, $t_{\text{off}}$ (s)</th>
<th>Linear concentration range a</th>
<th>Sensitivity b</th>
<th>Limit of quantification c</th>
</tr>
</thead>
<tbody>
<tr>
<td>P/Cads</td>
<td>3.4</td>
<td>$1.6 \times 10^{-9} - 1.6 \times 10^{-7}$</td>
<td>$5.34 \times 10^{5}$</td>
<td>1.6</td>
</tr>
<tr>
<td>P/Cglossy</td>
<td>3</td>
<td>$1.6 \times 10^{-2} - 1.6 \times 10^{-4}$</td>
<td>$7.99 \times 10^{1}$</td>
<td>160</td>
</tr>
</tbody>
</table>

a $\mu$g/mL; 
b $s$ mg/mL; 
c pg/mL

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

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

<table>
<thead>
<tr>
<th>CEA, ng/mL</th>
<th>Microsensor based on</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P/Cads</td>
</tr>
<tr>
<td>Sample no</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.50 ± 0.04</td>
</tr>
<tr>
<td>2</td>
<td>2.30 ± 0.03</td>
</tr>
<tr>
<td>3</td>
<td>1.65 ± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>1.70 ± 0.02</td>
</tr>
<tr>
<td>5</td>
<td>1.20 ± 0.02</td>
</tr>
</tbody>
</table>

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

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