Volume 17, Number 4, 2024. pp. 437-442

Jordan Journal of Physics

ARTICLE

Investigation of Electrochemically Modulated Fluorescent Cresyl Violet Molecules for Biosensing Application Using an Electrochemical Surface Plasmon Resonance

Aymen H. Qatamin

Department of Applied Physics, Tafila Technical University, Tafila 66110, Jordan.

Doi: **https://doi.org/10.47011/17.4.6**

Received on: 20/02/2023; *Accepted on: 17/04/2023*

Abstract: In this work, the potential-modulated fluorescent of cresyl violet molecules was investigated under an applied electric step potential using the electrochemical surface plasmon resonance (EC-SPR) technique. The EC-SPR device employed in the study consisted of two detection units: a reflected optical intensity detection unit and a fluorescence detection unit. Both units were used simultaneously to optimize the fluorescence signal and achieve the highest fluorescence intensity. The results show that the fluorescence intensity of the cresyl violet molecules changes during the step potential. This study demonstrates that cresyl violet molecules are suitable candidates for biosensing applications using the EC-SPR technique based on the detection of fluorescence differences between their reduced and oxidized states. This research opens up a new avenue to using this class of dyes in electrochemically modulated SPR fluorescence-based biosensors.

Keywords: Electrochemical surface plasmon resonance.

1. Introduction

Electrochemical surface plasmon resonance EC-SPR is a technique that combines the principles of surface plasmon resonance SPR and electrochemistry to detect and analyze chemical and biological interactions at the interface between a metal surface and an electrolyte solution [1, 2]. This technique has been widely used in various fields such as biosensing, bioelectronics, and materials science [3-7].

Recently, we have developed an EC-SPR approach for detecting immune responses, which employs a sandwich assay and utilizes a redox probe to generate an optically modulated electric signal [8]. This technique enables the identification of the H5N1 strain of avian influenza A virus, with a minimum detection limit of 300 pM. We posit that combining a sandwich assay with an electrochemically modulated SPR fluorescent sensor could yield significant benefits, potentially leading to a breakthrough in the field of EC-SPR sensing. As opposed to modulated absorbance measurements, the fluorescent intensity grows from the negligible background, and the SPR enhances the electromagnetic field to more effectively excite the fluorophores. Additionally, the metal film acts as an efficient blocker to reduce the background interference from the excitation light source.

In this work, we have investigated the use of electrochemically modulated fluorescent cresyl violet molecules as candidates for biomarkers in an EC-SPR device. Cresyl violet, a member of the oxazine class of dyes, has been found to have high fluorescence quantum yields and long-lived excited states, making it a potentially useful compound for energy and electron transfer

Corresponding Author: Aymen H. Qatamin **Email**: aymen.qatamin@ttu.edu.jo

reactions [9]. Additionally, cresyl violet has been observed to exhibit reversible redox behavior, undergoing a quasi-reversible reduction/ oxidation reaction in an aqueous solution [10, 11]. The platform of the device was created using a layer of gold (Au, 35 nm) and indium tin oxide (ITO, 10 nm). The Au film was chosen to improve the sensitivity of the SPR and to ensure the SPR platform's stability for spectroelectrochemical measurements. On the other hand, ITO was employed to prevent the metal surface from quenching the fluorescence.

2. Experimental Methods

2.1. Experimental Setup

Figure 1 illustrates the EC-SPR device with two detection units.

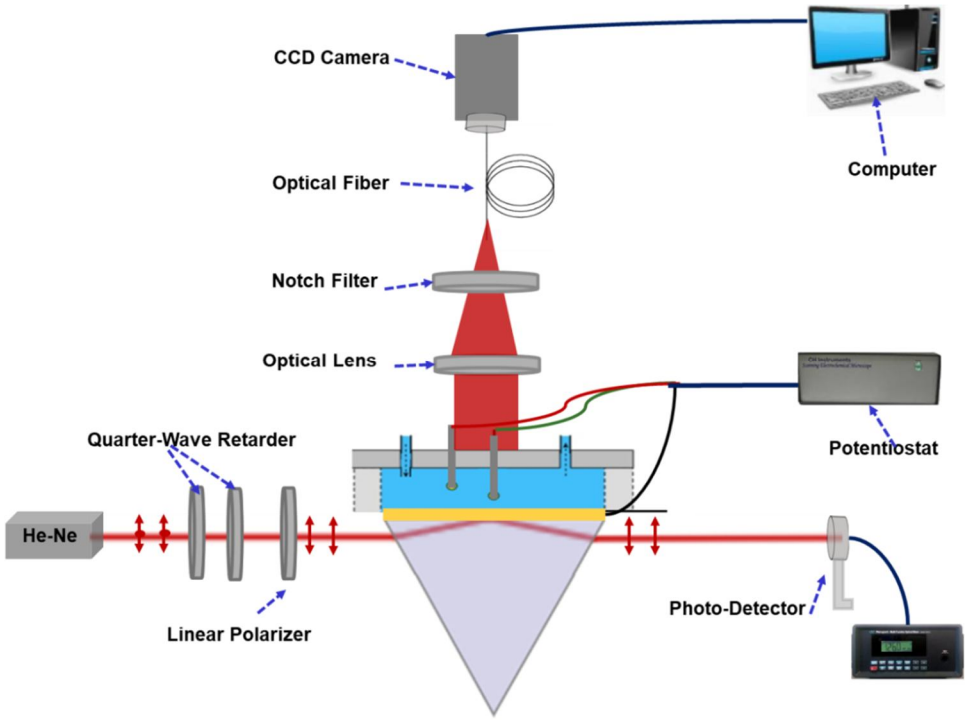

FIG. 1. Schematic of an EC-SPR device, where P-polarized light from a He-Ne laser is generated using two quarter-wave retarders and a linear polarizer to excite plasmons. The reflected optical intensity is measured by a photo-detector, and electrical control is achieved through a potentiostat with three electrodes. Additional setup for collecting fluorescence data includes a monochromatic CCD camera for spectrally resolved measurements.

An electrochemical flow cell was designed with three electrodes. The SPR surface served as the working electrode, while a platinum rod and silver/silver chloride (1 M potassium chloride) electrodes were utilized as the reference and counter electrodes. The flow cell was equipped with a 60-degree equilateral prism, which was optically connected to the SPR surface using index matching gel with a refractive index of 1.52. The setup was then fixed on a rotational stage to adjust the angle of incidence. A He-Ne laser beam with a wavelength of 595 nm (Newport Corporation, Irvine, CA USA) was utilized along with two quarter-wave retarders and a linear polarizing element to generate linearly polarized light with TM polarization. Then, the polarized light was directed towards the prism to excite the plasmon. This enables the fluorescent molecules close to the surfaces to be excited through the surface plasmons' evanescent field. The setup has two detection units: the reflected optical intensity detection unit and the fluorescence detection unit. The reflected optical intensity detection unit employed a photo-detector to monitor the reflected light at varying incidence angles. The fluorescence detection unit was mounted towards the base of the prism and rotated with the prism. The fluorescence emission from the sample surface was collected by a lens and passed through a notch filter to eliminate the transmitted laser light. The fluorescence emission was then directed to an optical fiber and coupled to the CCD camera (Hamamatsu, C5405-01). The electric potential applied to the working electrode was controlled by a potentiostat (CS350, CorrTest, China), and a computer with specialized software was used to acquire and process the data.

2.2. Preparation of the EC-SPR Surface

To assemble cresyl violet molecules on the surface, the surface was functionalized using self-assembled monolayers (SAMs) of MPA (3-mercaptopropionic acid from Sigma-Aldrich). Initially, the surface was immersed in a 10 mM MPA solution in ethanol for 48 hours [12, 13]. Afterward, the surface was rinsed with ethanol and DI water and then dried gently using N_2 gas. The surface coated with MPA was subsequently placed in an electrochemical flow cell. After that, to activate the carboxylate groups on the MPA coating, a mixture of 0.02 M EDC (1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride obtained from Sigma-Aldrich) and 0.04 M NHS (N-hydroxysulfosuccinimide, also from Sigma-Aldrich) was flowed into the flow cell for 1 hour [14]. Subsequently, a solution of 500 nM cresyl violet molecules was injected and kept in the flow cell for half an hour, after which any unbound cresyl violet molecules were removed by rinsing with a solution of 2.0 M PBS pH = 6.2 (phosphate buffered saline from Sigma-Aldrich). Finally, the adsorbed cresyl violet molecules on the platform were stabilized using cyclic voltammetry (CV) in the potential range of -0.75 to 0.2 V at a scan rate of 0.03 V/s.

2.3 EC-SPR Measurements

The study focuses on three specific measurements. The first measurement involved using CV modulation to confirm the presence of immobilized cresyl violet molecules at the device interface. A modulation of the potential was performed on the surface, ranging from -0.75 V to 0.2 V at a rate of 0.03 V/s, while a photo-detector was used to observe the reflected light that was electrically modulated at a fixed angle. In the second measurement, angular reflectance experiments were performed to obtain the SPR curve, while the fluorescence detection unit was used for the optimization of the fluorescence signal and getting the highest fluorescence intensity. A rotation stage was used to control the incident angle of light, while the reflected light was monitored at different incidence angles with the photo-detector. Simultaneously, the fluorescence intensities were measured at six different incident angles. In the third measurement, fluorescence measurements were performed with modulated potential, while ensuring that the angle of incidence was set to maximize fluorescence intensity. The electric potential applied to the working electrode was regulated using a potentiostat, while the fluorescence detection unit monitored the fluorescence intensities at a fixed angle.

3. Results and Discussion

3.1 CV Scan

The immobilization of cresyl violet molecules in the EC-SPR surface was demonstrated using a CV technique with a potential range of -0.75 to 0.2 V at a scan rate of 0.03 V/s.

Figure 2 illustrates the reflectance response obtained for cresyl violet molecules absorbed onto the EC-SPR surface during the CV scans.

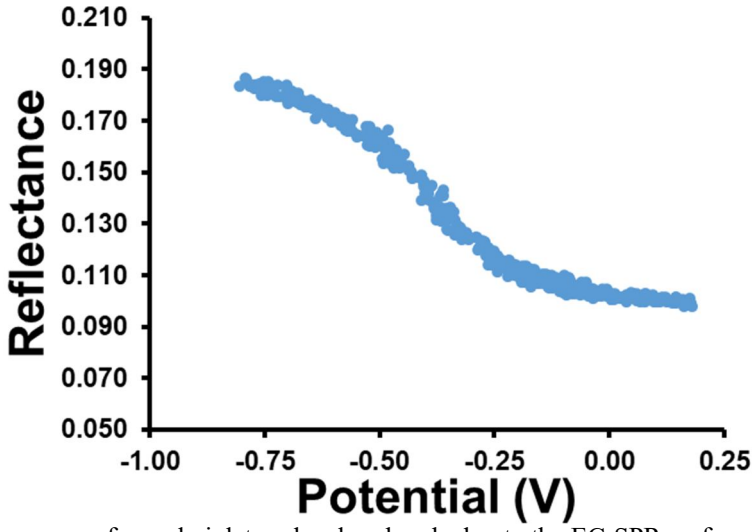

FIG. 2. Reflectance response of cresyl violet molecules absorbed onto the EC-SPR surface during a CV scan at a rate of 0.03 V/s.

The results demonstrate an evident and reversible change in the reflectance light when the modulation potential reaches the formal potential of cresyl violet molecules (around -0.35

V), providing evidence for the existence of immobilized cresyl violet molecules at the interface of the device.

3.2 Optimization of Fluorescence Detection

To optimize the SPR curve and enhance fluorescence detection, the reflected optical intensity and fluorescence detection units were used simultaneously. After demonstrating the successful immobilization of cresyl violet molecules in the EC-SPR surface, the SPR curve was determined. Figure 3 (left y-axis) shows the reflectance over a range of different incident angles.

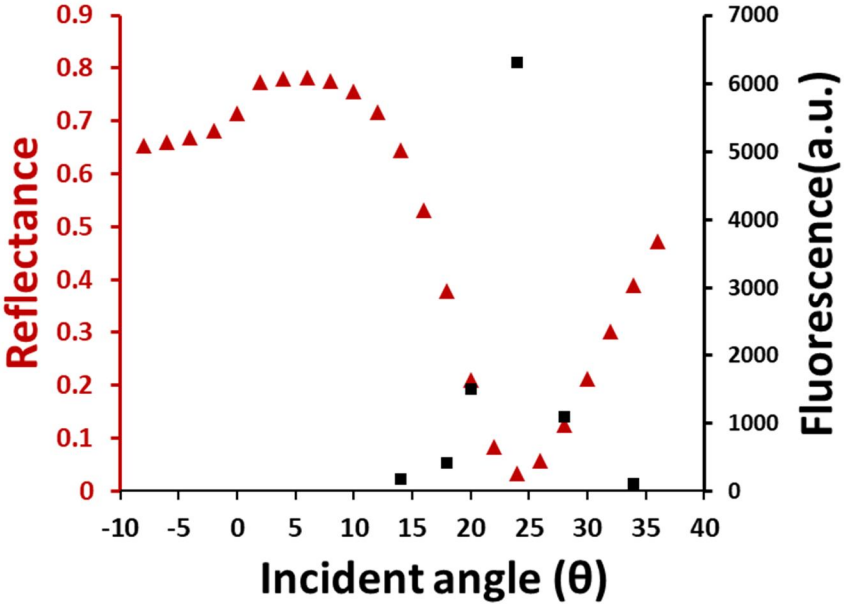

FIG. 3. The full curve of the SPR reflectance is shown on the left y-axis, while the fluorescence signal at six different incident angles is displayed on the right y-axis.

Then, the fluorescence signal at six different incident angles was measured. Figure 4 illustrates the measurement of fluorescence, covering a spectral range of 580 to 740 nm.

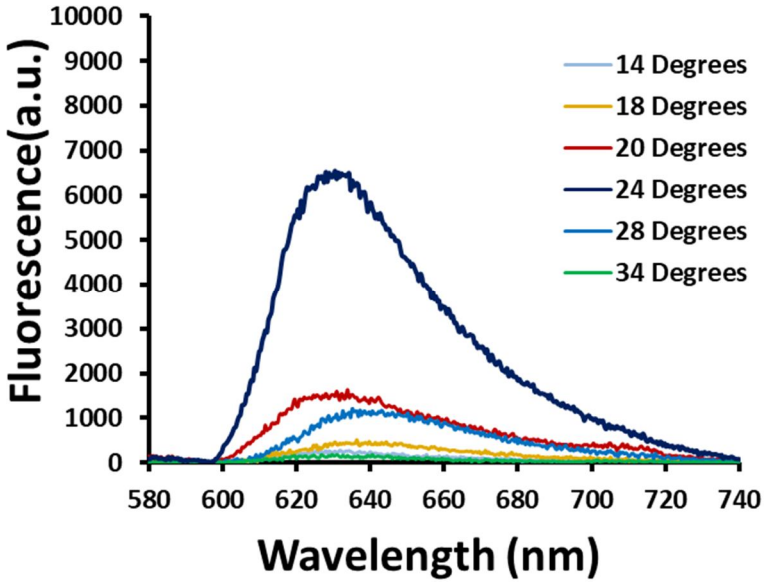

FIG. 4. Fluorescence signals at six different incident angles, covering a spectral range from 580 to 740 nm.

To better understand the relationship between the fluorescence intensity and the incident angle, the values of the highest intensity of fluorescence at six different incident angles were

plotted against the incidence angle on the same SPR curve graph (Fig. 3, right y-axis). The results demonstrate that the fluorescence intensity is influenced by the incident angle and that the highest value of the fluorescence signal was observed at the resonance angle (24 degrees). These findings confirm that maintaining a fixed incident angle at the resonance angle is crucial for improving and enhancing the sensitivity of fluorescence detection.

3.3 Potential-modulated Fluorescence Spectroscopy Using EC-SPR

To determine if cresyl violet molecules could potentially be used as a modulated fluorescent probe for biosensing applications, a potential step technique was applied. The incident angle was fixed at the resonance angle (24 degrees), which produces the highest fluorescence intensities. First, a 20 mM PBS pH 6.2 solution was introduced to the flow cell to measure the background, and the fluorescence spectrum was recorded. Next, a 500 nM cresyl violet solution in 20 mM PBS pH 6.2 was injected into the flow cell for half an hour. After rinsing the flow cell, fluorescence spectra were recorded at different electric potential values: 0.1, -0.1, -0.2, -0.3, and -0.6 V. Finally, the background was subtracted from the fluorescence spectrum at each specific electric potential step. The data are shown in Fig. 5.

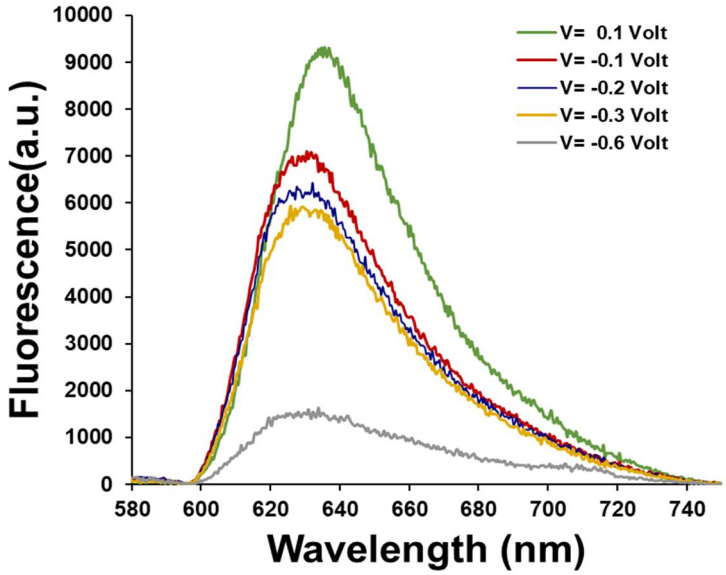

FIG. 5. Fluorescence spectrum of 500 nM cresyl violet under step potential modulation on EC-SPR surface.

The results indicated that the fluorescence intensity of cresyl violet changed during the potential step. A strong fluorescence spectrum was recorded at 0.1 V (green curve), indicating that most of the cresyl violet was in the oxidized state, as the oxidized state of cresyl violet produces very strong fluorescence [12]. In contrast, a weak fluorescence spectrum recorded at -0.6 V (grey curve) indicated that most of the cresyl violet molecules were in the reduced state, as the reduced state of cresyl violet produces very weak or no fluorescence.

The ability to detect and measure the change in fluorescence intensity of cresyl violet using the EC-SPR technique will establish a new technology for immuno-biosensor-based approach that allows for direct, highly sensitive detection of human pathogens with minimal background signal. This has the potential to be a significant contribution to the field of biosensing, as the ability to directly detect and quantify human pathogens can have important applications in medical diagnosis and public health. Additionally, reducing background signals can improve the sensitivity and specificity of biosensors, making them more reliable and accurate.

4. Conclusion

In conclusion, the study presents the investigation of electrochemically modulated fluorescence of cresyl violet molecules under applied electric step potentials. The results show that the fluorescence intensity of the cresyl violet molecules changes during the step potential, indicating the reversible redox behavior of these molecules. The capability of detecting a modulated fluorescence signal from the cresyl violet molecules makes them a promising candidate for bio-sensing applications using the EC-SPR technique.

References

[1] Lu, J., Wang, W., Wang, S., Shan, X., Li, J., and Tao, N., Anal. Chem., 84 (1) (2012) 327.

[2] Liang, W., Wang, S., Festa, F., Wiktor, P., Wang, W., Magee, M., LaBaer, J., and Tao, N., Anal. Chem., 86 (19) (2014) 9860.

[3] Fang, Y., Wang, W., Wo, X., Luo, Y., Yin, S., Wang, Y., Shan, X., and Tao, N., J. Am. Chem. Soc., 136 (36) (2014) 12584.

[4] Fang, Y., Wang, H., Yu, H., Liu, X., Wang, W., Chen, H.-Y., and Tao, N. J., Acc. Chem. Res., 49 (11) (2016) 2614.

[5] Shan, X., Díez-Pérez, I., Wang, L., Wiktor, P., Gu, Y., Zhang, L., Wang, W., Lu, J., Wang, S., Gong, Q., Li, J., and Tao, N., Nat. Nanotechnol., 7 (10) (2012) 668.

[6] Dallaire, A. M., Patskovsky, S., Vallée-Bélisle, A., and Meunier, M., Biosens. Bioelectron., 71 (2015) 75.

[7] Ribeiro, J. A., Sales, M. G. F., and Pereira, C. M., TrAC Trends Anal. Chem., 157 (2022) 116766.

[8] Qatamin, A. H., Ghithan, J. H., Moreno, M., Nunn, B. M., Jones, K. B., Zamborini, F. P., Keynton, R. S., O'Toole, M. G., and Mendes, S. B., Appl. Opt., 58 (11) (2019) 2839.

[9] Rurack, K. and Spieles, M., Anal. Chem., 83 (4) (2011) 1232.

[10] Kreller, D. I. and Kamat, P. V., J. Phys. Chem., 95 (11) (1991) 4406.

[11] Lei, C., Hu, D., and Ackerman, E. J., Chem. Commun. (Camb), (43) (2008) 5490.

[12] Michota-Kaminska, A., Wrzosek, B., and Bukowska, J., Appl. Spectrosc., 60 (7) (2006) 752.

[13] Mendes, R. K., Freire, R. S., Fonseca, C. P., Neves, S., and Kubota, L. T., J. Braz. Chem. Soc., 15 (2004) 849.

[14] Wilson, A. J., Molina, N. Y. and Willets, K. A., J. Phys. Chem. C, 120 (37) (2016) 21091.