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Abstract—**A microstrip antenna based sensor for the detection of the concentration of Horseradish peroxidase (HRP) is presented. The sensor is composed of a microstrip antenna connected to a stub with a gap and a graphene film deposited in the gap. The graphene film is deposited by doctor blading it into a designated position. The surface of the film is functionalized for HRP. In this paper, high concentrations of HRP are detected by the variation of the reflection coefficient of the antenna.**

Keywords—**microwaves sensors, patch antennas, graphene films, Horseradish peroxidase (HRP).**

I. Introduction (*Heading 1*)

In recent times, radiofrequency biosensing for the detection of molecules has caught significant attention. [1-6]. Research on nanomaterial based films shows that they can be adopted for the detection of biomolecules that can be of high importance for evaluating a range of different markers in the human body. They can help in early diagnosis of different types of cancers and other body chemicals like glucose.

Conventional methods for the detection of various biomolecules are invasive in nature, for instance, electro-impedance spectroscopy, enzyme oxidation, time domain reflectometer, and surface plasma resonance [7].

The use of radio frequency biosensors based on active and passive components is an emerging field of research, the functionality of whom can be augmented by incorporating nanomaterials [8-10]. Therefore, chemically functionalized nanomaterial films based RF sensing has high potential in diagnostics due to its sensitivity and selectivity to target unique molecules [11-14].

As an enzyme, Horseradish peroxidase (HRP) can be used as marker. It requires the use of a substrate to be oxidized, in the presence of hydrogen peroxide. The oxidizing agent in this case is hydrogen peroxide. A characteristic change in colour of the substrate is reached. Several different substrates can be used for resulting in this colour change e.g., tetramethylbenzidine (TMB), diaminobenizidine (DAB) and azinodiethylbenzthiazolone sulfonic acid (ABTS) etc.

In this paper, the concentration of HRP is detected by varying electrical properties of a graphene film connected to a microstrip antenna. The varying electrical properties of the graphene film results in the variation of the current passing through it which results in a variation of the resonant frequency of the antenna. The antenna is designed by standard PCB fabrication techniques, with the graphene film realized by doctor blading it in the designated position on the antenna. The film is chemically functionalized for HRP concentration detection.

II. Sensor Design and Realization

A. Design

The sensor is composed of a microstrip antenna connected to a stub on its radiating edge. The stub has a gap where the graphene film is deposited. The design of the antenna is simulated by Ansys HFSS. The design frequency of the antenna is 4.5 GHz.

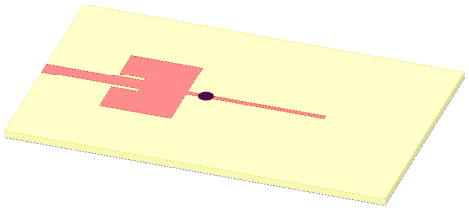

Fig. 1. Geometry of the microstrip patch antenna with stub and graphene film

The sensor is designed on a standard dielectric substrate Kappa 438 from Rogers. The dielectric substrate has permittivity, $\varepsilon_r = 4.38$, loss tangent $\tan\delta=0.005$ and thickness, h=1.52 mm. The metal part of the sensor is in copper with thickness, t=32 μm. The length of the antenna is L= 15 mm and width is w=20 mm. The length of the feed line is 5.5 mm and width is 3.2 mm corresponding to 50 Ω. The antenna is inset-fed with length of the inset optimized for reduced reflection coefficient. The antenna is connected to a stub with a gap; the first part of the stub has a length of 3 mm and the second part of 24 mm. The stubs are of a similar width of 1 mm corresponding to 100 Ω characteristic impedance. A graphene film is deposited in the gap. The length of the designated position for the deposition of graphene film is 2 mm. The antenna is simulated by Ansys HFSS. The resonant frequency of the antenna without the film is 4.52 GHz.

B. Realization

The sensor is fabricated by standard PCB photolithographic procedure. The graphene film is composed of a filler and a binder in the ratio of 9:1. The filler is commercial graphene nanoplatelets provided by Nanoinnova (Spain). The nanoplatelets have a nominal surface area of approximately 45 m^2/g and a carbon content of more than 98.9 wt%.

The binder is polyvinylidene fluoride (PVDF) and it binds the filler into a matrix providing it the required mechanical stability and adhesive properties. It makes the film stay in its designated position on the dielectric substrate.

The filler and binder are mixed in a solvent N-methyl-2-pyrrolidone (NMP) in order to help in the dispersion of the filler in the mixture. The mixture is a slurry which is mechanically mixed in order to get a good dispersion and to acquire a homogeneous film. The binder and the solvent are electrical insulators and given their very small proportion in the slurry, they do not influence the electrical properties of the film to a great extent.

Once a homogeneous slurry is obtained, it is doctor bladed onto the dielectric substrate by the help of a mask. The mask has a circular shape with a diameter of 5 mm and a thickness of 500 µm. The substrate is then dried under hood convection for several days. This removes the solvent from the film. Once dry, the mask is removed from the dielectric substrate.

The selectivity and sensitivity of the biosensing is ensured by the chemical functionalization of the surface of the graphene film. Controlled immobilization of molecules is performed on the surface with different concentrations of HRP and that are correlated to the different radiofrequency responses.

III. Results

The sensor is measured by a vector network analyser (Keysight, E8361A) after standard one-port calibration (see Fig. 2). The measurement is performed without the graphene film and with film, before and after functionalization and with two different concentrations of HRP (0.3 mM, 0.6 mM).

The antenna without the film resonates at the frequency of 4.54 GHz. The simulated and measured results of the antenna without the graphene film are in good agreement with each other as shown in Fig. 3.

The chemical process takes place on the surface of the graphene film. The film is functionalized for HRP. As shown in Fig. 4, there is a very slight variation in the reflection coefficient of the antenna with and without functionalization. In the case of the functionalized film, the antenna resonates at a frequency of 3.63 GHz, with return loss of almost -3 dB.

Different concentrations of HRP are dropped on the film. The quantity of HRP dropped over the film is 30 µL. When changing between different concentrations of HRP, the surface is washed with a buffer solution to remove the impact of the tested concentration.

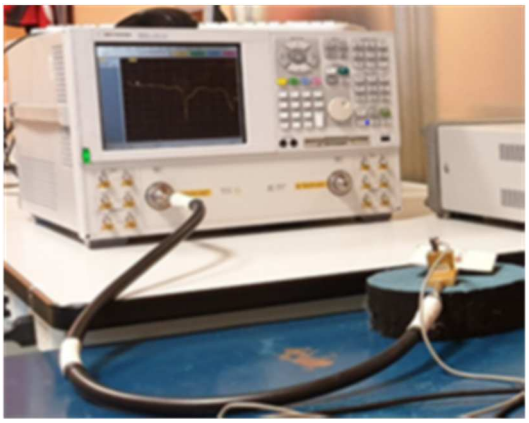

Fig. 2. Measurement setup of the HRP sensor.

As is shown in Fig. 5, when HRP with a concentration of 0.3 mM is dropped over the functionalized graphene film, the resonant frequency of the antenna changes to 3.36 GHz with a return loss of -4dB. Further increasing the concentration of HRP to 0.6 mM results in a resonant frequency of 3.29 GHz with a return loss of -4dB. The frequency shift between the 0.3 mM and 0.6 mM concentration is 70 MHz. This shows that the sensing capability of the sensor is quite good.

Thus, there is great potential for the sensor being used in other chemical markers. Other experiments are required with different concentrations to further test the capability of the sensor for lower and higher concentrations to find the limits of the system.

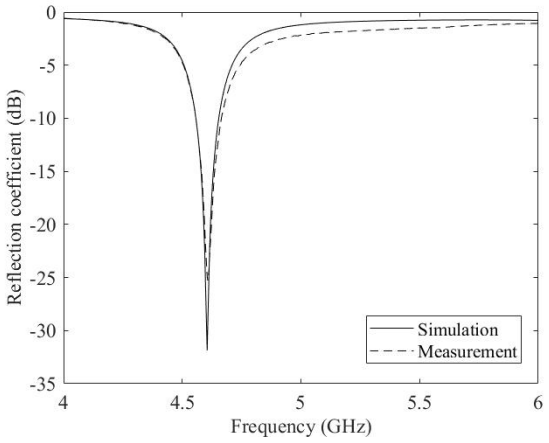

Fig. 3. Simulations (solid line) and measurements (dashed line) of a prototype without film in the gap.

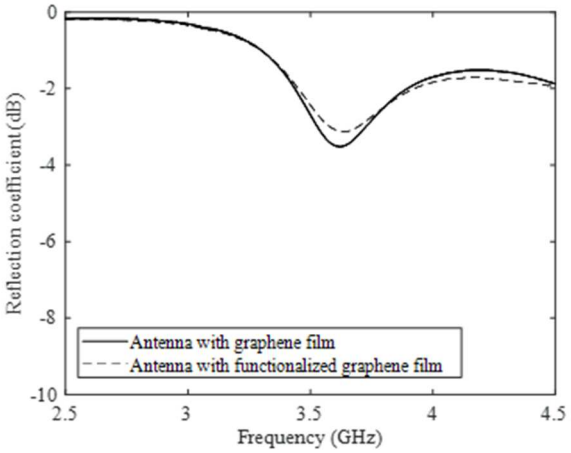

Fig. 4. Comparison between antenna with graphene film without functionalization and the antenna with functionalized film.

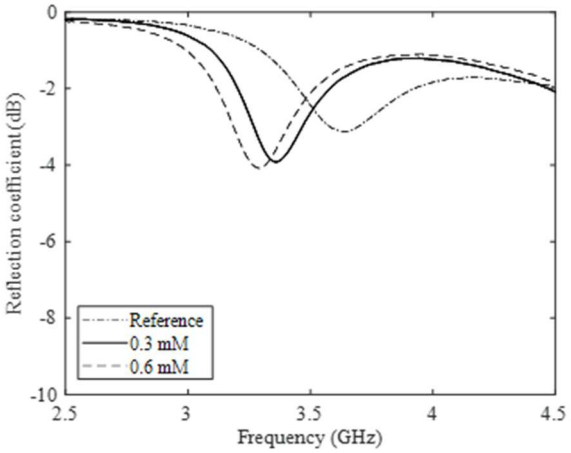

Fig. 5. Reflection coefficient as a function of HRP concentration.

IV. Conclusions

In addition to spectrophotometric methods for concentration detection of HRP, electrical parameters testing could provide additional information with good selectivity and sensitivity. Radiofrequency biosensor comprising of a microstrip antenna connected to a stub with gap and a graphene film deposited in the gap with functionalized surface is tested.

The resonant frequency and return loss of the antenna varies as a result of the concentration variation of HRP+TMB dropped on the surface of the graphene film. A frequency shift of 70MHz is obtained between the 0.3 mM and 0.6 mM concentration of HRP.

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