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Research Article

Biosensing Using a Simple Resistor: The Effect of Functionalization on Sensing Devices

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Biosensors can play an important role in early disease detection, a reason why they are gaining more attention in the world of biomedicine. Functionalization of the material used in the detector is of a great importance since it maintains the molecule's structure of interest with minimal changes. We report on sensing BSA molecules, solutions, and concentrations using a functionalized commercial resistor in a simple electric circuit. Our results demonstrate the outstanding utility of functionalization in biosensing devices; while sensing is not possible with a naked resistor, a BSA covered resistor can detect a very low solution concentration around 0.1 fM. A smaller molecule like tryptophan was also used in order to functionalize the resistor. After proving that tryptophan is more effective in covering the resistor before sensing, BSA molecules in other solvent conditions were detected, and a threshold of 1 μM was obtained. This can prove that sensing depends on the choice of the functionalizations of the material used for sensing and on the conformation of the molecule of interest in its solutions. This method of detection may be of great interest in triggering and sensing biological molecules using simple-based devices.

1. Introduction

Electric biosensors are devices combining a biological part (enzymes, antibiotics, phages, aptamers, or ssDNA) with an electric part (electrochemical, optical, thermometric, piezoelectric, or magnetic) [1]. Biosensors track biomarkers; the presence or absence of a biomarker is an indication that can be used to diagnose a disease, so the lower the concentration of detection during screening is, the earlier the detection and subsequent treatment can commence [2]. For maximal detection, nanostructures are being used in biological detection as they offer many advantages such as broadening the area available for electrochemical detection, accelerating detection by propelling the electrons faster, and/or acting as a landing dock or attachment site for biomolecules [3], and the field effect transistors (FETs) based on ion selectivity (ISFETs) are the most attractive models [4].

Early detection can prove to be detrimental in most of the diseases. Scientists have been struggling to find methods and techniques that allow detection at the lowest concentration possible with minimal biological modification. The delicacy of biomolecules has steered scientists into designing alternative detection models that involve minimal chemical manipulation such as using currents or radiation to conserve biological integrity [5]. One of the available options is to use electric detection as an alternative to chemical treatment [6]. Chemical manipulation is delicate and often requires a complex manipulation. With the use of electric conductors, semiconductors, and superconductors, electric current can be used in a way to detect the molecule of interest.

In addition to this, functionalization of the material used in the detection is possible. This manipulation is supposedly gentler and does not overly disrupt the biological molecules of interest. When dealing with biological molecules, it is

important to maintain their conformation during detection. As the surface of the inorganic materials used for detection is always toxic and can denaturate or change the structure of the molecule of interest, functionalization with biomolecules can solve this problem. This latter requires the biomolecule to be adsorbed or attached to the surface of the material used for sensing before detecting the molecule of interest. This justifies the importance of functionalization. There have been several studies that proved that functionalization is a viable option for biomolecular sensing [7, 8].

Proteins are the most diverse molecules found in nature. They are made up of various lengths and recombination of 20 amino acids that serve various structural and functional purposes inside the cell [9]. The biological materials tested were the bovine serum albumin (BSA) and tryptophan. BSA is the most abundant protein within the blood making up to 60% in some situations weighing 66.5 KD. Structure wise, BSA is formed of 3 similar structural domains where each is divided into two subdomains. It is a highly stable molecule as each domain is 70% alpha helixes, 9 loops, and 17 disulfide bonds [10]. The abundance of BSA is marked by the wealth of studies that involve it despite already been extensively researched before. Phan et al. [11] studied BSA attachment on self-assembled monomers and discovered that charged surfaces allow for great BSA adsorption. They also noted that adsorption happens below pH = 2 which is lower than the isoelectric point of BSA. Li et al. [12] also studied the interaction of BSA with Cy3 dye and managed to detect BSA at concentrations as low as 5×10^{-8} M. So despite being extensively used, BSA is still a very attractive molecule for detection and for functionalization of materials since its electric charge allows its adsorption. The second molecule used was tryptophan or (2S)-2-amino-(1H-indol-3-yl) propanoic acid, an essential amino acid that must be obtained as it plays a critical role in protein synthesis. It is the least abundant amino acid, so it is a determinant for the rate of protein synthesis [13]. Its deficiency can cause several diseases such as dementia, coeliac disease, Parkinson's and much more, so it is a likely candidate for detection studies and functionalization.

As mentioned before, resistors were used in this study. Resistance follows Ohm's law that relates resistance to voltage (V; unit voltage) and current (I; unit A) as follows: V/I. During our study, owning to the fact it is still exploratory, we conducted the experiments using commercial resistors. The wirewound resistor is made up of a wire wrapped around an insulating material with desirable heat properties [14]. The carbon composition resistor is made by rendering carbon into a fine powder that is later mixed with resins to obtain the desired resistance. Carbon film resistors are another example of the commercial type that is made by applying a very thin layer of carbon on a ceramic rod.

The aim of this study is to realize an electric biosensor using a simple commercial resistor with the aid of functionalization performed with two different molecules in a way to study its effect. The molecule of interest, BSA, is detected with the resistor in an electric circuit including a power supply and two multimeters.

2. Materials and Methods

The aim of this paper is to sense biomolecules, obtain low threshold values, and verify whether or not functionalization can improve the detection of biomolecules after adsorption on resistors. Therefore, the equipment, reagents, solutions, and measurements used are relatively simple and nonspecific.

2.1. Apparatus

2.1.1. Commercial Resistors (5W 270RJ). They are the surface where the biomolecules will be absorbed on. When bought, they were coated as seen in Figure 1(a). They were carefully removed from their casing by the aid of a knife followed by immersion in 0.1 dilution of HCl. HCl has two roles: cleaning and deoxidization of any residue. The end result is the resistor seen in Figure 1(b).

In our study, more than 100 of the concerned resistors were used, and the current was measured every time on the naked resistors before any treatment; results show 3.6 ± 0.2 mA at 1 V, 7.6 ± 0.3 mA at 2 V, and 14.4 ± 0.2 mA at 4 V. So, manufacturing repeatability is very good, and the errors seen on the naked resistors are within the errors of our detections.

2.1.2. Metrix Digital Power Supply Model AX 503. The voltages used are low and range from 1 to 4 V. The digital power supply used is adequate for the purpose. The characteristics of the chosen model are listed below:

(i) Designed for indoor use below 2000 m

(ii) Temperature range 0-50°C

(iii) <80% humidity up to 40°C

(iv) Supply current: 115 V or 230 V ± 10%

(v) Frequency: 50-60 Hz

(vi) 2 displays: voltage in green and current in red

2.1.3. YFE Digital Multimeter Model YF-3503. The characteristics are listed below:

(i) Temperature range 0-40°C

(ii) <80% humidity

(iii) LCD 3 1/2 digits with a maximum reading of 1999

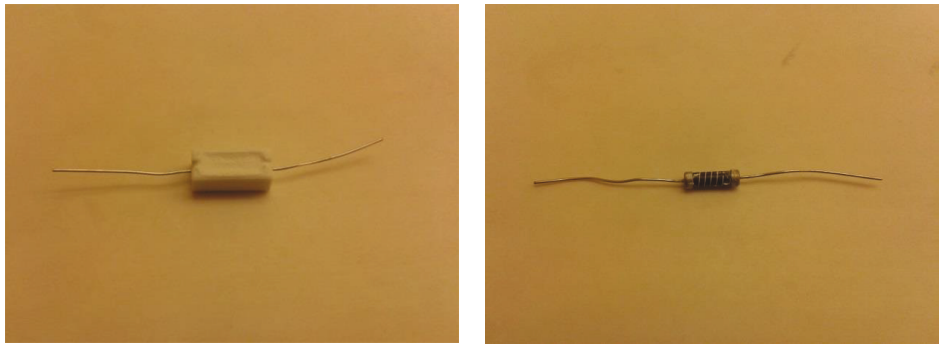
(iv) Power supply 006P DC 9 V

(v) AC/DC measurements

(vi) DC range < 1000 V and AC range < 750 V

2.2. Reagents. All listed chemicals were of the analytical reagent grade.

2.2.1. Bovine Serum Albumin (BSA) 66,000 g/mol. BSA is the molecule of interest that was tested; it is also used to functionalize the resistor before sensing. BSA is very close to the human albumin which makes it an attractive molecule



(a) (b)

Figure 1: Used resistor before (a) and after (b) cleaning and deoxidization.

for testing. The characteristics of the BSA used are listed in Table 1.

2.2.2. Tryptophan. Tryptophan is one of the essential amino acids that cannot be synthesized by the body, but is the building block of many important neurotransmitters. It is much smaller than BSA and chemically different as well. This makes it an attractive choice for functionalization. Table 2 is a list of the characteristics of the tryptophan used.

2.2.3. NaCl. NaCl was used as a solvent to prepare dilutions of BSA to be checked for current values. The characteristics are listed below in Table 3.

2.3. Solutions and Dilutions. The experimental part consisted of creating different dilutions of BSA, tryptophan, and NaCl. Three stock solutions were prepared in a first round, and all others are dilutions from the stock ones:

(1) 9 g/L NaCl solution in ultrapure water used as a solvent

(2) 6 g/L of BSA in NaCl

(3) 6 g/L of tryptophan in NaCl

The dilutions were made as follows:

(i) 10^{-3}, 10^{-5}, 10^{-7}, 10^{-9}, 10^{-11}, 10^{-12}, and 10^{-14} dilutions of BSA stock solution dissolved in NaCl

(ii) 10^{-3} dilution of tryptophan stock solution dissolved in NaCl

(iii) 10^{-3}, 10^{-5}, 10^{-9}, 10^{-11}, 10^{-12}, and 10^{-14} dilutions of the stock NaCl solution dissolved in ultrapure water

The 10^{-14} dilution of NaCl stock in ultrapure water is used as a solvent for another stock solution of 6 g/L of BSA and its dilutions. The dilutions (1/2, 0.1, 0.01, and 0.001) of this latest stock solution are made also using the10^{-14} dilution NaCl solvent.

2.4. The Electric Circuit. A uniformed resistor (5W, 270RJ) was placed as seen in the circuit of Figure 2. Resulting current of the different dilutions was collected at varying voltages. This was done by dipping the resistor in the solution and then slowly increasing the voltages according to desired values. The resulting currents were recorded from the multimeters.

2.5. Obtaining Measurements. Two types of measurements were done. The aim is to see if there is a difference between with and without functionalization. The first is without functionalization to assess if there is a specific relationship among the concentration of the BSA solution and the current values. Should it be proven that there is none, the next step is to functionalize and see if any relationship has been obtained.

2.5.1. Measurements without Functionalization. This method is to verify whether a valid relationship exists between current and concentration without functionalization. Before any measurement, the outer casing of the resistor must be removed. The resistor is then incubated in a 0.1 dilution HCl solution for two minutes. The resistor would be deoxidized. After washing the resistor with ultrapure water, it is now ready to be used.

(1) Assemble the circuit as shown above in Figure 2

(2) Drying the resistor after the HCl treatment and measure the current without solutions

(3) Insert the resistor in the prepared BSA solution

(4) Calibrate the voltmeter to get the most accurate reading

(5) Measure the current at 1, 2, and 4 V

2.5.2. Measurement with Functionalization. This method will necessitate the functionalization of the resistor. In this case, physical adsorption and self-assembly were adopted. This means incubating the resistors enough time for the molecules to attach themselves to the surface. After getting the resistor out of the casing and HCl treatment as shown before, the resistor is functionalized and then ready to detect the BSA dilutions:

(1) Assemble the circuit as shown in Figure 2

Table 1: Characteristics of BSA.

Category	Status
Assay	>96% agarose gel electrophoresis
Form	Lyophilized powder
Molecular weight	~66 KDa
Solubility	H_2O soluble 40 mg/mL
Stability	Suitable for cell culture
Storage temperature	2-8°C

Table 2: Characteristics of tryptophan.

Category	Status
Grade	Certified reference material
Form	Neat
Applications	HPLC suitable
	Gas chromatography suitable

Table 3: Characteristics of NaCl.

Category	Status
Assay	>99.5% AT
Impurities	Filter test compliance
	Insoluble matter compliance
pH	5.0-8.0
Melting point	801°C
Solubility	H_2O soluble 1 M clear and colorless

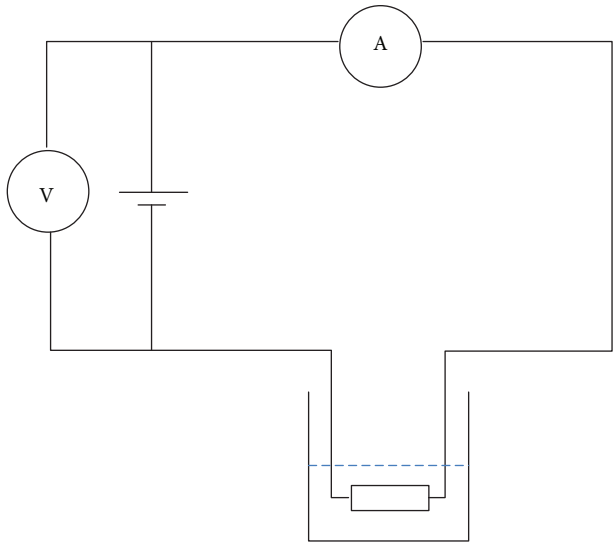


Figure 2: Schema of the circuit used to obtain measurements.

(2) Dry the resistor after the HCl treatment and measure without solutions

(3) Functionalize the used resistor: incubate the resistor in 10 mL of 10^{-3} dilution of the BSA (or tryptophan following the desired experiment) stock solution for two hours and then wash with ultrapure water several times

(4) Dry and measure to see the effect of the functionalization

(5) Repeat steps 3 to 5 from method 1

3. Results and Discussion

The first step involved measuring the current values without any prior functionalization. This is done in order to see if there is a clear trend for the variation of the resistor's current while using different concentrations of BSA in NaCl stock solution. After obtaining the deoxidized naked resistor, the resistor was then immersed in different dilutions of BSA solutions each of 10 mL volume, and the current was measured first at 1 V for each solution, then for 2 V, and finally for 4 V. Immersion starts from the more diluted (10^{-12}) to the more concentrated (10^{-3}) solution of BSA. The recorded currents obtained are shown in Figure 3.

The results in Figure 3 show that there are no specific trends of variations in terms of the different concentrations for any of the chosen voltages. The measured currents for different dilutions at a fixed voltage are random despite that all the dilutions originate from the same stock. It is also clear that at 4 V, the differences between BSA solutions are the highest even if it is not significant because there is no specific trend.

The next step was to assert whether the functionalization of the resistor prior to immersion in the solutions will modify the obtained results. In order to functionalize the resistor, the latter was first deoxidized, second incubated in 10 mL of 10^{-3} dilution of the BSA stock solution for two hours, and finally rinsed. After all these steps, the functionalized resistors were ready to be used in the electric circuit in a way to detect the different concentrations of the BSA solution. Before the solution measurements, the current for the naked and the functionalized resistors without any solution or immersion was measured at 1, 2, and 4 V. The recorded current values are shown in Table 4.

Table 4 shows that the measurements after functionalization were very different. The difference is more expressed at 4 V where the naked resistor current was at 14.3 ± 0.1 mA, and after functionalization, it was increased to 15.7 ± 0.5 mA. This can prove that the BSA molecules are well adsorbed on the resistor surface even after rinsing with ultrapure water.

From now on, the functionalized resistors are used in order to detect the different BSA solutions. Steps of the nonfunctionalized methods are repeated, and the resistors after functionalization were then immersed in different dilutions of BSA solutions each of 10 mL volume, and the current was measured at 1, 2, and 4 V. Figure 4 shows the recorded currents and their variations with respect to the measurements obtained after functionalization.

It can be noted from Figure 4 that there is a clearer trend of variation in the current between the different dilutions and hence concentrations. As the solution becomes more concentrated moving from 10^{-12} to 10^{-3}, the current will

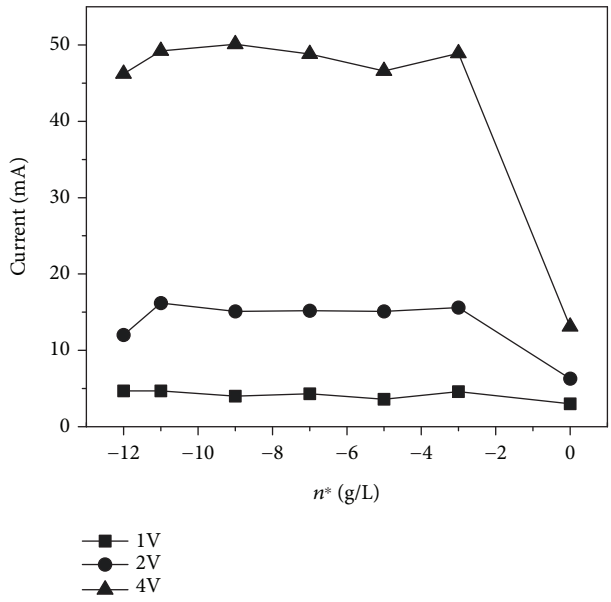


Figure 3: Current measured before and after immersion of the nonfunctionalized resistor in the different dilutions of BSA at 1 V, 2 V, and 4 V. *The graph is shown in terms of n, but the corresponding dilutions are in the form of (10^n) of the stock solution.

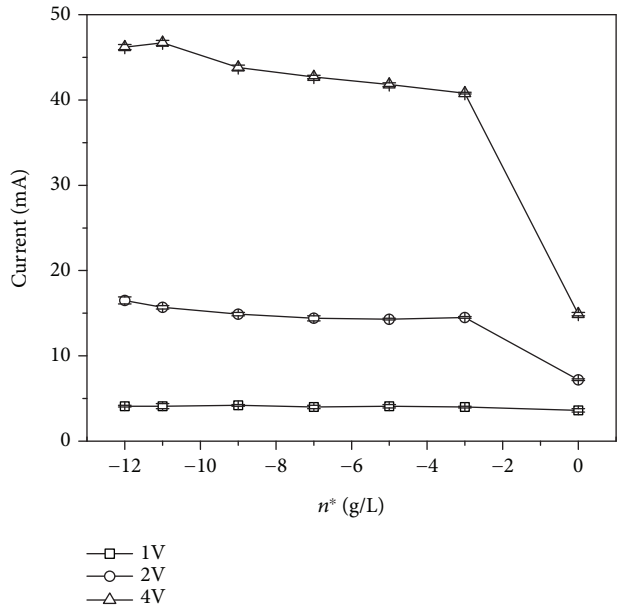


Figure 4: Current measured before and after immersion of the BSA functionalized resistor in the different dilutions of BSA at 1 V, 2 V, and 4 V. *The graph is shown in terms of n, but the corresponding dilutions are in the form of (10^n) of the stock solution.

Table 4: The measured current before and after BSA functionalization of the resistor.

	Voltage (V)	Current (mA)
Naked resistors	1	3.6 ± 0.1
Naked resistors	2	7.2 ± 0.1
Naked resistors	4	14.3 ± 0.1
Functionalized resistors	1	3.8 ± 0.1
Functionalized resistors	2	7.6 ± 0.2
Functionalized resistors	4	15.7 ± 0.5

decrease, and this is more expressed at 4 V (from 46.2 ± 0.3 to 40.8 ± 0.2 mA) and to a less extent for the 2 V. The decrease in the current when solutions are added to the bio-FET was also reported by Kang et al. and Estephan et al. [15, 16]. It can be acclaimed that at 4 V, where the difference between solutions is maximal, after the 10^{-12} dilution, the current become stable. So after calculating the concentrations corresponding to our dilutions, detection was possible at the impressive low concentration of 0.09 fM. As expected, the trend of variation was clear as functionalization is used. The method combines the advantage of having physical adsorption and self-assembly with minimum structural modification and lessened possibility of protein denaturation while testing [8]. The technique used is much simpler than other more sophisticated as that done in Kang, 2007 where the gate and the drain of a transistor were both covered, including the use of specific antibodies and the more sophisticated HEMTs. The minimum detection was 10 pg/mL in 1 μg/mL compared to the obtained 0.09 fM. It is also much higher than the 0.02 mM detection of glucose using graphene-chitosan nanocomposite [17]. This is an indication that this technique can be used as a cheaper and more accurate model for biomolecular detection.

Even the stock NaCl solvent solution without BSA molecules cannot change the current in more than 0.6 mA at 4 V when compared to a normal value without any solution. This can prove that values recorded at Figure 4 (difference around 30 mA at 4 V) correspond to the BSA solution and conformation in the solvent. Even though, and in a way to rule out any interference with the results, the effect of the NaCl was studied, several dilutions were done from the stock NaCl solution; we ran different dilutions in the circuit until the multimeter detected no current value. The results showed that the minimal detection (no current variation) was at 10^{-14} dilution.

Functionalization with BSA was not very accurate in terms of the large error (0.5 mA at 4 V (Table 4)) measured in the current after the BSA coverage. The potential reason is that the functionalization with these big molecules does not cover the resistor in a uniform manner, so the number of molecules adsorbed on the resistor is very random which is why the measured current is different from one resistor to another (maximum difference of 1 mA between functionalized resistors). For this specific reason, tryptophan was chosen in a way to replace the BSA molecule for the functionalization steps. Tryptophan has a much smaller size than BSA, and it can provide better coverage for the resistor, so it was used for covering the resistor, because in general, smaller molecules tend to be a better choice for functionalization [8]. As for the BSA, the effect of the tryptophan functionalization is studied: the current for the naked and the functionalized

Table 5: The measured current before and after tryptophan functionalization of the resistor.

	Voltage (V)	Current (mA)
Naked resistors	1	3.6 ± 0.1
Naked resistors	2	7.2 ± 0.1
Naked resistors	4	14.3 ± 0.1
Functionalized resistors	1	3.7 ± 0.1
Functionalized resistors	2	7.3 ± 0.1
Functionalized resistors	4	15.9 ± 0.1

resistors without any solution or immersion is measured at 1, 2, and 4 V. The recorded current values are shown in Table 5.

As for the BSA, an increase in the current is observed after tryptophans were adsorbed on the resistor surface (Table 5). This is a proof of the validity of functionalization with tryptophan. The error at 4 V in the table shows that tryptophan is more elegant: the narrow range of variation observed after adsorption is a proof that tryptophan is a better choice for functionalization over BSA.

In this last experiment, tryptophan was used to functionalize the resistor prior to BSA solution detection. Different concentrations and dilutions of BSA were prepared in the 10^{-14} dilution of NaCl in order to rule out the effect of the NaCl. As mentioned before, functionalization was done with tryptophan, and measurements were done in the BSA dilutions. Current difference between the functionalized resistor and the one recorded in the solution is reported in Figure 5 for every dilution only at 4 V.

There was an increase in the current differences as the concentrations increase (Figure 5). As the dilution decreases from 1/1000 to 1/2, the current difference steadily increased from 0 ± 0.1 to 1.4 ± 0.3 mA. Minimal detection was at 1/100 within the range of 1 μM.

With tryptophan resistor functionalization and a much diluted NaCl solvent for the BSA dilutions, detection can be read at approximately 1 μM while it was previously 0.09 fM with the BSA functionalization and the concentrated NaCl solvent. Minimum detection and increasing or decreasing of the current will depend on the molecules used for functionalization, the solvent, and the conformation of the molecule of interest in the considered solvent due to its electrochemical properties [18–20]. 1 μM is still a significant detection value considering commercial resistors were used. All is consistent with the results obtained by Kang (2007) and Estephan et al. [16] where using specific antigens and peptides, respectively, will aid in better functionalization and detection.

Finally, if we compare our results to previous works on BSA detection performance, we find that in works that showed their results visually, the minimum detection was several μM via optical detection and 10 pM via electrical detection (Table 6) whereas in this study, the minimum detection reached for BSA first using the tryptophan functionalized resistor without any interference for the NaCl solvent is 0.09 μM, and second using BSA functionalized

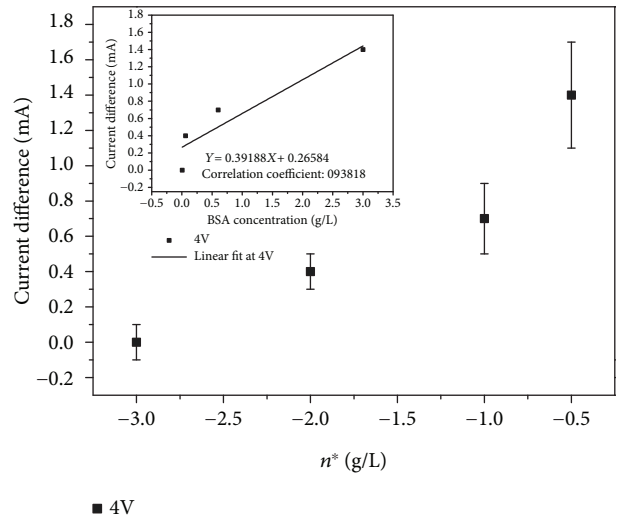


Figure 5: Current difference measured before and after immersion of the BSA functionalized resistor in the different dilutions of BSA diluted in 10^{-14} NaCl at 4 V. *The graph is shown in terms of n, but the corresponding dilutions are in the form of (10^n) of the stock solution except for the -0.5 that correspond to a dilution of 1/2 of the stock solution. The inset graph represents the linear range on the x axis in addition to the equation of the linear fit and the correlation coefficient.

resistor with 2.6% interference for the NaCl solvent is 0.09 fM. (The interference is calculated considering that the current difference detected after the NaCl solvent is only 0.6 mA).

Other previous works have stated the linear range of detection and calculated the lower limit of detection (LOD) using the standard deviation and slope method (SDS) as shown in Table 6. The best result among these studies was a LOD of 2×10^{-11} g L^{-1}. Our linear range using the tryptophan functionalized resistor and no solvent interference is $6 \times 10^{-3} - 3$ g L^{-1} with a LOD of approximately 2×10^{-3} g L^{-1} (inset of Figure 5), whereas using BSA functionalization with 2.6% interference of NaCl solvent (Figure 4), our linear range is $6 \times 10^{-12} - 6$ g L^{-1} with a LOD of approximately 1×10^{-13} g L^{-1}.

The current difference for BSA detection is changing in terms of solvent concentration and molecules used for functionalization. So, we believe that the functionalization of the resistor is a powerful tool in detecting molecules and that the detected current difference is in correlation with the conformation of the BSA molecule in its solvent.

4. Conclusion

We report on biomolecular detection using functionalization and electric biosensing via resistors. The results show that these latter are a viable option for functionalization of biomolecules. Two types of functionalization were used, and tryptophan was more successful in terms of accuracy while BSA had lower detection in the presence of the concentrated NaCl solvent. The lower minimum concentration detection

Table 6: Comparison of BSA detection with previous works. [LOD, SDS, CV, DPV, and CA stand for lower limit of detection, standard deviation and slope method, cyclic voltammetry, differential pulse voltammetry, and colorimetric assay, respectively.].

Ref.	Sensor type	Materials used	Linear range	LOD calculation	LOD
[21]	Electrical	Electrolyte-gated graphene FET	—	Visual	0.3 nM
[22]	Optical	Fluorescein derivatives	—	Visual	Several μM
[23]	Electrical	ZnO nanosphere	—	Visual	10 pM
[24]	CA, Bradford	—	0.781–12.500 g L^{-1}	SDS	0.006 g L^{-1}
[25]	CV	Carbon nanotubes, polymer film	$1 \times 10^{-4} - 1 \times 10^{-1}$ g L^{-1}	SDS	2.6×10^{-5} g L^{-1}
[26]	DPV	Liquid-graphene modified glassy carbon electrode	$1 \times 10^{-10} - 1 \times 10^{-4}$ g L^{-1}	SDS	2×10^{-11} g L^{-1}
[27]	DPV	Chitosan-coated magnetic nanoparticles modified multiwalled carbon nanotubes	$1 \times 10^{-1} - 1 \times 10^{-7}$ g L^{-1}	SDS	2.8×10^{-8} g L^{-1}

of 0.09 fM promises even more sophistication because the use of specified resistors instead of commercial ones is expected to considerably lower that threshold. Using tryptophan for functionalization and a diluted solvent for the different solutions gave a minimal detection at approximately 1 μM which is an indication that the biomolecular detection limit will vary with the molecule used for functionalization, the nature and the concentration of the solvent, and the conformation of the molecule needed to be detected.

Our work demonstrates the utility of the functionalization with biomolecules prior to detection. These results open the way to much more to be done in future studies. Different types of commercial resistors can be used to figure out which can give better results. Several other molecules can be studied and functionalized. Specific peptides and antibodies can be used for a specific functionalization.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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