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خودفرونشانی فلوئورسانس داروی دوکسوروبیسین در زوایای مختلف در محلول های آبی

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چکیده- در این پژوهش خودفرونشانی فلوئور سانس داروی دوکسوروبیسین به روش طیف سنجی فلوئور سانس القایی لیزری مورد تحلیل و بررسی قرار گرفته است. همچنین مشخص شد که فرونشانی شدت فلوئورسانس داروی دوکسوروبیسین به شدت به زوایه وابسته است. از جمله پارامترهای اصلی که در شکل گیری این تغییرات زاویه ای در نظر گرفته می شود غلظت دارو و تعامل بین آهنگ جابه جایی استوکس و همپوشانی بین طیف جذب/گسیل داروی دوکسوروبیسین است.

کلید واژه- داروی شیمی درمانی، طیف سنجی فلوئورسانس القایی، خودفرونشانی، منحنی فرونشانی اشترن-وولمر

Fluorescence self-quenching of Doxorubicin at different angles in aqueous solutions

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Abstract- In this study, the fluorescence self-quenching of Doxorubicin (DOX) was investigated by Laser-induced fluorescence (LIF). Furthermore, it was demonstrated that the fluorescence quenching of DOX strongly depends on the detection angles. In fact, DOX concentration and the interplay between Stokes shift rate and the overlapping between absorption/emission spectra of the chemo-drug are taken into account as the major parameters to form the angular distribution.

Keywords: Chemo-drug, Laser-induced fluorescence, self-quenching, Stern-Volmer formalism

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1. Introduction

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Nowadays, fluorescence imaging and spectroscopy have led to a wide range of applications in biosciences and pathology to identify the carcinoma in early stages [1, 2]. The fluorescence anisotropy upon excitation of a given chromophore by polarized light acts as an innate property for spectroscopic purposes. The method fails to perform in scattering media mainly due to fluorescence quenching by impurities and high photon scattering events. Moreover, fluorescence quenching has been widely studied both as a fundamental phenomenon, and as a source of information about biochemical systems which offers an authentic method to determine the optical properties of hybrid media [3].

Recently, the fluorescence quenching of dye molecules is demonstrated in the vicinity of the gold nanoparticles [4]. Furthermore, the fluorescence quenching of Rhodamine 6G (Rd6G) by graphene oxide (GO) was investigated [5]. On the other hand, it was shown that some chemo-drugs, such as doxorubicin, paclitaxel and bleomycin, act as fluorophores. In addition, DOX is a potent anti-tumoral agent that is widely employed for the purpose of the cancer therapy with good fluorescence characteristics. Moreover, GO/ND Nano quenchers in DOX solution attests the fluorescence ratio versus the quencher density according to Stern- Volmer formalism [6, 7]. To the best of our knowledge, the systematic study of self-quenching of DOX in different angles is rarely carried out.

Here, at first, Variations of maximum intensity at different angles have been studied and then the Stern-Volmer diagram of DOX has been plotted in different angles and self-quenching of DOX has been investigated.

2. Fluorescence quenching

Fluorescence quenching refers to any process that decreases the fluorescence intensity of a sample. The fluorescence quenching process is classified into static and dynamic regimes.

2.1. Dynamic quenching

The dynamic quenching is defined based on the collision events between the fluorophores and quenchers. The collision of quencher with an excited unbound fluorophore leads to the dissipation of radiative energy. In the case of static quenching, a nonfluorescent complex is formed by the fluorophore and the quencher. Dynamic quenching allows the well-known Stern-Volmer equation, given by:

$$\frac{I_o}{I} = 1 + K_D[Q] \qquad (1)$$

Where I_o (I) is the fluorescence intensity in the absence (presence) of quencher, [Q] is the concentration of quencher. One believes the I_o (I) versus [Q] plot is linear having slope K_D, a characteristic constant for dynamic quenching.

2.2. Static quenching

In static quenching a complex is formed between the fluorophore and the quencher, and this complex is nonfluorescent. Static quenching featuring by

$$\frac{I_o}{I} = 1 + K_S[Q] \qquad (2)$$

where K_D is replaced with the constant K_S despite the linear dependence of I_o (I) on [Q].

2.3. Self-quenching

Self-quenching is another process that occurs in large fluorophore concentrations leading to the reduction of the emission intensity due to de-activation events for a given exited fluorophore by the other non-exited dye molecule that named re-absorption and it takes place because of overlapping

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between the absorption and emission spectra of fluorophores [3].

3. Materials and methods

The second harmonic generation (SHG) of CW-Nd:YAG laser 150 mW at 532 nm is utilized to excite DOX molecules in a thick cylindrical glass cell with 1.3cm radius and 4cm height. Emission spectra are measured in different detection angles of 30°-90°. The spectrophotometer (Avantes Ava Spect, wavelength resolution 0.4 nm) is employed to detect the fluorescence emission. Moreover, the high grade DOX is supplied by EBEWE Pharma Ges.m.b.H. Nfg.KG. DOX is dissolved in deionized water. The aqueous solutions are steadily homogenized using a magnetic stirrer during the laser irradiation to prevent the destruction of DOX. Figure 1 illustrates the schematic arrangement for LIF spectroscopy in different detection angle.

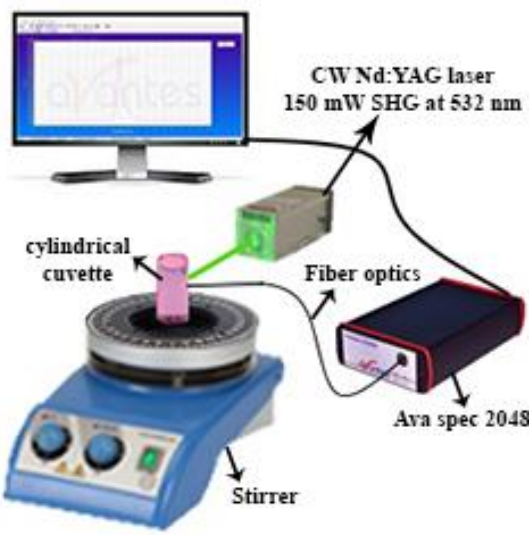


Fig. 1: Schematic of LIF setup for measurement of the fluorescence spectrum DOX

4. Result and discussion

Figure 2 depicts the LIF spectra for various concentrations using the excitation line at 532 nm. Note that the maximum intensity occurs at ~0.04 mg⁄ml.

In general, the signal intensity linearly increases with C_{DOX} in the dilute solutions, whereas it nonlinearly alters in the case of the dense solutions as shown in Figure 3.

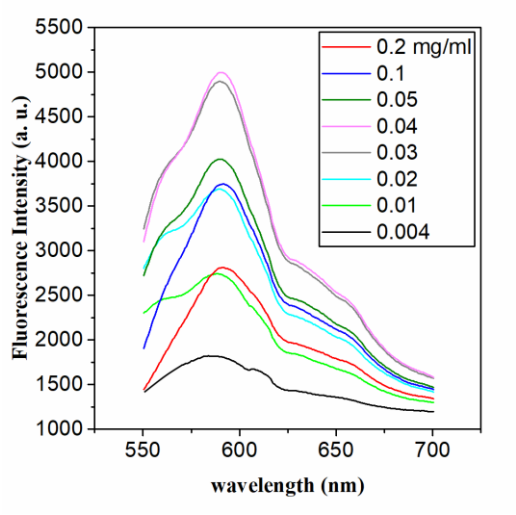


Fig. 2: LIF emission spectra due to the various C_{DOX} (0.004–0.2 mg/ml)

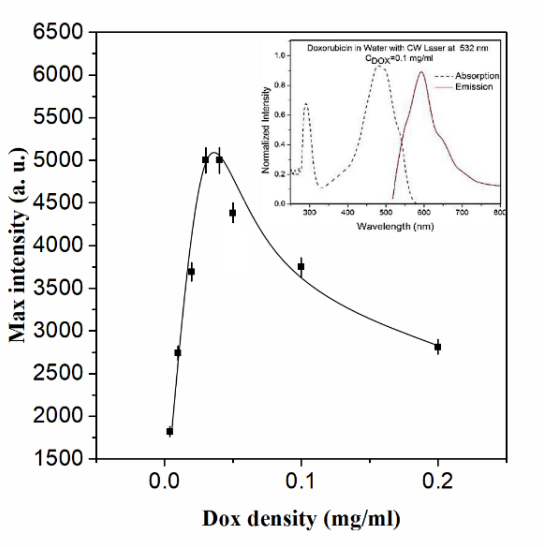


Fig. 3: The peak intensity as a function of DOX concentration. Inset: The normalized absorption-emission spectra for DOX [7]

On the other hand, we concentrated on the fluorescence intensity in different angles (the angle between laser direction and detector). It was shown in Figure 4 that the fluorescence intensity non-linearity decrease with detection angles in all concentrations. Moreover, not only self-quenching happens in all concentration, but also it takes place for higher concentrations of DOX in higher rate due to increasing non-excited population in high concentration.

Figure 5 represents fluorescence signal ratio I_o/I as a function of DOX concentration [Q] at the smaller and the larger angles of detection respectively.

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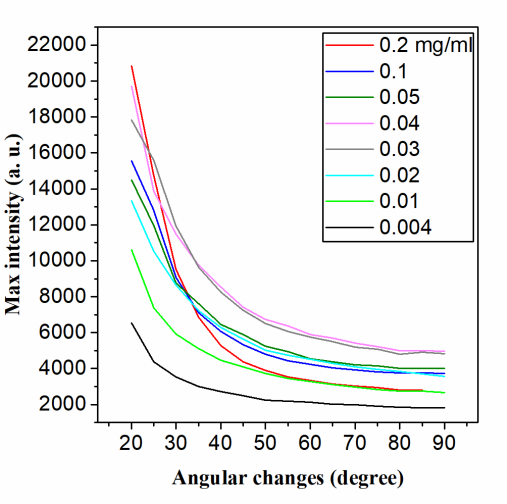


Fig. 4: Maximum fluorescence intensity versus different angles for various DOX concentration

At initial concentration of DOX (until 0.04 mg/ml) the slope is negative due to increasing the fluorescence intensity. Then, when the concentration of DOX passes the threshold concentration the Stern-Volmer formalism is established and we can determine the Stern-Volmer slope as an inherent characteristic of the aqueous solution. In addition, when we focus on variation of self-quenching in different angles, it was recognized that the larger slope was observed in the larger angles which the values are listed in Table 1. Consequently, these observations are confirmed the important role of re-absorption fluorescence spectra in large angles because of the increasing the non-excited molecules of DOX.

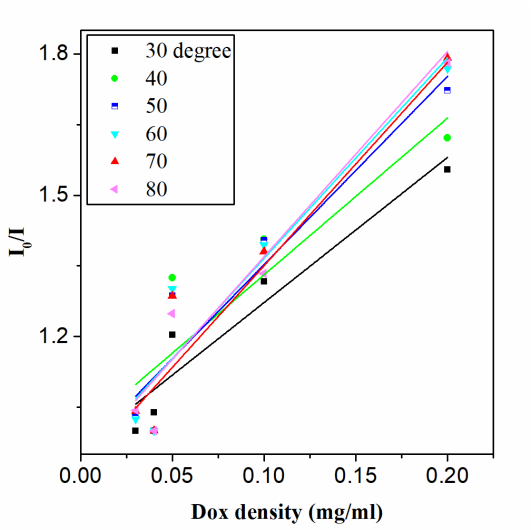


Fig. 5: fluorescence ratio (I_o/I) versus the DOX concentration.

Table 1. Self-quenching coefficient for DOX

Detection angle (Degree)	**K_{self-Q} ((mg/ml)$^{-1}$)**
30	3.081
40	3.327
50	3.955
60	4.258
70	4.318
80	4.356

5. Conclusion

The Stern-Volmer formalism of DOX in aqueous solution has been investigated in order to determine the corresponding self-quenching. The re-absorption events take place due to shorter Stokes shift. It was demonstrated that the larger angles, the more self-quenching happens in the fluorescence emission and the slope of Stern-Volmer, as a unique properties of an aqueous solution, greater in larger angles.

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