ELECTROANALYSIS OF MASPIN IN WHOLE BLOOD

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Maspin is a recently introduced biomarker that is utilized in the diagnosis of gastric and colon cancers. Electroanalysis of maspin was performed using an electrode constructed by modifying a graphite paste decorated with spheroidal Cu with ß-cyclodextrin. A high sensitivity (185.69 A mol^{-1}mL) was obtained, coupled with extremely low determination limits (5.12 pg mL^{-1}) and detection (2.05 pg mL^{-1}) were obtained. The electrode can be reliably employed in order to do the analysis of maspin in samples of whole blood.

Keywords: Maspin, electroanalysis, whole blood

1. Introduction

The biomarker known as Maspin, which was first identified in 1994, belongs to the serpin family [1]. Thus far, it has been utilized as a biomarker for the purpose of diagnosing breast, prostate, skin, lung, stomach, and colon cancers [1]. Since its initial identification as a biomarker for cancer detection, several clinical studies have been conducted to elucidate its significance in both diagnostic applications [2-4] and customized therapeutic interventions [5].

Subsequent investigations have shown evidence that maspin fulfills an important function in the development of malignancies, making it a valuable tool in research activities aimed at comprehending the genesis of tumors [6]. The genetic association of maspin with gastric cancer, namely the intestinal form of gastric adenocarcinoma, has been shown in previous studies [7,8]. Recent studies have indicated that the measurement of maspin concentrations in various biological fluids, such as whole blood, saliva, and urine, can potentially offer valuable insights into several important aspects of gastric and colon cancers. The maximal size of the tumor as well as its location, the pN values produced from the TNM staging system, the existence of budding and mucinous characteristics, and the pT values generated

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from the TNM organizing system are some of these factors. Furthermore, maspin concentrations may also provide information regarding the molecular subtype of these cancers [9,10]. Several devices and methodologies have been previously introduced for the measurement of maspin levels in biological samples, including whole blood. This study utilized stochastic sensors in two-dimensional (2D) and three-dimensional (3D) designs as screening devices for the purpose of determining the maspin concentrations present in whole samples of blood [11,12]. ELISA is widely utilized as the conventional method for quantifying maspin in clinical laboratory settings, with several pharmaceutical firms producing distinct ELISA kits for this purpose.

Although traditional methods have their own merits, innovative approaches provide improved features such as rapid reaction, cost-effectiveness in terms of reagents, and user-friendly equipment. Sensors, as compared to conventional methods, provide a range of benefits, making them a feasible choice for quantitative measurements. The effectiveness of a sensor is dependent on the basic parts utilized in its development. The capacity to identify the analyte at extremely low concentrations with a heightened level of sensitivity, together with the ability to discern it selectively from other entities within a biological sample, are essential characteristics for sensor materials. The utilization of electrodes for the electroanalysis of maspin in whole blood is supported by the following advantages: Sampling is unnecessary as the electrode can be directly inserted into the blood sample to determine the levels of biomarkers, such as maspin. The analysis is completed rapidly, typically within minutes. The utilization of miniaturized instrumentation enables efficient screening of large populations. Moreover, this method proves to be cost-effective due to the electrode's high level of robustness and reliability, as supported by previous studies [13,14].

The originality of this study lies in the use of an electrode constructed by modifying a graphite paste decorated with spheroidal Cu with ß-cyclodextrin, in the screening tests of whole blood for maspin.

2. Experimental

2.1. Materials and reagents

The following components were purchased from Sigma Aldrich in order to carry out the experiment: maspin (<0.1 EU/μg endotoxin), graphite powder (< 20 μm particle size), ß-cyclodextrin (≥97% purity), monosodium phosphate (99% purity), disodium phosphate (99% purity), and spheroidal copper (98% purity). In addition, the company Fluka was responsible for the procurement of the paraffin oil (d_4^{20}, 0.86 g × cm^{-1}). Both disodium and monosodium phosphate were utilized in the production of the buffer solution, a phosphate solution with a concentration of 0.1 mol L^{-1} and a pH value of 7.4. Deionized water that had been obtained by using the Millipore Direct-Q3 System was employed in the step of preparation so

that all of the solutions could be made. The serial dilution technique was employed to generate a range of maspin solutions with concentrations of up to 100 ng mL^{-1}.

The Maspin Human ELISA Kit was acquired from Aspira Chemical, a supplier based in the United States. The study employed an enzyme-linked immunosorbent test (ELISA) kit that was readily available for the purpose of determining the presence of maspin in various samples, including human serum and cell culture media. The identification of maspin antigen targets in samples is facilitated by the utilization of the ELISA analytical biochemical method provided by the kit. This approach depends on the interactions between maspin antibodies and antigens, employing immunosorbency. Additionally, it utilizes an HRP colorimetric detecting technology.

2.2. Equipment

Electrochemical measurements were conducted using a miniaturized PGSTAT instrument (PalmSens BV, Houten, The Netherlands). A connection had been established between the device and a laptop in order to facilitate the collection and analysis of data. The electrochemical cell consisted of three components: a working electrode composed of a cyclodextrin-based material, a reference electrode made of an Ag/AgCl wire, and a counter electrode consisting of a Pt wire.

The Stratasys objet 24 printer uses a technique known as polyjet to construct three-dimensional objects in a layer-by-layer manner. A hard white opaque polymer known as vero white plus was chosen as the material to be used. The fullcure 705 support material is an acrylic photopolymer that has the consistency of a gel and is nontoxic. It is also very easy to wash. The optimal working conditions involved 18-25 degrees Celsius and 30-70% relative humidity.

2.3. Preparation of the electrode

A homogeneous paste was obtained by combining 200 mg of graphite with 2 mg of spheroidal Cu, followed by the addition of paraffin oil to the mixture. A β-cyclodextrin solution with a level of concentration that is equal to 10^{-3} mol L^{-1} was introduced into the paste, resulting in the creation of a modified paste. After the paste was altered, it was placed inside a tube made of plastic that had been fabricated using 3D printing technology, with an internal diameter of 100μ. To build a link established between the used paste and the outside circuit, an Ag wire was utilized as an electrical contact. The electrode had been placed at room temperature while it was not utilized.

2.4. Procedure

Differential pulse voltammetry (DPV) was employed for all analyses. The utilized scanning range spanned from -1V to +1V, while maintaining a scan rate of 90 mV s^{-1}. The linear regression technique was employed to determine the parameters of the calibration equation. By entering the value for the current's

intensity into the equation of calibration for maspin, the concentrations of maspin in whole blood, which were previously unknown, were determined by calculation.

Sandwich enzyme immunoassay is the testing methodology that is implemented within this kit. The microtiter plate that is included in this kit already had an antibody that is specific to Maspin applied to it before use. After that, a biotin-conjugated antibody specific to Maspin is injected into the appropriate wells of the microtiter plate, and either standards or samples are added. The next step is to add Avidin that has been conjugated to Horseradish Peroxidase (HRP) to each well of the microplate and then incubate the mixture. After the addition of the TMB substrate solution, the color of the wells will only change if they contain Maspin, an antibody that is biotin-conjugated, and Avidin that has been enzyme-conjugated. The enzyme-substrate reaction is stopped when a solution of sulfuric acid is added, and the spectrophotometric analysis of the color change is performed at a wavelength of 450 nm +/- 10 nm. The paired sample t-test, also known as the dependent sample t-test, is a statistical method employed to assess if the mean difference between two sets of data is statistically significant, or in other words, whether it is likely to be zero. In the context of a paired sample t-test, it is customary to measure each subject or object on two separate occasions, generating pairs of observations. The statistical tool used for this test was Microsoft Excel (Data Analysis).

2.5. Samples

Patients with proven stomach and colon cancer had their whole blood collected. Prior to the analysis, there was no pretreatment. The samples utilized in this study were procured from two medical institutions: the Emergency Clinical Hospital of County Targu-Mures and the Clinical Hospital of County Targu-Mures. The research was conducted with the approval of the respective Ethics Committees, with the Emergency Clinical Hospital receiving permission on December 14, 2018 (approval number 32647/14.12.2018), and the Clinical Hospital obtaining permission on February 28, 2019 (approval number 3206/28.02.2019). All patients provided their informed consent.

3. Results and discussion

3.1. Response characteristics of the electrode utilized in the electroanalysis of maspin

All the response characteristics were obtained using DPV, at 25°C. A working concentration range between 5.12 pg mL^{-1} and 400 ng mL^{-1}, with a limit of determination, as defined by the lowest concentration observed within the linear concentration range in accordance with the updated IUPAC guideline (International Union of Pure and Applied Chemistry) (paragraph 3.36, Note 3) [15]), of 5.12 pg mL^{-1} and a limit of detection (calculated as the concentration for which the value of

the intensity of the current is 3 times the value of background current) of 2.05 pg mL^{-1} were determined. The equation of calibration of the electrode obtained using the DPV (Fig. 1 and 2) is:

$$I = 851.30 + 185.69 \times C_{maspin} \quad (1)$$

where I denotes the current's strength as measured in A and C_{maspin} denotes the maspin's concentration as measured in ng mL^{-1}. There is a 0.9982 correlation coefficient.

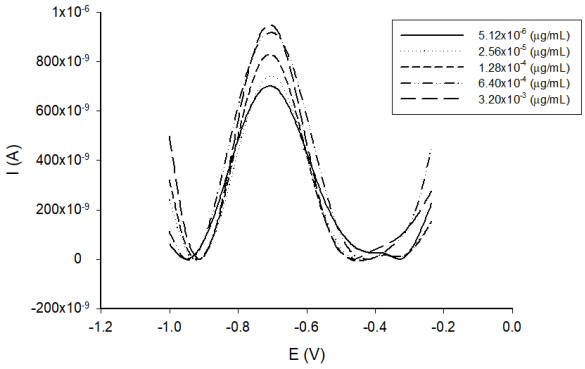

Fig. 1. Differential pulse voltammogram obtained for maspin at different concentrations.

The large linear domain of concentration (5.12 pg mL^{-1} - 400 ng mL^{-1}) facilitated the quantification of maspin in whole blood samples collected from patients at various stages of cancer.

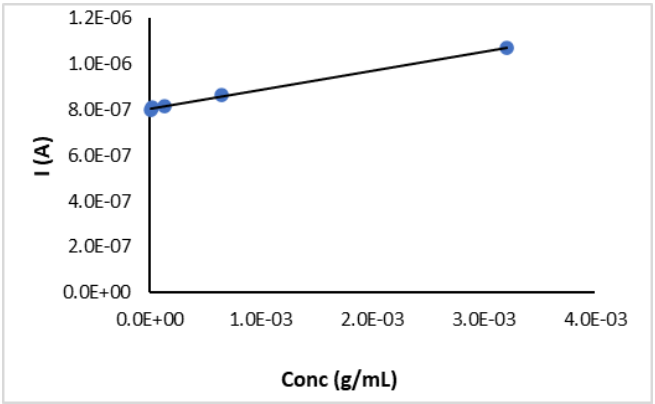

Fig. 2. Calibration of the electrode utilized in the analysis process of maspin.

As it can be seen from Fig. 2, the values we obtained for maspin for patients having different stages of cancer, are correlated with the values from the calibration curve.

Therefore, the achievement of a low determination limit for the electrode facilitated the early-stage detection of stomach and colon malignancies.

3.2. Selectivity of the electrode

HER3, HER4, L-serine, L-aspartic acid, L-tryptophane, D-leucine were considered as possible interference. In order to perform an investigation on the selectivity of the suggested electrode, the mixed solution technique was utilized. A ratio of 1:10 (mol/mol) between the maspin and the supposed interferent was used when synthetic mixed solutions were prepared. Amperometric selectivity coefficients were determined, and are: 5.2×10^{-4} for HER3, 3.0×10^{-4} for HER4, 3.1×10^{-4} for L-serine, 2.6×10^{-4} for L-aspartic acid, 2.9×10^{-4} for L-tryptophane, and 3.2×10^{-4} for D-leucine. These values shown that HER3, HER4, L-serine, L-aspartic acid, L-tryptophane, D-leucine interference in the determination of maspin was not observed.

3.3. Electroanalysis of maspin in whole blood

Fig. 3 displays a representative voltammogram acquired during the utilization of the electrode for the analysis of maspin in whole blood.

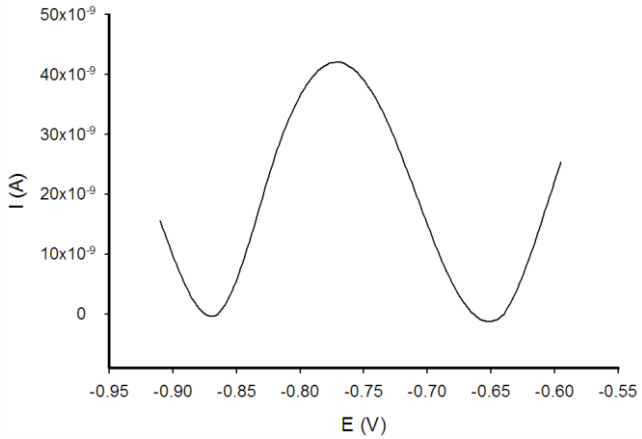

Fig. 3. Typical voltammogram recorded for measuring maspin levels in human blood.

The samples of whole blood were analyzed without any kind of preliminary treatment. A comparative analysis was conducted between the outcomes acquired through the utilization of the suggested electrode and those obtained by ELISA, which serves as the established technique applied in clinical laboratories. The findings are presented in Table 1.

Table 1

Electroanalysis of maspin in whole blood (N=10).

Sample number	DPV	ELISA
	Maspin, pg mL^{-1}	
1	144.97±0.12	143.20±0.65
2	253.03±0.17	254.00±0.87

3	296.50±0.23	295.70±0.43
4	170.52±0.14	170.30±0.67
5	279.36±0.15	280.00±0.34

The results of a paired t-test that had a degree of confidence of 99.00% (with a tabulated value of 4.13) was carried out to compare the results obtained from enzyme-linked immunosorbent assay (ELISA) with those obtained from the use of the suggested electrode in the differential pulse voltammetry (DPV) mode.

This comparison was created with the purpose of determining whether method produced more accurate results. Since the resulting value of 1.87 is lower than the value that was tabulated, this suggests there is a lack of discernible distinction that can be considered statistically significant between the two approaches. This suggests that the proposed electrode can be considered a reliable screening tool in the differential pulse voltammetry (DPV) mode to measure maspin levels in samples of blood.

4. Conclusions

A new electrode was constructed for the analysis of maspin in samples of whole blood. The proposed differential pulse voltammetry technique is able to determine reliable from the whole blood maspin, at concentrations that can be associated with early to later stages of colon and gastric cancer. The primary advantage of the approach that was suggested and electrode is the employment of them as mass screening test for colon and gastric cancers, especially that no sampling of whole blood is needed, and that this method is cost-effective.

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R E F E R E N C E S

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