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Energy Transfer and Photophysical Properties of Prodigiosin Dye Product from Serratia marcescens

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Abstract:

In this research displays, photophysical properties Prodigiosin dye product from Serratia marcescens through absorption and fluorescence spectra of this pigment. The absorption spectra of prodigiosin were studied and the highest absorption peak in the spectrum at a wavelength (537nm). The spectra of fluorescence of Prodigiosin molecules have been measured appear band at a wavelength (557 nm).
This metabolite mixing with Curcumin dyes dissolved in methanol at concentrations [10µM], [1µM] to achieve energy transfer respectively. The values of the Förster radii R0 were calculated from molar absorptivity of concentration [10µM] from spectral overlap by used the MatLab program, the quantity R0 for Förster resonance energy transfer (FRET) between given donor and acceptor molecules found amount 4.9834 °A.Via mix produced complex molecules, excited by a wavelength (430 nm). This leads to charge transfer between molecules consequently the stability of the group, by energy nonradiative. Found values the quenching factor (Q) and it found it depends on the value of concentration. Thus, the data obtained is useful enough to be relied upon as a database of spectra for Prodigiosin dye.

Keywords: *Serratia marcescens; Prodigiosin; Förster radii; Curcumin; Energy transfer.*

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I. INTRODUCTION

Prodigiosin, a Bio-dye, is a secondary metabolite that as a native red-colored pigment that associated with the group prodiginines (Li et al. 2018). It does attractive due to their immunosuppressive and anti-cancer effects (Anita Khanafari, Assadi, and Fakhr 2006). It has molecular weight (323.4) (Anita Khanafari, Assadi, and Fakhr 2006). It has a tripartite structure and is produced by multiple strains of bacteria Serratia marcescens and some of gram-negative and other independent organisms, prodigiosin dyes are as well important to have, it has many bio-activities such as active against bacteria, anti-fungal, anti-oxidant, bio-emulsification, anti-biofouling, etc. The prodigiosin set of physical products is a class of tripyrrole red colorant dyes that involves a standard (4-methoxy, 2-2 bipyrrole) ring mode as shown in fig. 1. Prodigiosin has been shown to be connected in extracellular vesicles, cell-associated or existing in intracellular granules (N and Y. 1991). Shown the fig.1 members of the prodigiosin family.

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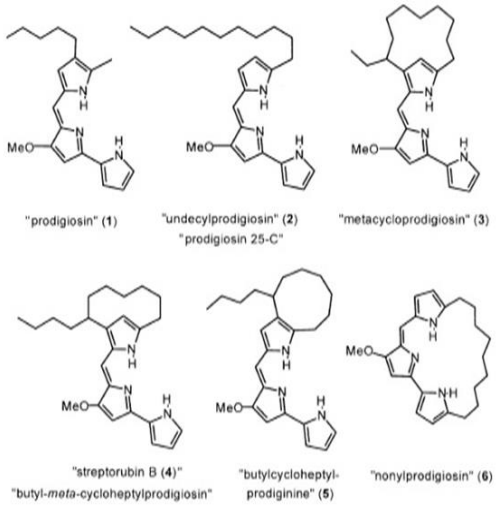

Figure 1 Representative members of the prodigiosin family (Fürstner 2003)**.**

In (1957), G. W. Monk was studied Spectral Absorption of Prodigiosin in cells and the effect of the medium, pH, and characteristics of the absorption spectrum (MONK 1957). Yoshida is interested in studying a water-soluble complex of Prodigiosin producing from biology element and purified by used precipitation with ammonium sulfate, several properties of the complex are described as absorption spectrum. this work described in years (1962) (Yoshida 1962).

Shahla Namazkar and Wan Azlina Ahmad in (2013) were used the encapsulation method. In this research, spray-dried microparticles including prodigiosin dye secured from Serratia marcescens was produced using κ- carrageenan. The result of spray-drying parameters on the encapsulation yield (EY), particle dimension and brightness intensity of the prodigiosin microcapsules were studied. The outcomes suggest that the spray-dried prodigiosin dye can be useful as a colorant basic (Namazkar and Ahmad 2013).

Andreyeva and T. Ogorodnikova in (2014) were studied the Pigmentation of S. marcescens and spectral characterization of Prodigiosin dyes The absorption spectrum of prodigiosin in the original dye-protein group was different from the spectrum of the dye dissolved in ethanol. The sensitivity of prodigiosin in the complex with an original protein is similar to the dye photosensitivity in the synthesis of a fit bacterial cell. they were found Prodigiosin extracted under removed conditions was low sensitive to illumination. Both the prodigiosin form of red and yellow in solution fluoresced at 560–565 nm; the in vivo fluorescence of the red pigment form was more marked(Andreyeva and Ogorodnikova. 2015).

In this report, spectroscopic properties and energy transfer of the prodigiosin molecule dissolved in methanol with the Curcumin molecule are presented.

II. Experimental and Method

Material

The culture was used from the laboratories of the Biotechnology Department, College of Science, University of Baghdad. A chemically defined liquid medium described by Chen and coworkers (Chen et al. 2013), All the components of the agricultural medium were equipped from the university mentioned above.

The medium was inoculated with S. marcescens at a level of 2% (v/v) and then incubated in an orbital shaker at 30°C and 200 rpm for 48h. After the incubation, samples were taken for the analyses of prodigiosin. For more reliability, each run was conducted either in triplicate or duplicate and the results were represented as the arithmetic average.

We will explain the extraction method later. Uses the compound; Curcumin molecule, it is [1, 7- bis (4-hydroxy- 3-methoxyphenyl) -1, 6– heptadiene -3, 5 -dione], it also is known as diferuloylmethane (Hewlings and Kalman 2017), with the chemical formula (C21H20O6) is a manufacturer of Curcumin used in our research is (HCD) Laboratory Chemicals in high purity from Central Drug House (P) Ltd, India.

III. Samples preparation

The result s have been prepared with different concentrations and that dissolution a given weight of compounds to be studied in a given volume of solvent which was used in the preparation of the

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solution. The compounds are weighted from the following relationship(Wadday, Saleh, and Al-Marjani 2019):

$$\mathbf{W} = \frac{[\mathbf{M}]\times\mathbf{V}\times\mathbf{M.W}}{\mathbf{1000}} \qquad (1)$$

Where:W is the weight of material in grams, M.W.: molecular weight g/ml.[M]: molar concentration (ml/L)**,** V: the volume of solvent used to dissolve the material in ml.The prepared solvent; were diluted according to the following equation:

$$[\boldsymbol{M}]_1\boldsymbol{V_1} = [\boldsymbol{M}]_2\boldsymbol{V_2} \qquad (2)$$

a. Measuring procedures

Dependent transmission of energy in organic material on the guest concentration (C_G). It can define the amount of numerical characteristic of the degree of energy transfer, a quenching factor (Q)(Northrop and Simpson 1956):

$$\mathbf{Q} = \frac{\mathbf{A}-\mathbf{A_d}}{\mathbf{A_d}} \qquad (3)$$

Where: A and A_d: are the areas under the curves represents the fluorescence spectrum of pure Curcumin and mixture respectively.

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The concentration of prodigiosin was calculated by using the molar extinction coefficient (*E* 530=7.07×10^{4}M^{-1}cm^{-1}):

$$\mathbf{prodigiosin}\left(\frac{\mathbf{gm}}{\mathbf{l}}\right) = \frac{\mathbf{O.D_{530}}*\mathbf{323.4}}{\mathbf{7.07}*\mathbf{10^4}} * \mathbf{D.f.} \qquad (4)$$

Where: O.D 530: Optical density at 530 nm, 323.4: Molecular weight of prodigiosin. E 530=7.07×10^4 M^{-1}cm^{-1} (Molar extension coefficient of prodigiosin at 530 nm), D.f. : dilution factor is equality final volume/ sample volume. Or, by using the Spekwin32 spectroscopy program of determination of prodigiosin concentration.

IV. Results and discussion

3.1 The absorption and fluorescence spectra of prodigiosin.

The absorption and fluorescence spectra of prodigiosin(Pro.), dissolved in methanol, were studied with different concentration at room temperature and optimal wavelength excitation (537 nm), this is almost agreement with (MONK 1957).

❖ Absorption spectra

The absorption spectra of Prodigiosinsolutions dissolved in methanol at concentrations [10 µM] and [1 µM] were measured at pH=7, the spectrum of Prodigiosin concentration [10µM] solutions consist of one peak as shown in figure (2). The peaks of spectra are respecting located at wavelengths (537 nm) this is an agreement with (Darshan and Manonmani 2016). The solution of Prodigiosin (Pro.) is a red redox-active slight color at the concentration of [10, 1 µM] and disappears at [0.1 µM] concentration.

it is illustrated from the figure the absorption spectra which are broad, structureless and do not contain a vibronic band and this is an agreement with (Drink et al. 2015). Differences in spectra are the result of the fact that extraction of rabid dyes in an organic solvent changes their surrounding and this often reason great shifts in their absorption limitation and this is an agreement with (Reichardt 1994), shows from all Figures that the Prodigiosin in methanol is red and exhibits a sharp spectral peak at 537nm. Dye spectra also showed a persistent shoulder at about 505 nm.The spectral properties of prodigiosin are in accord with the result obtained by (Andreyeva and Ogorodnikova. 2015). From Figure (2) it is clear that increasing the concentration of dyes led to increasing the absorption intensity and this is agreement with (Barzan and Hajiesmaeilbaigi 2018)

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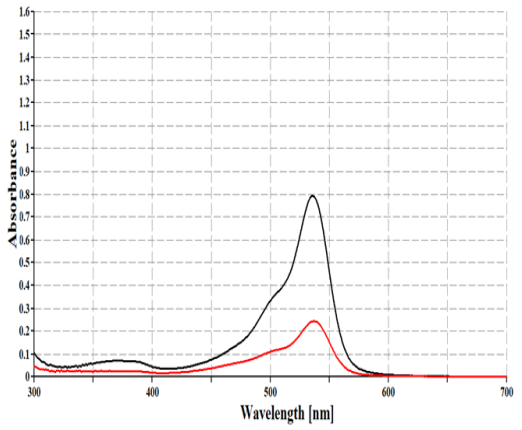

Figure 2

Absorption spectra of Prodigiosin dissolved in methanol at concentration [10µM] black line; at concentration [1 µM] red line.

❖ **Fluorescence Spectra**

The fluorescence spectra of Prodigiosin molecules which were dissolved in methanol have been measured for starting from[10µM], and ends with concentration [1µM], at optimal wavelength excitation (537 nm). The bandhas appeared at a wavelength(557 nm) as shown in figure(3), this is an agreement with (Andreyeva and Ogorodnikova. 2015). From figure (3), increasing the concentration of dyes led to lowering the fluorescence intensity this is agreement with (Barzan and Hajiesmaeilbaigi 2018). The decrease in relative fluorescence intensity and disappearance of the vibronic band are caused by the phenomenon of self-absorption and this is an agreement with (Misiak et al. 2011).

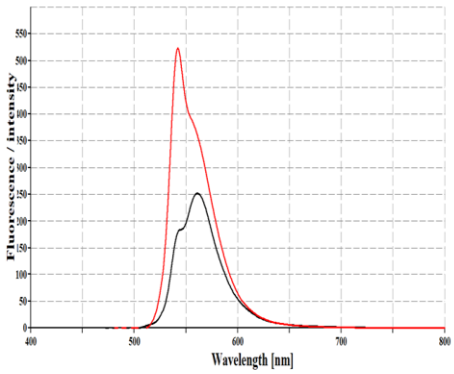

Figure 3 Fluorescence spectra of Prodigiosin dissolved in methanol at concentration: [10 µM] black line;at concentration [1 µM] red line.

3.2 The absorption and fluorescence spectra of Curcumin

As for the absorption and fluorescence spectra of Curcumin (CUR), dissolved in methanol, were studied with different concentrations at room temperature and peak of spectra are respecting located at wavelengths (430 nm) and this agreement with (Jasim and Ali 1989, Patra and Barakat 2011), and this agreement with conception (Mukerjee et al. 2010, Nardo et al. 2008) as shown in figure (4).

The solution is colored bright yellow at the high-concentration and disappears almost at [1µM] concentration. These figures also show that the absorbance increases with the molecular concentration of solution which increases according to Beer-Lambert law that increases in the molecular concentration of the solution and consequently leads to increases in the number of molecules in the solution and this lead to increases in the absorbance and this in agreement with (Barzan and Hajiesmaeilbaigi 2018).

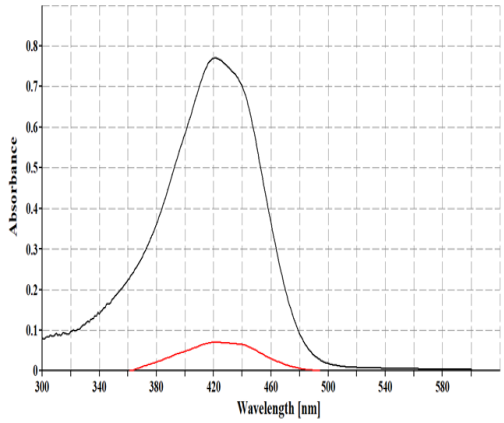

Figure 4

Absorption spectra of Curcumin dissolved in methanol at concentration: [10 µM] black line; [1 µM] red line.

The spectra of fluorescence of Curcumin molecules which were dissolved in methanol [10, 1 µM], had been measured at the excitation wavelength (λ_{ex}=430 nm) as shown in figure (5), the band has appeared at a wavelength (540 nm) and this is agreement with (Subhan et al. 2013). The decrease in relative fluorescence intensity and the disappearance of the

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vibronic band are caused by the phenomenon of self-absorption and the concentration quenching, this is an agreement (Misiak et al. 2011, Barzan and Hajiesmaeilbaigi 2018).

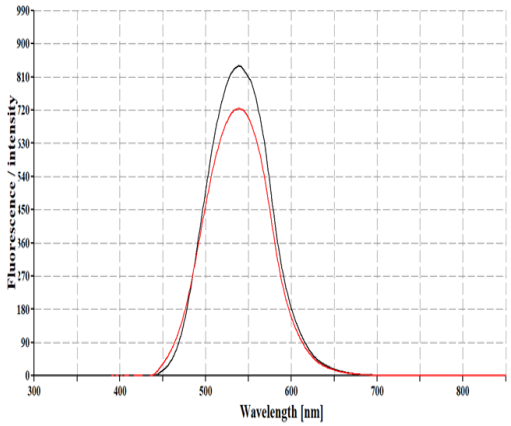

Figure 5

Fluorescence spectra of Curcumin dissolved in methanol at concentration: [10 µM] black line; [1 µM] red line.

V. Energy Transfer Calculations

This paragraphdisplays Calculation of R0 from Spectral Overlap by used MatLab program this routine was written by Joshua Vaughan, Fall 2000(Genevieve Kate Phillips 2017), used between Prodigiosin and Curcumin, Then, is calculation found that the value of the Förster radii which was calculated from molar absorptivity of concentration [10 µM].

The quenching factor (Q) results from absorption and fluorescence spectrum of Curcumin doping of Prodigiosin compounds dissolved in methanol with different concentrations [10µM], [1µM] respectively. the quantity R_o= 4.9834 ºA for FRET between given donor and acceptor molecules.

The fluorescence spectrum of the donor must overlap with the absorption spectrum of acceptor, as verified in figure (6), as in (Clegg 2009) .

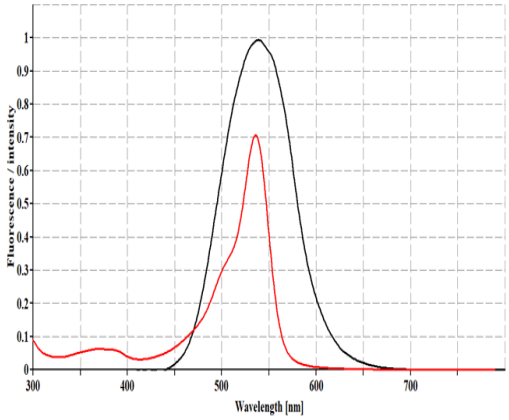

Figure 6 Fluorescence spectra of Curcumin (donor) and Absorption spectra of Prodigiosin (acceptor) dissolved in methanol at concentration [10 µM].

When mixing the Curcumin dissolved in methanol with Prodigiosinat were studied with at different concentrations [10, 1µM] at room temperature at ratio (v/v) =(1/99), (7/93), (11/89). Where a mixture solution of Prodigiosin as guest and Curcumin as a host, was calculated the following equation:

$$\frac{\text{guest}}{\text{host}} = \frac{1\%\ [10\mu M]}{99\%\ [10\mu M]} = 0.010$$

$$\frac{\text{guest}}{\text{host}} = \frac{3\%\ [10\mu M]}{97\%\ [10\mu M]} = 0.031$$

$$\frac{\text{guest}}{\text{host}} = \frac{7\%\ [10\mu M]}{93\%\ [10\mu M]} = 0.075$$

Figure (7), shows the absorption of a solution to the Curcumin mixing with Prodigiosin at various rates dissolved in methanol. It appears from the analysis sample, it consists of the shoulder as undifferentiated at wavelengths (534 nm) and the peak located at the wavelength of Curcumin peak at (420 nm).

The shoulder is for the Prodigiosin spectra and other peaks are for the Curcumin molecule.The change of absorption peak was caused by the change of doping ratio because of the addition of Prodigiosin in pure methanol and complex configuration, this is an agreement with(Guseva, Antina, and Ksenofontov 2019). The intensities of the absorption maxima change to Prodigiosin and Curcumin chromophores

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depended on the relative molar fractions of these two mixed moieties as shown in figure (7).

Upon addition of Prodigiosin, the band at 420 nm and 443nm progressively decreased, whilst a new band with a peak at 535nm formed, this is an agreement with (Kim et al. 2015).These differences further proved that the complex may be formed by intermolecular force between Prodigiosin and Curcumin molecules when Prodigiosin was added and this is very good agreement with (Wang et al. 2017).

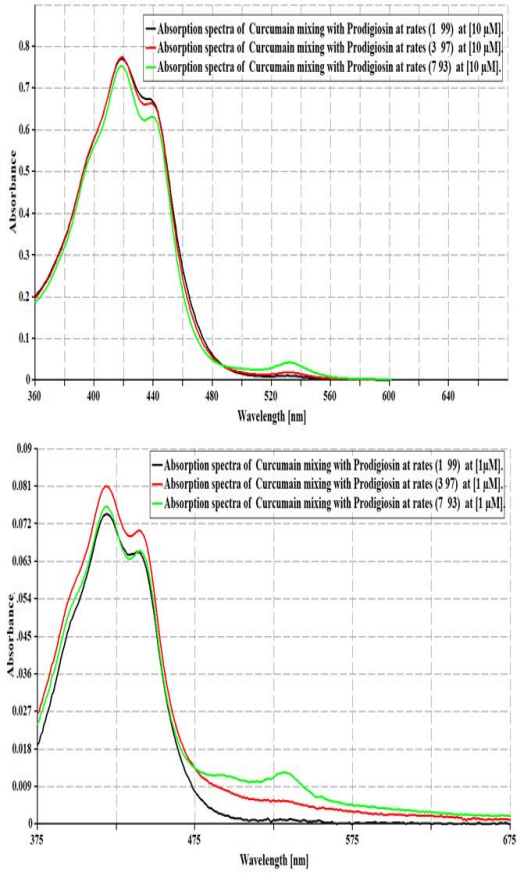

Figure 7 Absorption spectra of Curcumin mixing with Prodigiosin at all rates mixing at above [10 µM]; below [1 µM].

Figure (8) and figure (9) shows the fluorescence of a solution the Curcumin mixing with Prodigiosin at all rates mixing dissolved in methanol. Appear at concentration [10, 1µM] respectively, with sample analysis consist of a peak at (535nm),this peak of complex formation from molecules mixing; to which most of the energy was transferred as a result transfer of nonradiative energy and result of charge transfer between the Curcumin molecule and Prodigiosin molecule.

The fluorescence intensity of Curcumin also decreases upon addition of Prodigiosin without large obvious changes in emission wavelengthis a very good agreement with (Kim et al. 2016, Hao et al. 2017, Liu et al. 2019). i.e. fluorescence intensity of Curcumin and Prodigiosin gradually decreased with increasing rate mixing of Prodigiosin, indicating fluorescence quenching and suggesting the existence of interactions between Curcumin and Prodigiosin is a very good agreement with (Liu et al. 2019).

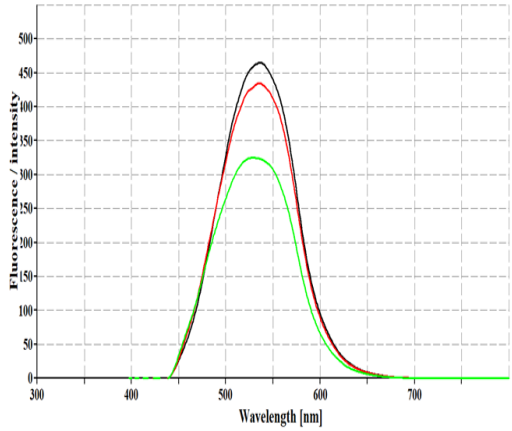

Figure 8 Fluorescence spectra of Curcumin mixing with Prodigiosin dissolved in methanol at concentration [10 µM] of rate mixing: black line 99:1; red line 97:3; green line 93:7.

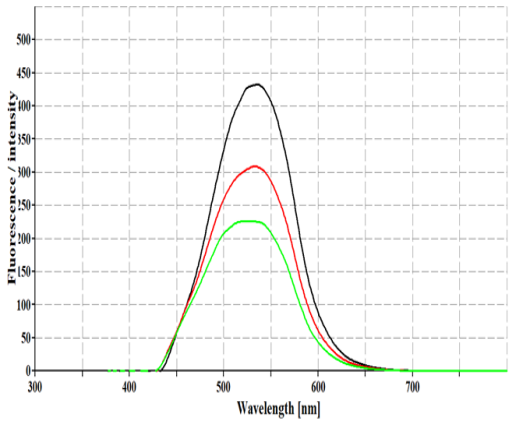

Figure 9 Fluorescence spectra of Curcumin mixing with Prodigiosin dissolved in methanol at concentration [1 µM] of rate mixing: black line 99:1; red line 97:3; green line 93:7.

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Due to the presence of three effective nitrogen atoms in a Prodigiosin molecule, we note in table (4.1) a decreasing in the intensity of fluorescence of Curcumin molecule as a result of increased existence Prodigiosin. The higher rate of guest presence, the greater rate of nonradiative energy and the increasing of the quenching process resulting from the presence of impurities, this is an agreement with (Kim et al. 2015).

Due to the most of energy was transferred as a result of the convergence of the tops of molecules through nonradiative energy between them. Thus, energy transfers between them were achieved. The value of the spectrum intensity of Prodigiosin solution at a concentration [1µM] was less than an intensity at concentration [10µM] because of concentration quenching fluorescence, as noted in figures (8, 9).

Table (1) Value of quenching factor (Q) at concentration [10µM] at all mixing ratio (v/v).

I_G/I_H	A	A_d	Q
0.0101	76211	44924.746	0.696414711
0.0309	76211	42980.578	0.773149724
0.0752	76211	33933.273	1.245907726

Table (2) Value of quenching factor (Q) at concentration [1µM] at all mixing ratio (v/v).

I_G/I_H	A	A_d	Q
0.0101	16303.95	10769.48	0.5139026
0.0309	16303.95	8217.76	0.9839885
0.0752	16303.95	6382.02	1.55466

We note in a table (2) a decreasing the fluorescence intensity of Curcumin molecule as a result of increased existence Prodigiosin. The higher rate of guest presence, causes the greater the rate of nonradiative energy and the increasing of the quenching process resulting from the presence of impurities.

VI. Conclusion

The objective of this work is achievement energy transfer between Curcumin as donor and Prodigiosin as acceptor. The results of this research showed that:

The Prodigiosin molecule absorption spectrum clearly marked with and sharp edges of the spectrum at high concentrations refer to the strong self-absorption of this molecule, and also relative to the Curcumin molecule at diluted concentrations .

Decreasing the rate of energy transfer is due to the strong self-absorption of the Guest and to defects resulting from the addition of impurities and concentration quenching when comparing [10,1 µM]. At adding (1%, 3%, 7%) of Prodigiosin to the Curcumin dye at [10,1 µM], the complexes had led to an increase in the absorption intensity of the shoulder as undifferentiated and quench the fluorescence intensity of Curcumin dye. As a result, energy transfers are nonradiative.

The overlap between the absorption spectrum of Curcumin and the emission spectrum of Prodigiosin supports the fact that the Prodigiosin molecules accept the excitation energy from Curcumin molecules. But, which consists of our molecular complex is composed of the collisions between molecules, one is excitation by a wavelength (430 nm), which leads to charge transfer between molecules consequently. The stability of the group, by nonradiative energy from dipole-dipole interaction, is clear in the fluorescence spectrum of the solution of different proportions and noted the overlap between donor emission spectrum and acceptor spectrum. Furthermore, the fluorescence relative intensity was taken as an indicator of energy transfer efficiency.

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