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Fast Screening Method of Biological Samples Based on Needle Stochastic Sensors for Early Detection of Gastric Cancer

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Abstract: *Early detection of cancer is essential for saving the life of patients. Needle stochastic sensors were proposed as new tools for the fast screening of biological samples. The design of the stochastic sensors was based on the immobilization of protoporphyrin IX in pastes of S-doped graphene. The selected biomarkers were CEA and CA19-9. High sensitivities, and wide linear concentration ranges as well as low limits of quantification were achieved. The proposed sensors were validated; student t-test shown that the proposed needle stochastic sensors can be reliably used for the pattern recognition and quantification of CEA and CA19-9 in whole blood, gastric tumor tissue, saliva, and urine.*

Keywords*: needle stochastic sensors, fast screening test, gastric cancer, CEA, CA19-9*

1. Introduction

Patients with gastric disease have no undeniable side effects, and a large number of them are in the center or propelled stages while accepting investigation in an emergency clinic, in this manner passing up on the best open door for treatment [1,2]. Gastroscopy joined with obsessive conclusion is the best quality level for analysis of gastric malignant growth, however poor resilience to gastroscopy, high monetary expenses and low worthiness among patients make it unsatisfactory for fundamental screening [3,4]. Metabolites straightforwardly created by tumor cells and discharged into body liquids or tissues, or expert deuced by ordinary cells when battling against tumors fill in as tumor markers, and normally exist as antigens, compounds or hormones [5]. In the typical condition, such markers show no presence or amazingly low substance in the body. Their quality or changes in amount can mirror the event and movement of tumors, with points of interest of advantageous detection, solid adequacy and low costs, which improves the location pace of essential tumors, screen tumor repeat or metastasis, and assess the anticipation impact [6].

The forecast of early gastric malignant growth is good after revolutionary gastrectomy, with a 5-year by and large endurance rate surpasses 97% [7]. An assortment of elements have been perceived as prognostic elements for early gastric malignant growth, including tumor size, separation status, tumor profundity, LNM and vessel association [8]. Also, tumor markers including carcinoembryonic antigen (CEA) [9], cancer-related antigen 19-9 (CA19-9) [10], and AFP [11] were exhibited to be prognostic variables for gastric malignancy. CEA and CA19-9 are some of the biomarkers that are present in gastric cancer. When these biomarkers are found in the human body in an increased concentration, they indicate the presence of gastric cancer. CA 19-9 is a well-known biomarker of digestive tract malignancies. Although tumor biomarkers are an invaluable asset to medical practice, their role in screening, diagnosis and oncologic treatment remains poorly standardized [12,13]. In gastric cancer, the most used tumor markers are: CEA and CA 19-9 [12].

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To date, the methods proposed for the assay of these biomarkers include fluorescence [14], spectroscopy [15], chemiluminescence [16], radioimmunoassay [17], electrophoresis [18] and enzyme-linked immunosorbent assays (ELISA) [19]. These methods are very expensive, laborious, and are only designed for one biomarker assay per run.

Stochastic sensors have been used to date for the molecular recognition and quantification of several biomarkers used in the diagnosis of different illnesses such as diabetes, cancer, and obesity [20-22]. Their main advantages are the possibility to perform a reliable qualitative and quantitative analysis of various biomarkers at very low concentrations in complex samples, no treatment (sampling) was needed on the biological samples before performing the analysis, and the results of the molecular recognition and quantification were not influenced by the complexity of the matrix from which the biomarkers were determined.

In this study, protoporphyrin IX (PIX) was used as the recognition molecule for the design of the needle stochastic sensors for the simultaneous recognition and quantification of CEA and CA19-9 in biological samples such as: whole blood, saliva, urine, and tissue samples.

2. Materials and methods

2.1. Materials and reagents

CA19-9, CEA, monosodium phosphate and disodium phosphate were purchased from Sigma Aldrich (Milwaukee, USA); paraffin oil was purchased from Fluka (Buchs, Switzerland). Monosodium phosphate and disodium phosphate were used for the preparation of phosphate buffer, *p*H=7.5. Deionized water obtained from a Millipore Direct-Q 3 System was used for the preparation of all solutions. Dimethylsulfoxide (DMSO) and sodium sulfide (Na_2S) were purchased from Merk (Germany); sulfur was acquired from Remed Prodimpex (Romania).

2.2. Apparatus and methods

For all measurements, a potentiostat/galvanostat AUTOLAB/PGSTAT 302 (Methrom, Utrecht, The Netherlands), connected to a personal computer with a GPES software installed was used. An electrochemical cell, containing a three electrode system was also employed. The three electrode system is made out of: the counter electrode (platinum wire), the working electrode (proposed needle stochastic sensors) and the reference electrode (Ag/AgCl electrode).C/H/S contents in the prepared samples were obtained using elemental analysis (FLASH EA 1112, Italy) and EDX. Samples were morphologically characterized by TEM (H-7650 120 kV Automatic TEM, Hitachi, Japan). The structural data were obtained from XRD patterns (Bruker D8 Advance diffractometer, Germany).

2.3. S-doped graphene synthesis

The S-doped graphene samples were prepared by solvothermal method using three different doping agents, namely: dimethylsulfoxide (for S-dop-1); sodium sulfide(for S-dop-Gr-1-S) and sulfur (for Gr-S5-TT).

2.4. Preparation of S-dop-1

The preparation of S-dop-1 was conducted by a solvothermal method using DMSO as reducing/doping agent. Briefly, 150 mg of GO, previously synthesized [23] were dispersed in 55 mL DMSO and kept at 180°C for 18 h. The obtained material was washed with ethanol and water, to remove the unreacted DMSO and the unpleasant odored by-products, then dried by lyophilization.

2.5. Preparation of S-dop-Gr-1-S

For the preparation of S-dop-Gr-1S we used 240 mg GO dispersed in 80 mL H_2O then 240 mg of Na_2S were added; the mixture was put in an autoclave and kept in oven for 10 h at 200°C. After cooling to room temperature the sample was washed with double-distilled water, filtered and dried by lyophilization.

2.6. Preparation of Gr-S5-TT

700 mg GO were sonicated for 25 min in 140 mL toluen, then 500 mg S was added and sonicated another 30 min. The obtained dispersion was kept for 12 h at 160°C. The prepared material was washed with toluene, filtered, and dried by lyophilization. For further purification the sample was thermally treated, 15 min at 450°C.

2.7. Design of the needlestochastic sensors

Three types of S-doped graphene powders were mixed with parrafin oil, until three homogeneous pastes were obtained. A 10^{-3} mol L^{-1} protoporphyrin IXsolution was added to each paste (100µL solution:100mg graphene paste). Each paste was placed in a non-conducting plastic tube with internal diameter of 100µm.The conection between the paste and the external circuit was made using a silver wire. Before and after each measurement, the sensors were cleaned with deionized water, and dryed. When not in use, the three stochastic sensors were kept in the refrigerator, in a dark box.

2.8. Stochastic mode

The stochastic mode was used for all measurements. The principle of the stochastic sensors is based on the channel conductivity. Chronoamperometry was selected and a constant potential of 125 mV was applied. The parameters read in the diagrams were t_{off} and t_{on}. The biomarkers: CEA and CA19-9 were identified in the diagrams (Figures 1-4) based on their signatures(t_{off} values), while reading the corresponding t_{on} value in between two t_{off} values, the determination of the concentration of the biomarker was possible by inserting the t_{on} values in the equation:

$$1/t_{on} = a + b \times C_{biomarker} \quad (1)$$

where *a* is the intercept, *b* is the slope and *c* is the concentration of the biomarker.

The equations of calibration for each sensor, and each biomarker were statistically calculated using the t_{on} values obtained after measurements of the standard solutions (of different concentrations) of CEA, and CA 19-9.

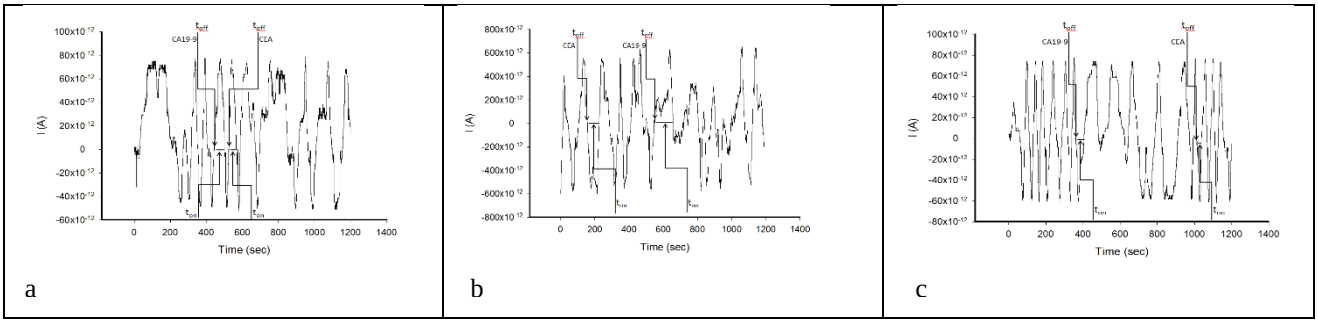

Figure 1. Pattern recognition of CEA and CA19-9 in whole blood samples, using stochastic sensors: (a) PIX-S-dop-1, b) PIX-S-dop-GR1-S, c) PIX-GR-S5-TT

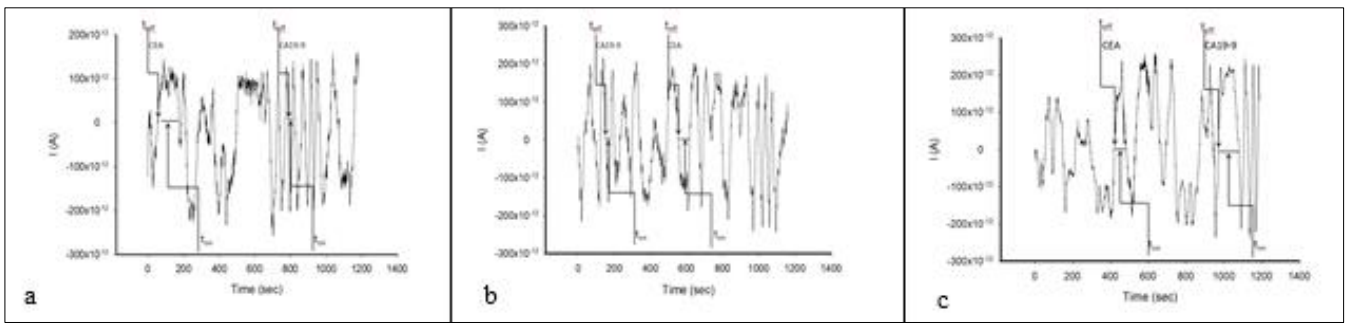

Figure 2. Pattern recognition of CEA and CA19-9 in saliva samples using stochastic sensors: (a) PIX-S-dop-1, b) PIX-S-dop-GR1-S, c) PIX-GR-S5-TT

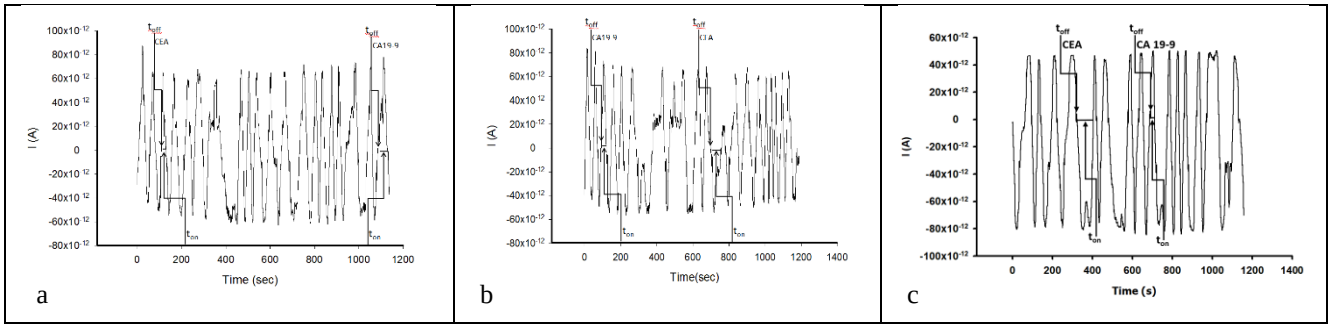

Figure 3. Pattern recognition of CEA and CA19-9 in urine samples using stochastic sensors: (a) PIX-S-dop-1, b) PIX-S-dop-GR1-S, c) PIX-GR-S5-TT

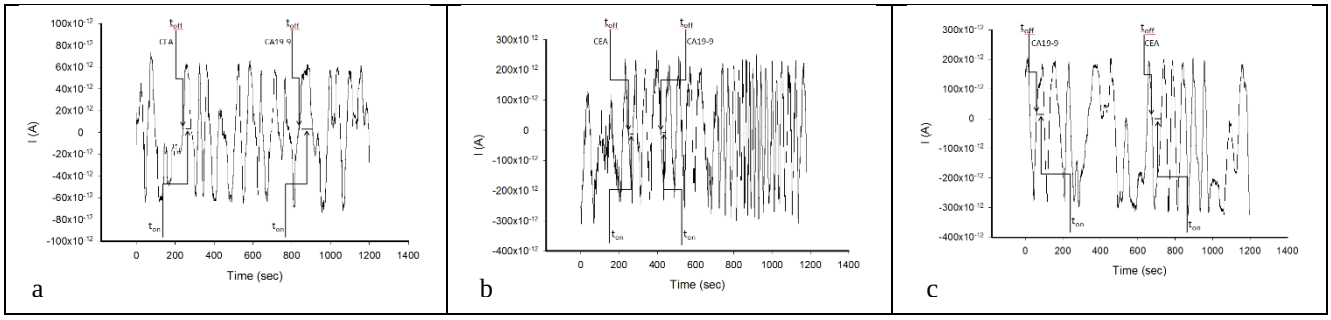

Figure 4. Pattern recognition of CEA and CA19-9 in tissue samples using stochastic sensors: (a) PIX-S-dop-1, b) PIX-S-dop-GR1-S, c) PIX-GR-S5-TT

2.9. Samples

Whole blood, saliva, urine and tissue samples were recieved from the County Emergency Hospital from Targu-Mures. Samples from confirmed patients with gastric cancer were collected according to the procedures specified in the Ethics committee approval number 32647/2018 awarded by the County Emergency Hospital from Targu-Mures. Written consent was obtained from all patients. No sample pre-treatment was performed.

3. Results and discussions

3.1. Characterization of the S-Gr samples

According to the elemental analysis, the samples prepared with sulfur (*Gr-S5-TT*) and sodium sulfide (*S-dop-Gr-1-S)* as doping agents have low content of sulfur whereas the other sample, *S-dop-1*, prepared in DMSO, has a higher content of sulfur, 5.42 wt% (Table 1).

Table 1. C/S/H contents obtained from elemental analysis

sample	wt%		
	C	**S**	**H**
S-dop-1	78.04	**5.42**	0.55
S-dop-Gr-1-S	80.78	**1.18**	0.55
Gr-S5-TT	82.85	**1.71**	0.55

In Figure 5 a-c are shown representative TEM images of the prepared samples. It can be observed the wrinkled graphene surface with narrow dark lines which represents the edges of the folded graphene.

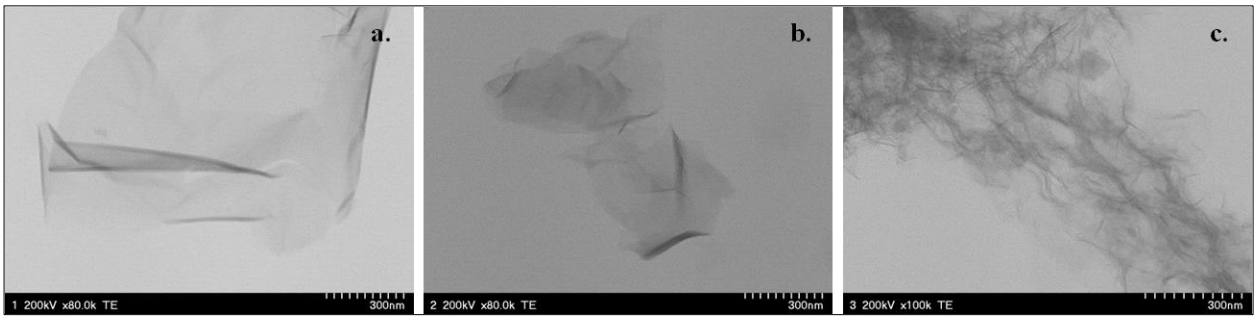

Figure 5. TEM images of S-dop-1 (a); S-dop-Gr-1 S (b) and Gr-S-5-TT (c)

TEM-EDX mapping was also employed to determine the elemental distribution within the samples. Sample GRS-5-TT contains 1.5 wt% sulfur, in good agreement with the elemental analysis (1.71 wt%) (Figure 6).

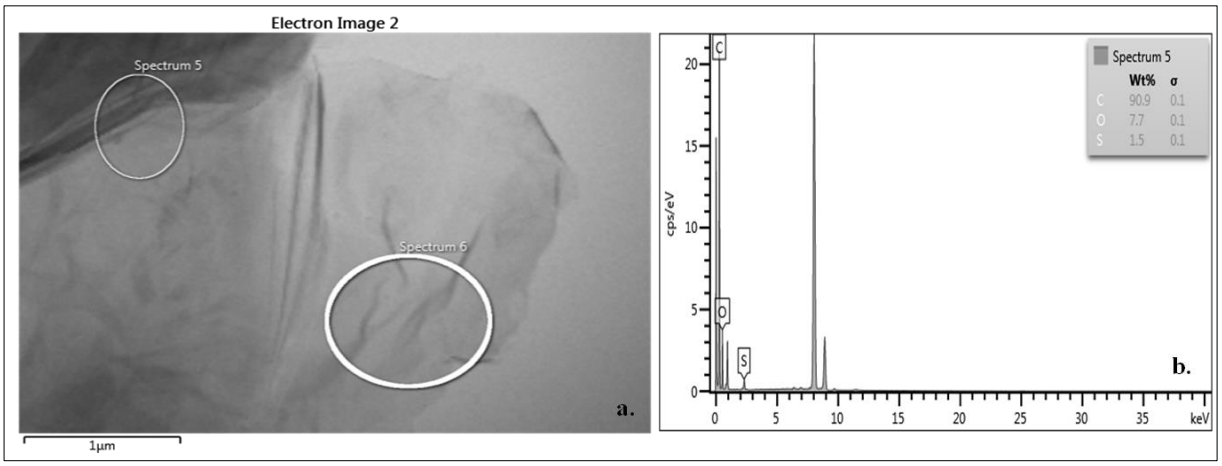

Figure 6. TEM image (a) of Gr-S-5-TT and EDX analysis (b)

3.2. Response characteristics of the stochastic sensors

The process of current development in stochastic sensors comprised two phases [22]:the first phase is known as the pattern recognition phase; the biomarker is extracted from the solution into the membrane-solution of the interface and blocks the channel; because of this, the intensity of the current decreases to 0 value for a certain period of time, which is known as t_{off} and it represents the signature of the biomarker; its value is used for the qualitative analysis of the biomarker; the second phase is known as the binding phase and it takes place when the analyte interacts with the wall channel, and the redox processes take place. During the second phase, the following equations of binding take place:

$$Ch_{(i)} + CEA_{(i)} \Leftrightarrow Ch \bullet CEA_{(i)} \quad (2)$$

$$Ch_{(i)} + CA19\text{-}9_{(i)} \Leftrightarrow Ch \bullet CA19\text{-}9_{(i)} \quad (3)$$

where *Ch* represents the channel and *i* represents the interface. The quantitative parameter, also known as t_{on}, represents the equilibrium time needed for the interaction between the biomarker and the wall channel/redox processes.

Table 2 shown the response characteristics of the proposed needle stochastic sensors. The highest sensitivity for the determination of CEA was obtained when the needle stochastic sensor based on Gr-S5-TT was used; while for the assay of CA19-9, the highest sensitivity was obtained when the sensor based on Sdop1 was used. The linear concentration range for the assay of CEAwas obtained when the sensor based on S-dop-Gr-1-S was used while for the assay of CA-19-9 the widest linear concentration range was obtained when the sensor based on Gr-S5-TT was used. The lowest limit of quantification for

the determination of CEA was obtained using the sensor based on S-dop-Gr-1-S while for the assay of CA-19-9 the lowest limit of quantification was obtained when the sensor based on Gr-S5-TT was used.

3.3. Selectivity

Different t_{off} values, also known as the signatures of the biomarkers of interest, were recorded for CEA and CA19-9, using the same sensors (Table 2). When checking p53, maspin, D-glutamine as possible interference, for all sensors, signatures higher than 2.4s were obtained, proving that the proposed needle stochastic sensors are selective.

Table 2. The response characteristics of needle stochastic sensors used for the assay of CEA and CA19-9

Needle stochastic sensor based on PIX and	Calibration equation* and correlation coefficient (r)	Linear concentration range	t_{off} (s)	Sensitivity	Limit of quantification
CEA*					
Sdop1	$1/t_{on}=0.01+6.24xC$ r=0.9983	$6.40x10^{-11}$ – $1.00x10^{-6}$	2.0	6.24	$6.40x10^{-11}$
S-dop-Gr-1-S	$1/t_{on}=0.01+4.26x10^{2}xC$ r=0.9999	$2.56x10^{-12}$ – $1.00x10^{-6}$	1.3	$4.26x10^{2}$	$2.56x10^{-12}$
Gr-S5-TT	$1/t_{on}=0.02+9.71x10^{2}xC$ r=0.9992	$1.28x10^{-11}$ – $3.20x10^{-6}$	1.3	$9.71x10^{2}$	$1.28x10^{-11}$
CA19-9**					
Sdop1	$1/t_{on}=0.02+3x10^{-4}xC$ r=0.9999	$1.60x10^{-1}$ – $1.00x10^{2}$	1.1	$3x10^{-4}$	$1.60x10^{-1}$
S-dop-Gr-1-S	$1/t_{on}=0.03+4.37x10^{-5}xC$ r=0.9998	$1.31x10^{-10}$ – $5.00x10^{2}$	0.6	$4.37x10^{-5}$	$1.31x10^{-10}$
Gr-S5-TT	$1/t_{on}=0.02+1.13x10^{-4}xC$ r=0.9999	$2.10x10^{-13}$ – $5.00x10^{2}$	0.6	$1.13x10^{-4}$	$2.10x10^{-13}$

* All concentrations are expressed in g mL^{-1}.
**All concentrations are expressed in UmL^{-1}.

3.4. Stability

The needle stochastic sensors have been used continuously for one year for measurements. Comparing the sensitivities obtained every day, each with the other, a relative standard deviation value lower than 1.00% was obtained. Accordingly, the proposed needle sensors are highly stable.

3.5. Determination of CEA and CA 19-9 using a fast screening method of biological samples

Biological samples: urine, whole blood, saliva and gastric tissue samples, were collected from confirmed patients with gastric cancer. All samples were analyzed without any pretreatment. Molecular recognition based on the signatures of CEA and CA19-9 was first performed, followed by thereading of the t_{on}values, and the determination of the concentrations of CEA and CA19-9 accordingly with the stochastic mode described above.

The obtained results for the determination of CEA and CA19-9 in urine, whole blood, saliva and tissue samples from patients confirmed with cancer are shown in Tables 3-6. A good correlation between the obtained results using the proposed stochastic sensors were obtained for whole blood, gastric tissue samples, saliva, and urine samples, indicating that the proposed needle stochastic sensors may be reliably used for the screening tests used for the molecular recognition and determination of CEA and CA19-9 in biological samples. Student t-test were also performed at 99.99% level; all calculated t values were lower than 4.3 which is the tabulated value; these results also certified that the proposed needle stochastic sensors can be reliably used for pattern recognition and quantification in fast screening tests of whole blood, gastric tumor tissue, saliva, and urine samples.

Table 3. Determination of CEA, and CA19-9 in whole blood samples (N=10)

Sample	Needle stochastic sensor based on PIX and	CEA (ng mL^{-1})	CA19-9 (U mL^{-1})
1	Sdop1	4.19±0.02	18.23±0.08
	S-dop-GR1-S	4.15±0.07	19.07±0.07
	GR-S5TT	3.94±0.05	18.74±0.07
2	Sdop1	8.49±0.03	63.57±0.03
	S-dop-GR1-S	8.25±0.07	62.08±0.05
	GR-S5TT	8.76±0.08	64.43±0.07
3	Sdop1	8.88±0.03	47.98±0.08
	S-dop-GR1-S	8.80±0.05	45.47±0.03
	GR-S5TT	8.83±0.07	41.47±0.04
4	Sdop1	99.56±0.04	375.03±0.06
	S-dop-GR1-S	98.45±0.07	375.78±0.05
	GR-S5TT	98.41±0.05	375.25±0.05
5	Sdop1	1.46±0.04	643.43±0.03
	S-dop-GR1-S	0.99±0.07	640.60±0.04
	GR-S5TT	1.19±0.03	648.95±0.07
6	Sdop1	8.39±0.04	643.23±0.04
	S-dop-GR1-S	8.75±0.05	648.30±0.03
	GR-S5TT	8.26±0.07	648.95±0.05
7	Sdop1	4.85±0.08	20.74±0.03
	S-dop-GR1-S	5.30±0.03	20.19±0.05
	GR-S5TT	5.42±0.03	21.88±0.06
8	Sdop1	4.79±0.05	71.95±0.08
	S-dop-GR1-S	5.45±0.09	77.80±0.05
	GR-S5TT	4.87±0.07	70.37±0.05
9	Sdop1	1.64±0.03	18.23±0.03
	S-dop-GR1-S	1.69±0.05	18.16±0.04
	GR-S5TT	1.08±0.07	18.77±0.07
10	Sdop1	106.79±0.04	258.90±0.08
	S-dop-GR1-S	103.78±0.03	227.55±0.08
	GR-S5TT	104.51±0.04	272.67±0.05
11	Sdop1	16.83±0.07	332.27±0.05
	S-dop-GR1-S	16.87±0.05	326.43±0.08
	GR-S5TT	16.42±0.05	333.36±0.05
12	Sdop1	3.32±0.03	183.95±0.05
	S-dop-GR1-S	3.98±0.04	189.30±0.08
	GR-S5TT	3.46±0.04	183.42±0.04
13	Sdop1	10.68±0.03	658.98±0.03
	S-dop-GR1-S	10.90±0.05	657.08±0.05
	GR-S5TT	10.16±0.05	650.24±0.04
14	Sdop1	67.12±0.04	647.12±0.05
	S-dop-GR1-S	67.64±0.04	647.32±0.05
	GR-S5TT	65.69±0.03	648.95±0.03
15	Sdop1	88.08±0.03	35.35±0.04
	S-dop-GR1-S	87.10±0.03	36.38±0.05
	GR-S5TT	82.62±0.05	34.06±0.05
16	Sdop1	7.47±0.08	373.97±0.04
	S-dop-GR1-S	7.40±0.05	375.78±0.03
	GR-S5TT	7.37±0.05	374.48±0.03
17	Sdop1	6.37±0.04	187.40±0.08
	S-dop-GR1-S	6.89±0.04	189.30±0.05
	GR-S5TT	6.36±0.03	181.28±0.07
18	Sdop1	1.66±0.04	187.60±0.08
	S-dop-GR1-S	0.99±0.02	185.57±0.07
	GR-S5TT	1.04±0.05	184.82±0.07
19	Sdop1	2.61±0.08	648.40±0.03
	S-dop-GR1-S	2.80±0.05	657.21±0.04
	GR-S5TT	3.00±0.03	648.34±0.05
20	Sdop1	1.01±0.04	272.60±0.05
	S-dop-GR1-S	1.39±0.03	276.20±0.03
	GR-S5TT	1.34±0.03	272.67±0.04

21	Sdop1	4.91±0.08	200.94±0.08
	S-dop-GR1-S	4.67±0.07	202.51±0.05
	GR-S5TT	4.83±0.07	201.40±0.05
22	Sdop1	5.54±0.08	550.00±0.04
	S-dop-GR1-S	5.98±0.04	547.91±0.08
	GR-S5TT	5.80±0.05	548.84±0.05
23	Sdop1	3.76±0.06	62.85±0.03
	S-dop-GR1-S	3.87±0.05	62.74±0.04
	GR-S5TT	4.08±0.08	62.80±0.04
24	Sdop1	4.22±0.08	127.17±0.04
	S-dop-GR1-S	4.76±0.03	128.78±0.05
	GR-S5TT	4.78±0.04	127.88±0.05
25	Sdop1	4.22±0.05	278.46±0.03
	S-dop-GR1-S	4.64±0.05	274.49±0.04
	GR-S5TT	4.43±0.03	272.67±0.04
t-test	-	2.20	2.43

Table 4. Determination of CEA, and CA19-9 in saliva samples (N=10)

Sample	Needle stochastic sensor based on PIX and	CEA (ng mL^{-1})	CA19-9 (U mL^{-1})
1	Sdop1	4.34±0.03	93.60±0.08
	S-dop-GR1-S	4.66±0.04	93.28±0.05
	GR-S5TT	4.46±0.04	94.32±0.04
2	Sdop1	6.42±0.04	185.56±0.08
	S-dop-GR1-S	6.09±0.08	184.54±0.05
	GR-S5TT	6.59±0.05	186.01±0.05
3	Sdop1	5.49±0.04	88.29±0.04
	S-dop-GR1-S	5.47±0.05	83.99±0.07
	GR-S5TT	5.26±0.04	80.86±0.05
4	Sdop1	2.07±0.04	87.17±0.05
	S-dop-GR1-S	2.77±0.04	87.59±0.05
	GR-S5TT	2.15±0.07	88.61±0.04
5	Sdop1	4.82±0.04	51.04±0.08
	S-dop-GR1-S	4.70±0.03	50.94±0.03
	GR-S5TT	4.85±0.05	50.71±0.03
6	Sdop1	106.35±0.07	154.94±0.04
	S-dop-GR1-S	104.55±0.03	155.12±0.03
	GR-S5TT	104.14±0.03	153.37±0.03
7	Sdop1	382.19±0.04	272.57±0.07
	S-dop-GR1-S	380.95±0.05	279.55±0.03
	GR-S5TT	380.16±0.05	272.67±0.08
8	Sdop1	3.76±0.04	296.79±0.04
	S-dop-GR1-S	3.36±0.04	296.46±0.08
	GR-S5TT	3.80±0.08	296.78±0.07
9	Sdop1	4.85±0.05	66.78±0.05
	S-dop-GR1-S	4.02±0.04	67.21±0.04
	GR-S5TT	4.24±0.03	64.40±0.08
10	Sdop1	3.28±0.08	418.07±0.09
	S-dop-GR1-S	3.30±0.05	414.48±0.08
	GR-S5TT	3.80±0.06	413.42±0.07
11	Sdop1	8.88±0.06	157.12±0.05
	S-dop-GR1-S	8.42±0.08	156.03±0.06
	GR-S5TT	8.76±0.07	153.37±0.06
12	Sdop1	4.84±0.05	48.23±0.03
	S-dop-GR1-S	4.50±0.08	48.48±0.05
	GR-S5TT	4.38±0.07	48.34±0.05
13	Sdop1	17.42±0.05	221.72±0.05
	S-dop-GR1-S	17.59±0.04	225.92±0.07
	GR-S5TT	17.30±0.04	224.18±0.07
14	Sdop1	8.88±0.03	224.39±0.05
	S-dop-GR1-S	8.92±0.04	225.00±0.06
	GR-S5TT	9.00±0.04	224.18±0.05

15	Sdop1	526.14±0.07	223.23±0.05
	S-dop-GR1-S	528.93±0.07	219.39±0.06
	GR-S5TT	536.68±0.05	224.18±0.06
16	Sdop1	266.26±0.05	241.48±0.05
	S-dop-GR1-S	267.20±0.04	240.37±0.04
	GR-S5TT	264.96±0.04	224.18±0.03
17	Sdop1	13.31±0.05	164.12±0.07
	S-dop-GR1-S	13.17±0.08	161.95±0.05
	GR-S5TT	12.45±0.07	163.68±0.05
18	Sdop1	5.10±0.03	35.47±0.03
	S-dop-GR1-S	5.44±0.04	35.25±0.03
	GR-S5TT	5.07±0.04	34.70±0.05
19	Sdop1	3.30±0.03	501.27±0.03
	S-dop-GR1-S	3.56±0.05	501.78±0.04
	GR-S5TT	3.80±0.04	505.56±0.04
20	Sdop1	3.32±0.03	78.42±0.03
	S-dop-GR1-S	3.82±0.07	78.05±0.05
	GR-S5TT	3.24±0.02	78.26±0.04
21	Sdop1	3.28±0.02	326.28±0.02
	S-dop-GR1-S	3.21±0.03	325.98±0.03
	GR-S5TT	3.28±0.03	326.96±0.04
22	Sdop1	9.20±0.05	176.20±0.05
	S-dop-GR1-S	8.99±0.03	177.68±0.04
	GR-S5TT	8.33±0.04	178.33±0.04
23	Sdop1	17.78±0.05	138.21±0.08
	S-dop-GR1-S	17.59±0.05	137.66±0.05
	GR-S5TT	17.54±0.06	134.24±0.04
24	Sdop1	7.20±0.04	357.47±0.03
	S-dop-GR1-S	7.22±0.03	358.43±0.07
	GR-S5TT	7.62±0.03	350.22±0.07
t-test	-	2.87	2.53

Table 5. Determination of CEA, and CA19-9 in urine samples (N=10)

Sample	Needle stochastic sensor based on PIX and	CEA (ng mL^{-1})	CA19-9 (U mL^{-1})
1	Sdop1	7.53±0.03	271.89±0.07
	S-dop-GR1-S	7.03±0.05	273.96±0.07
	GR-S5TT	7.00±0.05	271.90±0.05
2	Sdop1	5.57±0.03	228.50±0.05
	S-dop-GR1-S	4.99±0.03	229.03±0.04
	GR-S5TT	5.12±0.04	224.26±0.04
3	Sdop1	5.53±0.03	227.17±0.07
	S-dop-GR1-S	5.63±0.04	229.35±0.07
	GR-S5TT	5.38±0.05	224.26±0.04
4	Sdop1	4.27±0.03	87.17±0.03
	S-dop-GR1-S	4.63±0.05	86.95±0.05
	GR-S5TT	4.25±0.05	86.87±0.04
5	Sdop1	6.37±0.03	593.00±0.07
	S-dop-GR1-S	6.98±0.02	597.26±0.04
	GR-S5TT	6.84±0.03	595.12±0.07
6	Sdop1	0.99±0.04	202.55±0.05
	S-dop-GR1-S	1.38±0.05	202.76±0.04
	GR-S5TT	1.38±0.04	202.82±0.05
7	Sdop1	13.16±0.02	58.29±0.05
	S-dop-GR1-S	13.61±0.02	55.60±0.05
	GR-S5TT	13.57±0.03	53.48±0.04
8	Sdop1	2.47±0.03	501.47±0.04
	S-dop-GR1-S	2.01±0.05	500.60±0.04
	GR-S5TT	2.21±0.05	498.93±0.05
9	Sdop1	8.88±0.02	867.67±0.03
	S-dop-GR1-S	8.59±0.03	863.93±0.04
	GR-S5TT	8.08±0.07	864.89±0.04

10	Sdop1	4.62±0.07	411.05±0.03
	S-dop-GR1-S	4.90±0.05	417.58±0.02
	GR-S5TT	4.95±0.05	411.82±0.04
11	Sdop1	4.82±0.04	13.48±0.08
	S-dop-GR1-S	4.34±0.04	14.60±0.06
	GR-S5TT	4.40±0.07	13.48±0.06
12	Sdop1	7.60±0.03	47.98±0.02
	S-dop-GR1-S	8.69±0.07	47.60±0.05
	GR-S5TT	7.54±0.04	47.47±0.03
13	Sdop1	2.94±0.03	860.12±0.03
	S-dop-GR1-S	2.01±0.04	860.78±0.04
	GR-S5TT	2.70±0.03	852.16±0.04
14	Sdop1	6.37±0.03	10.69±0.04
	S-dop-GR1-S	6.15±0.05	11.03±0.03
	GR-S5TT	6.50±0.04	10.47±0.03
15	Sdop1	4.27±0.03	105.01±0.03
	S-dop-GR1-S	4.60±0.02	104.94±0.04
	GR-S5TT	4.89±0.03	104.75±0.04
16	Sdop1	3.28±0.05	148.12±0.05
	S-dop-GR1-S	3.99±0.05	147.08±0.03
	GR-S5TT	3.27±0.03	148.53±0.04
17	Sdop1	2.32±0.05	190.14±0.05
	S-dop-GR1-S	2.21±0.06	193.01±0.04
	GR-S5TT	2.20±0.05	191.50±0.04
18	Sdop1	4.87±0.03	59.43±0.07
	S-dop-GR1-S	4.39±0.05	62.92±0.06
	GR-S5TT	4.15±0.05	64.43±0.05
19	Sdop1	8.88±0.02	308.29±0.03
	S-dop-GR1-S	8.76±0.03	304.15±0.05
	GR-S5TT	8.15±0.02	304.43±0.05
20	Sdop1	5.54±0.07	78.06±0.04
	S-dop-GR1-S	4.97±0.03	75.75±0.07
	GR-S5TT	4.81±0.03	75.46±0.04
21	Sdop1	5.54±0.08	219.20±0.08
	S-dop-GR1-S	4.37±0.03	224.18±0.05
	GR-S5TT	5.27±0.03	220.16±0.04
22	Sdop1	4.84±0.04	56.63±0.03
	S-dop-GR1-S	4.98±0.04	50.60±0.05
	GR-S5TT	4.04±0.08	57.80±0.05
23	Sdop1	5.81±0.07	149.42±0.05
	S-dop-GR1-S	5.96±0.06	140.30±0.05
	GR-S5TT	5.90±0.07	142.73±0.04
24	Sdop1	11.34±0.04	225.34±0.07
	S-dop-GR1-S	10.91±0.05	225.30±0.07
	GR-S5TT	11.91±0.03	237.06±0.08
25	Sdop1	3.28±0.04	236.13±0.05
	S-dop-GR1-S	4.04±0.03	237.08±0.04
	GR-S5TT	3.55±0.05	234.87±0.04
t-test	-	2.18	2.55

Table 6. Determination of CEA, and CA19-9 in gastric tumoral tissue samples (N=10)

Sample	Needle stochastic sensor based on PIX and	CEA (ng mL^{-1})	CA19-9 (U mL^{-1})
1	Sdop1	4.79±0.03	111.85±0.05
	S-dop-GR1-S	4.36±0.05	111.58±0.07
	GR-S5TT	4.54±0.03	112.31±0.03
2	Sdop1	13.16±0.03	661.16±0.05
	S-dop-GR1-S	12.25±0.07	668.56±0.07
	GR-S5TT	12.70±0.06	662.75±0.08
3	Sdop1	5.38±0.02	226.64±0.05
	S-dop-GR1-S	5.72±0.04	230.95±0.07
	GR-S5TT	5.83±0.05	243.70±0.03

4	Sdop1	3.26±0.04	313.79±0.03
	S-dop-GR1-S	3.88±0.04	320.39±0.08
	GR-S5TT	3.03±0.08	311.52±0.02
5	Sdop1	4.49±0.04	169.49±0.03
	S-dop-GR1-S	4.38±0.05	167.96±0.02
	GR-S5TT	4.05±0.05	168.26±0.03
6	Sdop1	4.14±0.04	131.50±0.05
	S-dop-GR1-S	4.28±0.03	130.94±0.04
	GR-S5TT	4.74±0.03	131.06±0.05
7	Sdop1	7.96±0.04	59.99±0.05
	S-dop-GR1-S	7.24±0.04	60.70±0.05
	GR-S5TT	7.82±0.07	60.32±0.04
8	Sdop1	4.39±0.03	195.05±0.04
	S-dop-GR1-S	3.46±0.05	197.04±0.03
	GR-S5TT	4.74±0.05	198.03±0.03
9	Sdop1	2.10±0.05	88.02±0.02
	S-dop-GR1-S	2.71±0.05	87.94±0.07
	GR-S5TT	2.88±0.05	85.93±0.07
10	Sdop1	8.73±0.03	169.26±0.03
	S-dop-GR1-S	8.72±0.04	169.26±0.02
	GR-S5TT	8.38±0.04	168.07±0.04
11	Sdop1	4.17±0.03	175.29±0.04
	S-dop-GR1-S	4.02±0.04	174.30±0.07
	GR-S5TT	4.84±0.04	171.57±0.07
12	Sdop1	4.54±0.07	271.30±0.08
	S-dop-GR1-S	4.04±0.05	274.94±0.06
	GR-S5TT	4.49±0.05	275.25±0.06
13	Sdop1	4.17±0.03	277.67±0.03
	S-dop-GR1-S	4.15±0.03	275.84±0.05
	GR-S5TT	4.88±0.02	275.25±0.05
14	Sdop1	7.47±0.03	30.95±0.05
	S-dop-GR1-S	7.15±0.05	26.84±0.04
	GR-S5TT	7.04±0.04	27.27±0.04
15	Sdop1	8.88±0.02	509.25±0.04
	S-dop-GR1-S	9.01±0.03	507.26±0.04
	GR-S5TT	8.26±0.04	509.60±0.05
t-test	-	2.08	2.01

4. Conclusions

Three needle stochastic sensors were used as new tools for the fast screening test of biological samples for two gastric cancer biomarkers: CEA and CA19-9. The needle stochastic sensors showed high sensitivity, low limits of quantification, and wide linear concentration ranges. Accordingly, they can be reliably used as tools in the fast screening test of biological fluids making possible the early diagnosis of gastric cancer.

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