Retraction for *Analyst*:

Retracted article: A new sensor for the assay of breast cancer antigen

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Analyst, 2011 (DOI: 10.1039/C2AN16305E). **Retraction published 6th September 2012**

We, the authors Raluca-Ioana Stefan-van Staden, Ileana Stefanescu, Jacobus F. van Staden and Marius Enachescu, hereby wholly retract this *Analyst* article. Due to confidentiality reasons and possible conflict of interest we cannot provide at this stage more data to support the conclusions of the article.

Signed: R. I. Stefan-van Staden, J. F. van Staden, National Institute of Electrochemistry and Condensed Matter, Bucharest, Romania, and I. Stefanescu, M. Enachescu, University "Politehnica" Bucharest, Romania.

This retraction is endorsed by May Copsey, Editor. Retraction published 6th September 2012.

Editor's Note: Significant concerns have been received from members of the community regarding the extent of the conclusions that have been drawn in the above *Analyst* article based on the data presented in the article. May Copsey, Editor.

Analyst

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Cite this: DOI: 10.1039/c0xx00000x
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ARTICLE TYPE

A new sensor for the assay of breast cancer antigen

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

A stochastic sensor based on maltodextrin (dextrose equivalent between 4 and 7), and diamond paste was designed and used for the screening of whole blood for a specific breast cancer biomarker. The sensor can be used for the assay of the breast cancer antigen in the linear concentration range: 5.0-500.0U/mL.

Early diagnosis of cancer is a priority in the medical world because patients detected at an early stage have the highest rate (80 to 100%) of survival if treated with correct medication. Screening is an option for this purpose, and can be performed using stochastic sensors, because this type of sensors can show the "signature" of the analyte (t_{off}) as well as the amount of the analyte in the sample (t_{on}).[1-3]

Studies were conducted by different scientists in order to identify the biomarkers for breast cancer with the highest incidents in patients.[4,5] Tests performed on human mammary cells reveals that these cells expressed at their surfaces polymorphic epithelial mucin (PEM) which was named breast cancer antigen and CA15-3.[4,5] Accordingly this was the substance to identify and quantify in the biological fluids in order to have the correct diagnosis of breast cancer. To date, different methods were proposed for detection of cancer, based on cancer cell detection using impedimetric transducers,[6] biosensors based on gold nanoparticles,[7] Raman maps,[8] fluorescence,[9] and detection of the specific biomarkers using calorimetry,[10] suspended microchannel resonators[11] and opto-fluidic ring resonator biosensors.[12]

In this article we propose a stochastic sensor based on maltodextrin with dextrose equivalent between 4 and 7 (this is the first dextrin used to develop a stochastic sensor), having a compact helix structure – as electrochemical active material. The maltodextrin was physically immobilized on a diamond paste matrix. We chose the natural diamond powder having a particle size of 1µm, because in previous experiments it gave the best sensitivity, selectivity and S/N ratio for amperometric measurements.[13] The choice of stochastic sensors for this type of determination was done because one can determine if the biomarker is in the biological fluid, and in which quantity, the values of t_{off} and t_{on} being independent on the matrix from where the biomarker is determined.[14]

Experimental

Natural diamond powder having a particle size of 1µm (99.9%), maltodextrin (dextrose equivalent 4-7) and breast tumor antigen (0.1mol/L solution in phosphate buffer, pH=7.4 containing 0.1% NaN_3) were purchased from Aldrich (Milwaukee, USA); paraffin oil was purchased from Fluka (Buchs, Switzerland). 0.1mol/L Phosphate buffer solution (pH=7.4), and NaN_3 were purchased from Merck. Deionised water obtained from a Millipore Direct-Q 3 System (Molsheim, France) having a conductivity of 0.05µS/cm, was used for the preparation of all solutions. The solutions of biomarkers were all made in 0.1 mol/L phosphate buffer (pH=7.4) containing 0.1% NaN_3 in order to have the medium from the vials on which the antigen was purchased. The range of concentrations for sensors' evaluation was obtained using serial dilution technique, from 0.005 to 5000U/mL.

Maltodextrin was physically immobilized in the diamond paste. The modified diamond paste was prepared as follows: 200 mg of natural monocrystalline diamond powder was mixed with 20 µL paraffin oil to form a diamond paste. 100µL from the solution of maltodextrin with dextrose equivalent 4-7 (10^{-3}mol/L) was added to the diamond paste. The modified paste was placed into a plastic tube (Fig.1). The diameter of the sensor was about 300µm. Electric contact was obtained by inserting an Ag/AgCl wire into the modified diamond paste. The surface of the sensor was wetted with deionised water and polished with alumina paper (polishing strips 30144-001, Orion) before using. When not in use, the sensor was stored in a dry state at room temperature.

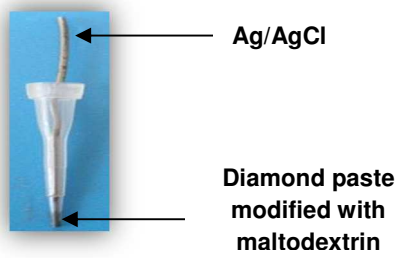


Fig. 1 Stochastic sensor based on maltodextrin.

A PGSTAT 12 and software Ecochemie (version 4.9) were used for all chronoamperometric measurements. A Pt electrode and an Ag/AgCl electrode served as the counter and reference electrodes in the cell. A Cyberscan PCD 6500 pH/mV-meter from Eutech Instruments was employed for all pH measurements.

A chronoamperometric technique was used for the measurements of t_{on} and t_{off} at 125mV. At this value of potential the sensor had a stochastic behavior. The electrodes were dipped into a cell containing solutions of biomarker (buffered with a

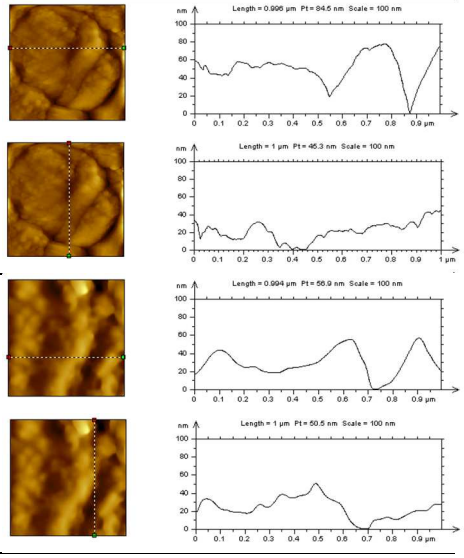
solution containing 0.1 mol/L phosphate buffer (pH=7.4) and 0.1% NaN_3) of different concentrations. Equations of calibration $1/t_{on}$ = f(Conc.) are determined using statistics. The unknown concentrations of biomarker in blood samples were determined from the calibration equations.

Results and discussions

High-resolution imaging using atomic force microscopy (AFM) has been applied to observe the differences of surface structures between unmodified diamond paste and modified diamond paste with maltodextrin. The topography of the surfaces was investigated by AFM in tapping mode in a range of scan lengths from 50µm to 1µm. These measurements were carried out with an Agilent 5500 SPM system, described by PicoSPM controlled by a MAC Mode module and interfaced with a PicoScan controller from Agilent Technologies, Tempe, AZ, USA (formally Molecular Imaging). A multipurpose large scanner and Point Probe Plus Silicon SPM Sensor cantilevers (PPP-FM cantilevers), n^+- silicon material with no coating, of about 227 µm length, 1.8 N m^{-1} spring constants, with the tips oscillated near their resonant frequencies in air, of about 64 kHz were used for all measurements. Scanning of the surface was done at a rate of 0.8-1.2 lines per second, at room temperature in the tapping mode.

Table 1 shows the AFM topographical images of plane and modified diamond paste based sensor. The surface roughness analysis was performed for both modified and unmodified diamond pastes. Because the roughness can be defined by either S_q(root mean square height) or S_a(arithmetical mean height) value, we determine these parameters using the PicoImage tool and accordingly with ISO25178, these values were automatically calculated. The values obtained for the unmodified diamond paste were S_q=13.6nm, and S_a=10.8nm, while for the diamond paste modified with maltodextrin were S_q=119nm, and S_a=88.2nm. The significant difference between roughness of plane and modified pastes as well as of images obtained proved that the maltodextrin was physically immobilized into the diamond paste.

Table 1 AFM topographical images of plane and modified diamond paste based sensor.

Material	Extracted profiles (1x1µm)
Diamond paste modified with maltodextrin	
Diamond paste	

No structural changes (for maltodextrin or diamond) are expected from this type of immobilization. The presence of the round channel in the modified paste justified the stochastic behavior of the sensor when a constant value of potential (125mVvs Ag/AgCl) was applied and the current value vs. time was recorded.

Breast cancer antigen is a transmembrane protein which contains two subunits that form a stable dimer. The signature of breast tumor antigen is given by the value of t_{off} which is 3.3s when both standard biomarker solutions and whole blood samples were measured. This value can be used for the qualitative analysis of the antigen in whole blood, because it did not change when the biomarker was assayed from whole blood samples (Table 2).

For the quantitative analysis, the measurements were performed for 6 months in order to determine the stability as well as the sensitivity of the sensor. The equation of calibration for the stochastic sensor was determined using the values of t_{on} - defined as the binding time[1] (binding constant (determined from the experimental data) between maltodextrin and breast tumor antigen on the channel being $0.011U^{-1}/mL^{-1}$), measured in sec. obtained for different concentrations of the breast tumor antigen, measured in U/mL: $1/t_{on} = 20.11 + 0.044 \times C$

The correlation coefficient, r for this equation was 0.9608. Calibration graph is shown in Fig.2.

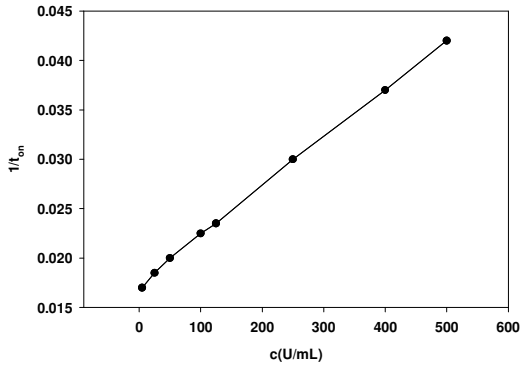


Fig. 2 Calibration graph of maltodextrin based stochastic sensor.

The linear concentration range was between 5.0 and 500U/mL, covering patients who are on an early stage as well as patients on late stages of breast cancer. The sensitivity was 0.044mol s/mL, with a limit of detection of 0.25U/mL. The relative standard deviation (RSD%) per sensor was less than 0.1% for the response/sensitivity of the sensor, values being recorded for the 6 months. Ten sensors were designed and tested during this period; while the results obtained in terms of sensitivity for 6 months had a RSD value less than 0.1%, proving the reliability of the design proposed.

Other biomarkers such as ovarian cancer antigen, gastrointestinal cancer antigen, and hepatitis B virus antigen were tested as possible interferences using separate solution method and mixed solution method. On both separate and mixed solution methods the tests have shown that each of the biomarkers mentioned had a specific signature given by the t_{off} value, which is different from the one recorded for breast cancer antigen. Accordingly, no interference was recorded. Furthermore, the biggest advantages of stochastic sensors is that the signature of the analytes (t_{off} values) are independent on the matrix from where the analytes are determined.

The proposed stochastic sensor was used for qualitative and quantitative analysis of the breast tumor antigen from whole

blood samples. The results obtained were compared with those provided by the clinical laboratories that used for the analysis of breast tumor antigen in serum samples ELISA technique (used in clinical laboratory as standard method for the assay of CA15-3).

Qualitative assay of the breast tumor antigen was done in whole blood samples using the value of t_{off}. Accordingly, t_{off} values specific for the breast cancer antigen were found in four out of five diagrams obtained for real whole blood samples. As a result, there were four positive results and one negative result for the screening test performed with the proposed stochastic sensor. The results obtained for qualitative assay are shown in Table 2. A very good correlation between the proposed method (based on stochastic sensor) and standard method (ELISA) was obtained.

Table 2 Qualitative analysis of breast tumor antigen

Sample Nr.	Stochastic sensor based on maltodextrin (whole blood samples)	ELISA (standard method, serum samples)
1	+	+
2	+	+
3	+	+
4	+	+
5	-	-

The results obtained for the quantitative assay of breast tumor antigen performed for samples 1-4 are shown in Table 3. The lowest values obtained when ELISA method was used are due to the fact that ELISA is using serum samples – separated from whole blood samples. Each separation process will induce a decrease of concentration of the analyte determined. The correlation between the values is good, proving that the stochastic sensor can be used for the quantification of the antigen in whole blood.

Table 3 Quantitative analysis of breast tumor antigen

Sample Nr.	Stochastic sensor based on maltodextrin (U/mL)		ELISA (standard method, serum samples) (U/mL)
	Whole blood samples	Serum samples	
1	98.0	96.2	96.0
2	69.5	67.7	68.0
3	45.2	42.8	43.0
4	56.3	53.5	54.0

The proposed method comparing with ELISA is faster, more selective and reliable, as well as cost effective (more than 100 determinations of the breast cancer antigen from whole blood samples can be performed continuously with the same sensor in the same day). A major advantage is also its possibility to determine directly from the whole blood samples the breast cancer specific antigen, while for ELISA, a separation of serum is needed in order to perform the analysis.

The results obtained proved that the sensor is a good screening tool for breast cancer antigen. Its utilization can solve a major problem for breast cancer: early detection. Early detection of breast cancer can avoid the cancer evolution by treating the patient immediately with available medication, making possible in most of the cases the 100% recovery of the patient; at the moment treatment at a very early stage is the only way to cure the patients.

Conclusions

This article opens a new and very important field in the use of stochastic sensors in clinical analyses. The possibility of determining the quality as well as the quantity of the analyte in one run made the method comparable with the best existing chromatographic methods, but at higher precision and lower quantification values. The proposed stochastic sensor was reproducible as design and response characteristics.

Notes and references

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Acknowledgements
This work has been supported by the Romanian National Programme PN II, Program Ideas 2011-2014, Contract 123/05.10.2011, by the European Union through the European Regional Development Fund, and by Romanian National Authority for Scientific Research, under project POSCCE-O 2.1.2-2009-2/12689/717.

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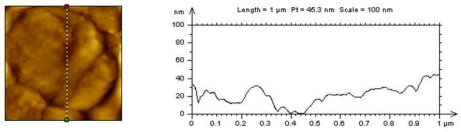

Stochastic sensors based on maltodextrin are new tools of screening for breast cancer antigen.