# A NEW POTENTIOMETRIC SENSOR FOR THE ASSAY OF P53 IN BLOOD SAMPLES

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*A potentiometric sensor based on graphite paste modified with a phthalocyanine-BODIPY conjugate was proposed for the assay of tumor suppressor P53. The working concentration range was between $1.17x10^{-9}$ and $2.93x10^{-8}$ μg/mL with a slope of 82.62mV/μg $mL^{-1}$ and a limit of detection was $3.37x10^{-10}$ μg/mL. The selectivity of the sensor was checked against possible interfering species from blood samples. The sensor was applied for the analysis of P53 in whole blood samples, without any pretreatment of the sample.*

**Keywords**: P53, colon cancer, potentiometric sensor, blood samples.

**List of Abbreviations**

MWCNT- multiwalled carbon nanotube
PA6 – doped nylon 6
PPy – polypyrrole
GCE –glassy carbon electrode
t-GO – thiolated graphene oxide

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bt - biotinilated
NPs – nanoparticles
BSA – bovine serum albumin
ABC – avidin-biotin-peroxidase complex
BODIPY - boron dipyrromethene
ECL - Electrochemiluminescence
DPV - Differential pulse voltammetry
IM-ECL - Immunomagnetic-electrochemiluminesce

## 1. Introduction

P53 is a well-known tumor suppressor gene that plays a vital role in the repairing of DNA, apoptosis and cell proliferation. It conserves the stability in cells by preventing genome mutation [1]. Moreover, the deactivation of p53 gene is the most commonly detected anomaly in human cancers and was noticed in more than half of the human cancers [2]. The cell proliferation is disrupted and tumors are formed once P53 is mutated. Also, p53 gene is mutated in 50% of human tumors [3]. Most studies have stated that mutant P53 is highly expressed and accumulated in cancer cells due to its prolonged half-life [4]. Furthermore, P53 mutation in serum could be a useful marker for the diagnosis of human colon cancer [5]. Consequently, the pathway of P53 plays a major role in regulation of cell growth and survival, in which the detection of P53 mutant level in human serum is an effective way for early diagnosis and prognosis of cancer. Numerous research works have been conducted regarding the quantitative detection of P53 for this purpose.

Several sensing techniques have been developed for detection of P53 such as surface plasmon resonance (SPR) [6], field-effect transistors [7], quartz crystal microbalance [8], colorimetry [9], chemiluminescence [10], enzyme-linked immunosorbent assay (ELISA) [11], and different electrochemical methods which are presented in Table 1. In this paper we proposed a potentiometric sensor based on graphite paste modified with a phthalocyanine-BODIPY conjugate (Figure 1) for the assay of P53 in blood samples.

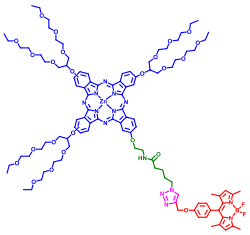

Fig. 1. Chemical structure of phthalocyanine-Bodipy conjugate

*Table 1*

**Different methods for the analysis of P53 tumor suppressor.**

| Method | Electrode type | LOD | LOQ | Linear concentration range | Biological sample | Ref. |
|---|---|---|---|---|---|---|
| DPV-*biosensor* | MWCNT-PA6-PPy/Au | 50 fmol/L | 100 fmol/L | 0.1 pmol/L – 100 pmol/L | - | 12 |
| ECL *ECL immunosensor* | Ru–silica nanoporous@gold nanocomposite/GCE | 22.8 fmol/L | 76.1 fmol/L | 0.2 pmol/L - 200 pmol/L | Serum Skin fibroblast cells | 13 |
| SPR *SPR chip* | Carboxymethylated-dextran modified Au sensor chip | 1.06 pmol/L | 0.01 nmol/L | 0.01 nmol/L – 1.06 nmol/L | Normal/ Cancer cells | 14 |
| DPV *immunosensor* | t-GO/Streptavidin-Au NPs/bt antibody /BSA/antigen/bt secondary antibody/ABC/GCE | 30 fmol/L | 115 fmol/L | 0.2 pmol/L - 200 pmol/L | Serum Skin fibroblast cells | 15 |
| (IM-ECL) | Pt Electrode | - | 0.01 ng/mL | 0.01 ng/mL - 1000 ng/mL | Serum | 16 |
| Potentiometry *potentiometric sensor* | Phthalocyanine-boron dipyrromethene /Graphite | 0.34 fg/mL | 1.17 fg/mL | 1.17 fg/mL – 29.3 fg/mL | Whole blood | This work |

## 2. Experimental

### 2.1. Reagents and materials

Tumor suppressor P53, graphite powder, monosodium phosphate and disodium phosphate were purchased from Sigma Aldrich (Milwaukee, USA). MEG107porphyrin ($C_{106}H_{139}BF_2N_{14}O_{24}Zn$) was synthesized in the house by the group of Professor Mahmut Durmus. Paraffin oil ($d_4^{20}$, 0.86 g x $cm^{-1}$) was purchased from Fluka (Buchs, Switzerland). Monosodium phosphate and disodium phosphate were used for preparation of phosphate buffer 0.1 mol/L, pH=7.5. Deionized water obtained from a Millipore Direct-Q3 System (Molsheim, France) was used for the preparation of all solution. Standard solution of P53 was prepared in PBS pH=7.5 to a concentration of 7.14μg/mL. Serial dilution technique was used for the preparation of solutions of different concentration for P53. All solutions were of analytical grade.

### 2.2. Apparatus

For all potentiometric measurements an Ivium potentiostat/galvanostat was used. The potentiometric cell is composed from a two-electrode system: reference and working electrode. Ag/AgCl electrode served as reference electrode in the cell.

### 2.3. Sensor`s design

Modified graphite paste was prepared as follows: 100 mg of graphite powder was mixed with 30 μL of paraffin oil to form a homogenous paste. 100 μL of $10^{-3}$ mol $L^{-1}$ phthalocyanine-BODIPY conjugate was added to this paste, to obtain the modified paste. Electric contact was made using a silver wire (0.5 mm in diameter). Between the measurements, the sensor was washed with deionized water. When not in use, the graphite potentiometric sensor was kept in a dry place at room temperature.

### 2.4. Recommended procedure

Direct potentiometry was used for the measurements of the potential of each solution of known concentration (1.17 fg/mL – 7.14 μg/mL). The working electrode was placed in stirred standard solution while the potential was recorded. The equation of calibration was obtained using statistics, and used for calculations of unknown concentrations of P53 in whole blood samples.

### 2.5. Sample preparation for whole blood

Whole blood samples were obtained from the University Hospital in Bucharest (Ethics committee approval nr. 75/2015) from 5 different patients diagnosed with colon cancer. These samples were used for the assay of P53 without any pretreatment. The cell was filled with the whole blood and the potential was measured for 5 minutes for each sample. The unknown concentrations were determined from the calibration curve as described in the direct potentiometry method.

## 3. Results and discussions

### 3.1. Characteristic response of potentiometric sensors

The mechanism of P53 recognition by phthalocyanine BODIPY is based on formation of host-guest compound between the analyte (P53) and ligand (phthalocyanine BODIPY) at the membrane-solution interface [17].

The sensor based on graphite paste modified with phthalocyanine-BODIPY conjugate was tested using direct potentiometric method for the analysis of P53. The mechanism of potential development was explained by the interaction between P53 and phthalocyanine-BODIPY conjugate at the membrane-solution interface. The proposed sensor showed linear and near-Nernstian response, so that it can be used for the determination of P53. The sensor based on graphite paste modified with phthalocyanine-BODIPY conjugate can be used for the assay of P53 in the linear concentration range 1.17 fg/mL – 29.3 fg/mL. The response time of the sensor was 5 minutes. The limit of quantification reached with the sensor based on phthalocyanine-BODIPY conjugate/graphite for P53 was 1.17 fg/mL. The proposed sensor covered the range on which P53 can be found on patients not

clinical ill as well as for patients presenting stages 1 - 3 of colon cancer. The equation of calibration recorded was:

$$E = -617.4 + 82.6 \times pP53; r = 0.9999 \quad (1)$$

where pP53 is $-\log C_{P53}$ and r is the correlation coefficient. The standard potential was -617.4±2.47mV, and the slope of the sensor was 82.6±0.93 mV/decade of concentration. The limit of detection was 0.34 fg/mL. Calibration graph is shown in Figure 2.

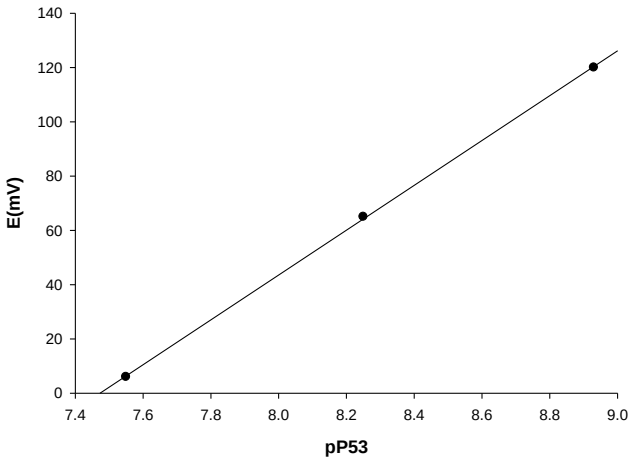


Fig. 2. Calibration graph of the potentiometric sensor

The sensor was used for more than one month for measurements, and calibrated every day. The RSD of the slope of the proposed sensor was 1.25% after 30 days of daily use. Also, 10 such pastes were made and used in sensors' bodies for measurements – when the values of RSD of the slopes obtained was 0.24%, showing good reproducibility of sensor's design.

The response characteristics of the proposed sensor made possible the detection of P53 in whole blood at a very early stage, when its concentration is less than 10ng $mL^{-1}$ [12-16].

The advantages of the proposed sensor and method for the determination of P53 are versus the other methods proposed to date [12-16]: it has the lowest determination and detection limits; the range of the working concentration favorized its early detection in the biological samples; it is the only one that was used for whole blood screening.

### 3.2. Selectivity

Dopamine (DA), norepinephrine (NE), epinephrine (Epi), acetylcholine (Ach), kirsten rat sarcoma viral (KRAS), human epidermal growth factor receptor

1 (HER1) and carcinoembryonic (CEA) have been chosen as possible interfering species in the detection of P53. Mixed solution method was employed to determine the potentiometric selectivity coefficient. The ratio between the concentration (mol/L) of the interfering species and P53 was 10:1. The results are shown in Table 2. The values obtained for potentiometric selectivity coefficients shown that the selected substances are slightly interfering with the determination of P53.

*Table 2*

**Potentiometric selectivity coefficients for the sensor based on phthalocyanine-BODIPY conjugate/Graphite**

| Potentiometric sensor based on | $K_{sel}^{pot}$ | | | | | | |
|---|---|---|---|---|---|---|---|
| Phthalocyanine-BODIPY conjugate /Graphite | DA | Epi | NE | Ach | KRAS | HER-1 | CEA |
| | $2.1x10^{-3}$ | $1.2x10^{-3}$ | $1.2x10^{-3}$ | $1.8x10^{-3}$ | $1.5x10^{-3}$ | $1.5x10^{-3}$ | $7.7x10^{-3}$ |

Taking into account the mechanism of potential development specific for potentiometric sensors, the main explanation for high selectivity of the proposed sensor versus selected possible interferences is that the ligand is forming less stable complex compared with those formed by P53.

### 3.3. Analytical applications

The characteristics of response recorded for the sensor based on phthalocyanine-BODIPY conjugate/Graphite (linear concentration range of determination, selectivity, and sensitivity) revealed that the sensor could be used for the evaluation of P53 in biological fluids. Consequently, the proposed sensor was used for the analysis of P53 in whole blood samples. The five samples of blood were supplied from the hospital, and used as collected, for the evaluation of P53 by using the proposed sensor. Therefore, the results obtained by using the sensor based on phthalocyanine-BODIPY conjugate/Graphite show that it is a reliable tool for the evaluation of P53 in whole blood samples (Table 3). The proposed sensor based on phthalocyanine-BODIPY conjugate/Graphite can detect P53 at lower concentrations.

*Table 3*

**Recovery tests of P53 in blood samples.**

| fg/mL, Recovery of P53 | | | | | |
|---|---|---|---|---|---|
| Sample no. | 1 | 2 | 3 | 4 | 5 |
| | 5.62±0.07 | 6.76±0.09 | 5.00±0.07 | 4.68±0.08 | 1.17±0.08 |

A control sample taken from a healthy patient showed no P53 in the blood sample.

## 4. Conclusions

The proposed sensor shown very good results for the recovery test which makes it a reliable tool for measuring P53 in whole blood samples, and this is very important for early detection of colon cancer. The proposed method was highly sensitive, exhibited good selectivity for the detection of P53 from blood samples. The advantages of the proposed method versus techniques like ELISA and chromatography are the following: it is a simple and easy method performed with low cost, short analysis time, and low limit of quantification (1.17 fg/mL), being able to detect at an early stage the colon cancer.

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