

Fluorescence Quenching Reaction of Chlorophyll a by Tris(acetylacetonate)Iron(III) in Various Solvents

(Tindak Balas Pemelindapan Kependarfluoran Klorofil a oleh
Tris(asetilasetonat)Iron(III) dalam Pelbagai Pelarut)

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ABSTRACT

Chlorophyll a is known as the prevailing light absorbing pigment giving a strong absorption and fluorescence emission in visible region. Quenching reactions of the chlorophyll a fluorescence by $Fe(acac)_3$ were precisely investigated in various organic solvents which are benzene toluene, ethanol, methanol, dmf, dmsO and acetonitrile. Electron transfer performance of chlorophyll a by $Fe(acac)_3$ was investigated from oxidative quenching reaction. Herein, the simplified Rehm-Weller relationship was used to calculate the free energy change of the photo-induced electron transfer reaction. Emission intensity decreased when the concentration of $Fe(acac)_3$ quencher was increased. Non-linear Stern-Volmer plots are found to be affected by inner filter effect more than the ground state complex formation. Rate of quenching reactions (k_q) were determined from the Stern-Volmer equation with corrected inner filter effect. The rates of quenching reactions occurred faster in high viscous solvents.

Keywords: Chlorophyll a; oxidative quenching reaction; Stern-Volmer plotting

ABSTRAK

Klorofil a dikenali sebagai pigmen cahaya penyerap yang memberikan penyerapan yang kuat dan pelepasan kependarfluoran yang boleh dilihat. Tindak balas pemelindapan klorofil a oleh $Fe(acac)_3$ dikaji secara tepat dalam pelbagai pelarut organik seperti benzena toluen, etanol, metanol, dmf, dmsO dan asetonitril. Prestasi pemindahan elektron klorofil a oleh $Fe(acac)_3$ telah dikaji daripada tindak balas pemelindapan oksidatif. Di sini, hubungan Rehm-Weller dipermudah telah digunakan untuk mengira perubahan tenaga bebas tindak balas pemindahan elektron teraruh-foto. Keamatan pelepasan menurun apabila kepekatan pelindap $Fe(acac)_3$ meningkat. Plot Stern-Volmer tak linear terjejas oleh kesan turas dalaman lebih daripada pembentukan keadaan asas yang kompleks. Kadar tindak balas pemelindapan (k_q) telah ditentukan daripada persamaan Stern-Volmer kesan turas dalaman diperbetulkan. Kadar tindak balas pemelindapan berlaku dengan lebih cepat dalam pelarut likat yang tinggi.

Kata kunci: Klorofil a; tindak balas sepuh lindap oksidatif; plot Stern-Volmer

INTRODUCTION

Chlorophyll a is one type among four derivatives (chlorophyll a-d) which is the most abundant in blue-green algae, cyanobacteria and general plants. Chlorophyll a harvests light in visible region at longer wavelength comparing with other types and give an emissive fluorescence in longer wavelength close to near IR region. Because of its structure and photo-physical properties, chlorophyll a is widely used to be a donor molecule in photochemistry frameworks. One of its interesting applications is being a sensitized molecule in dye sensitized solar cell. The electron transfer can occur from chlorophyll a to metal oxide semiconductor (Amao et al. 2004; Durrant et al. 2004; Liu et al. 2008; Wang et al. 2006). Furthermore, chlorophyll a can be used in artificial photosynthetic model (Jennings et al. 1989; Ngweniform et al. 2007). Photo-induced electron transfer of chlorophyll a to several acceptor molecules has been widely investigated via quenching reaction (Falco et al. 2015; Gazdaru et al. 2001)

owing to its obvious fluorescent property. Chlorophyll a, the central magnesium coordinated with four nitrogen atoms of the pyrrole rings can be both electron donor due to presence of ketone (C=O) groups and electron acceptor via its central Mg atom (Trifunac & Katz 1974). Chlorophyll a and its derivatives are not just only frequently used in electron transfer but also being a donor molecule in energy transfer process in plants (Xiaoqing et al. 2007). Lots of researches try to replace the metal center of Mg with other transition metal ions in order to explore photo-physical properties of its derivatives (Li & Inoue 1991; Medforth et al. 1997; Nonomura et al. 1994; Yamashita & Inoue 1991). Nevertheless, only few researches focus on electron transfer of chlorophyll a to Fe(III). As such Fe(III) is a biologically important metal ion. It plays an important role for most organisms including oxygen transportation, respiration, synthesis and reparation of DNA and primary metabolism (Zheng & Nolan 2012) which are relevant to electron transfer process. An imbalance of Fe(III) can

cause various diseases. There are some publications that stated the detection of Fe(III) by using the synthesized dyes which are harmful to the living cells (Nandre et al. 2014). In this approach, the preliminary results of using chlorophyll a, an ecofriendly green pigment, to detect the Fe(III) from quenching reaction of outer sphere electron transfer is derived. It is owing to the properties of Chlorophyll a in light absorption and being an excellent electron transfer assistant from water to carbon dioxide in order to produce the carbohydrate and oxygen in photosynthesis system make the Chlorophyll a to be an appropriate agent in photo-induced electron transfer (PET) process to Fe(III) acceptor. The results from this study can be a model of utilizing the electron transfer process of the plant pigment to the metal ion sensor with simple and inexpensive techniques which can be further applied to Fe(III) detection in other medicinal frameworks.

In this present work, the chlorophyll a is a donor molecule transferring electrons to Fe(III). In order to avoid problems of ionic strength of the studied system, a forming of Fe-Chl a complex at ground state, the acceptor molecule of Fe(III) must be uncharged molecule. The tris(acetylacetonate)iron (III), an $[\text{Fe}(\text{acac})_3]$, is selected as an acceptor complex owing to its neutral charge. Therefore, the ionic strength can be neglected. Besides, the replacing of Fe^{3+} in the porphyrin of chlorophyll a yielding Fe-Chl a complex is least possible to occur owing to the strength of *tris*-complex of Fe(III). In addition, $\text{Fe}(\text{acac})_3$ can dissolve all types of solvent while the Chlorophyll a cannot dissolve in water but completely well soluble in organic solvent. In this research, the electron transfer of chlorophyll a fluorescence to $\text{Fe}(\text{acac})_3$ in different polarities of solvents like benzene, toluene, methanol, ethanol, acetonitrile, tetrahydrofuran, dimethylformamide and dimethylsulfoxide solvents were investigated by steady-state measurement. Quenching reactions were measured. The photo-physical properties of both fluorophore and quencher were explored. The rates of reactions were determined in term of quenching rate constant (k_q). High k_q value is presumable high electron transfer.

METHODS

MATERIALS

All Chemical reagents were analytical reagent grades and purchased from the following commercial sources; Chlorophyll a (spinach) from Fluka, $\text{Fe}(\text{acac})_3$ and ferrocenemethanol from Sigma-Aldrich, acetonitrile and methanol from Lab Scan, benzene and DMSO from BDH, ethanol, toluene and colloidal silica from MERCK, DMF from Fluka Riedel-deHäen and THF from Fisher Scientific. Chlorophyll a was used as received without further purification. All solvents were dried by 4 and 5 Å of molecular sieves over two nights before using. UV-Vis absorption spectra were monitored with a UV-Vis spectrophotometer model Specord S100. Emission spectra were recorded with a luminescence spectrometer LS55

manufactured by Perkin Elmer. Luminescence cuvette is a standard transparent cuvette with a screw cap. Path length of the cuvette is 1 cm. Excitation and emission slit widths are 5 nm. Luminescence lifetimes of Chlorophyll a in various solvents were measured by modulation technique with digital storage oscilloscope (Landgraf 2004) by using LED 370 nm as a light source, gray filter U 340 nm as an excitation filter, RG 610 as a long pass filter and analyzing data by LD_CALC program (version 2.04). A scattering sample (colloidal silica or Ludox, Aldrich, Austria) was applied which acts as reference. Cyclic voltammetry measurement was measured by MacLab (4e AD Instruments with potentiostat/Serial No. p068). Software of the MacLab is EChem program version 1.5. Glassy carbon was used as a working electrode, SCE was used as a reference electrode and Pt liked flag was used as a counter electrode. Ferrocenemethanol (Fc-MeOH) was used as an internal standard and showed a redox couple at -0.382 V versus saturated calomel electrode (SCE) in DMF.

METHODS

Chlorophyll a and $\text{Fe}(\text{acac})_3$ stock solutions were prepared with the concentrations of 50 μM and 1 mM, respectively. All chlorophyll a solutions were removed using O_2 by purging Ar for 5 min before the absorbance or emission intensity was measured. For quenching experiment, the absorbance of chlorophyll a was fixed at 0.05 in each solvent in order to prevent the re-absorption and self-quenching reaction. Then, a certain amount of chlorophyll a stock solution was added into 10 mL volumetric flask. The concentration of chlorophyll a was 1 μM at 0.05 absorbance. This concentration was used for all samples while concentrations of $\text{Fe}(\text{acac})_3$ were varied from 10 μM to 0.2 mM by quantitatively added certain amounts of volume into chlorophyll a solutions and adjusted total volume of solvent up to 10 mL. The integrated areas of luminescence bands were recorded. Redox potential of $\text{Fe}(\text{acac})_3$ was measured by cyclic voltammetry technique which was taken for Gibbs free energy in Rehm-Weller calculation. The 0.1 M of tetrabutylammonium hexafluorophosphate (TBAP) was used as supporting electrolyte in CV experiments. Cyclic voltammograms were scanned from -2 V to +2 V with the scan rate of 50, 100, 250, 500, 1000 and 2000 mV, respectively.

RESULTS AND DISCUSSION

PHOTO-PHYSICAL PROPERTIES AND SOLVENT EFFECT OF CHLOROPHYLL A AND $\text{Fe}(\text{ACAC})_3$

Absorption spectra (Figure 1(a) and 1(b)) of chlorophyll a in various solvents were recorded in UV and visible region. Chlorophyll a displays two obvious absorption bands in visible range due to extended π -delocalization at the edge of cyclic tetrapyrrole (porphyrin) part. These two bands are a Soret band in Ultraviolet-Visible and a Q band in visible region (Zvezdanovic & Markovic 2008).

The Soret and Q bands are an intense absorption from $\pi-\pi^*$ transition around 430 nm ($\epsilon = 1.5 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$) and charge transfer band around 669 nm ($\epsilon = 1 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$), respectively. There is an interesting behavior of Soret absorption bands of chlorophyll a in different polarity of solvents like benzene, methanol, acetonitrile, DMF and DMSO. Their maximum wavelengths of absorptions show the red shifting toward higher solvent polarities (Figure 1(a)) implying to the stabilized of π^* orbital. However, the Q bands are independent from the solvent property (Figure 1(b)).

Fluorescence emission spectra (Figure 2) of chlorophyll a in various solvents are observed strong bands with maximum wavelength around 670 nm and shoulder bands around 720-730 nm at room temperature. The excitation wavelength is 430 nm which is the Soret band. The emission spectra are devoid of vibrational structure. An increasing of viscosity and polarity of solvents leads the emission spectra appear at longer wavelengths (Table 1). Stoke shifts between absorption Q bands and emission spectra are small which is a characteristic for homo-energy transfer phenomena. Nevertheless, the stoke shifts between

Soret absorption and emission bands provide larger energy values. No overlap between these two bands is observed. In this case, the controversy of energy transfer can be neglected. Then the maximum wavelength of Soret band is chosen to be the excitation wavelength instead of Q band. Another important parameter is the excited lifetimes which were measured from phase modulation technique with digital storage oscilloscope. In phase modulation technique the sample was excited with a sinusoidally modulated continuous radiation of LED. Hence the observed emission was also modulated with the same frequencies as the excitation. For a monoexponential decay, the lifetime is independent from modulated frequencies. In our case, chlorophyll a has short-lived excited electronic state within the range of 5.5 to 7.5 ns. From the studied system, there is no trend between the solvent properties and the emission lifetimes either solvent polarity or solvent viscosity as shown in Table 1. In the different solvents, the emission lifetimes are different because the effects of solvent and environment on energy relaxation are complex and are due to several factors for example rate of solvent relaxation, changes in radiative and non-radiative decay rates,

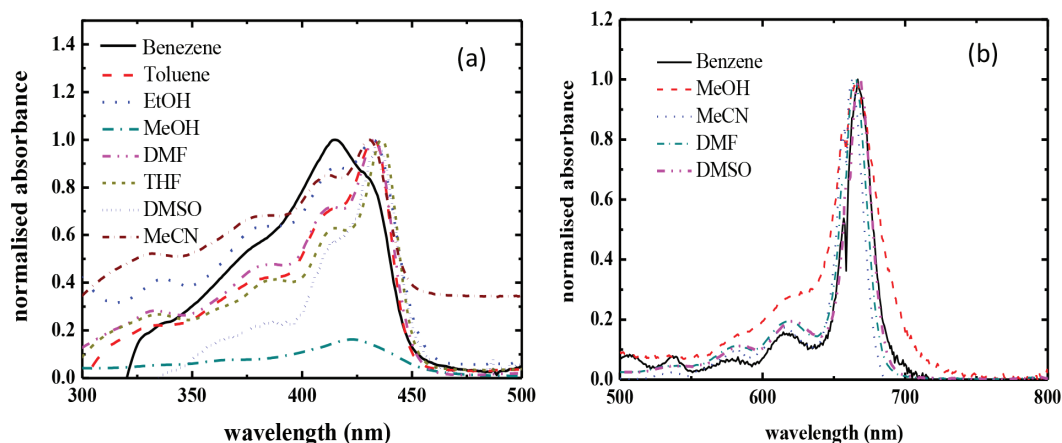


FIGURE 1. Normalized (a) absorption soret bands and (b) absorption Q bands of chlorophyll a in various solvents

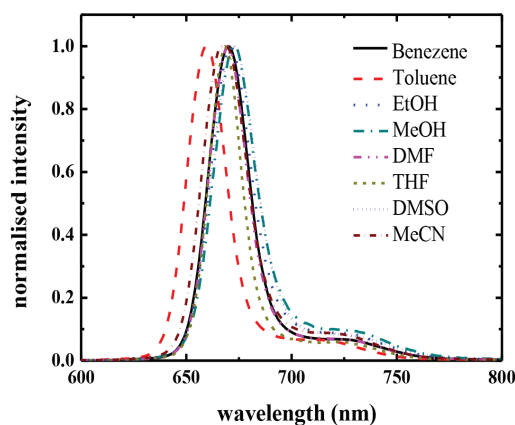


FIGURE 2. Normalised emission spectra of chlorophyll a in various solvents

TABLE 1. fluorescence emission data with related energies of chlorophyll a in various solvents

Solvents	Properties		Wavelengths of (nm)		Stoke shift of Soret band (eV)	E_{00} (eV)	τ^0 (ns)
	Polarity	Viscosity at 25°C (cP)	Soret abs band	em			
Benzene	2.4	0.604	437	670	2.87	2.37	5.5
Toluene	2.7	0.560	438	659	2.99	2.42	7.5
Ethanol	5.0	1.074	432	671	2.84	2.35	6.1
Methanol	5.1	0.544	434	673	2.87	2.36	5.5
DMF	5.8	0.802	436	670	2.93	2.39	7.5
DMSO	6.4	2.140	438	673	2.88	2.37	6.8
Acetonitrile	7.2	0.352	438	668	2.86	2.35	6.8

rigidity of the local environment and solvent orientation polarizability (Lakowicz 2006).

In this work, investigation of the oxidative quenching fluorescence of chlorophyll a by $\text{Fe}(\text{acac})_3$ was carried out. The electron acceptor is $\text{Fe}(\text{acac})_3$ which is non-emissive species, no chemical interaction between chlorophyll a and $\text{Fe}(\text{acac})_3$ and no hetero-energy transfer between excited chlorophyll a and $\text{Fe}(\text{acac})_3$. However, there is a strong overlapping between absorption of chlorophyll a and $\text{Fe}(\text{acac})_3$ (Figure 3) which causes the inner filter effect.

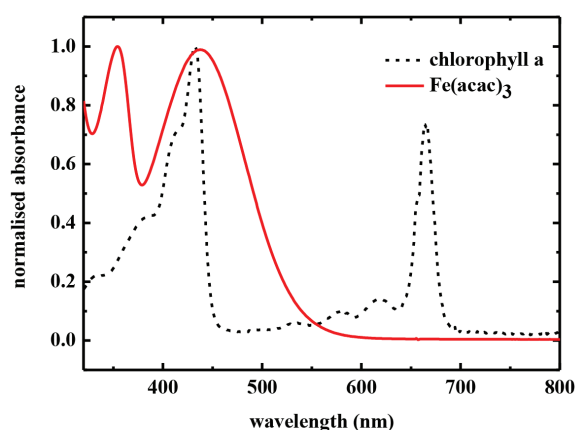


FIGURE 3. Normalised absorption spectra of $\text{Fe}(\text{acac})_3$ and chlorophyll a in a solution

THERMODYNAMICS PARAMETER: DRIVING FORCE

In order to specify the possibility of electron transfer between chlorophyll a and $\text{Fe}(\text{acac})_3$, the driving force or Gibbs free energy has to be identified. The fundamental thermodynamic condition for spontaneous electron transfer between neutral reactants must give $\Delta G^0 < 0$. The thermodynamic driving force of electron transfer can be predicted by Rehm-Weller equation (1):

$$\Delta G^0 = E_{1/2}(D/D^+) - E_{1/2}(A/A^-) - E_{00}(D^*) + w_p - w_r, \quad (1)$$

where $E_{1/2}(D/D^+)$ and $E_{1/2}(A/A^-)$ are the half wave oxidation potential of the ground state donor and the half wave oxidation potential of acceptor, respectively. The terms w_p and w_r are the Coulombic work terms of the products and reactants, respectively. The Coulombic work terms are usually small and zero for neutral species. In this study, the system is neutral charge and work term parameters can be neglected. Herein, we report an example of Gibbs free energy calculation in DMF as an example. The parameter of $E_{00}(D^*)$ is zero-zero (0-0) spectroscopic transition energy gap estimated from the half of the absorption and emission energies. The $E_{00}(D^*)$ was obtained at 1.84 eV (Richert 1989). The oxidation potential of chlorophyll a (Mg is center atom) in DMF is taken at 0.58 V (Kathiravan et al. 2009; Nanomura et al. 1997) while the reduction potential of $\text{Fe}(\text{acac})_3$ was measured by cyclic voltammetry technique and given the half wave potential in DMF at 0.66 V (Figure 4). Therefore, the thermodynamic driving force is -1.93 eV. Electron transfer from chlorophyll a to $[\text{Fe}(\text{acac})_3]$ can spontaneously occur.

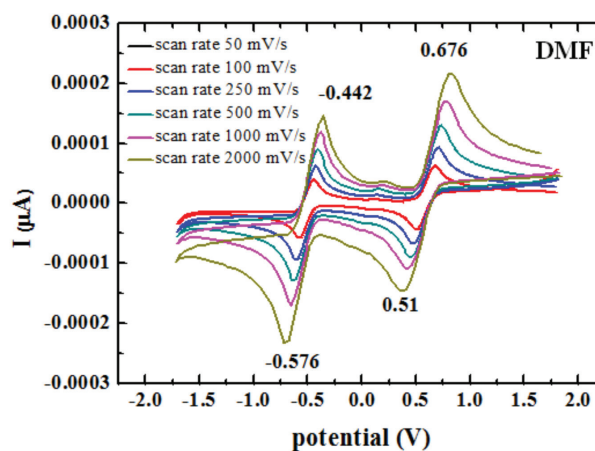


FIGURE 4. Cyclic voltammograms of reversible couple of $\text{Fe}(\text{II/III})$ in $\text{Fe}(\text{acac})_3$ comparison with ferrocene methanol (at E_{pa} -0.576 V and E_{pc} -0.442 V) in DMF

QUENCHING REACTION OF CHLOROPHYLL A BY MOLECULE ELECTRON ACCEPTOR $\text{Fe}(\text{acac})_3$

The steady-state fluorescence quenching is a common experimental method for determining the reaction rate constant obtained from the Stern-Volmer plot: $I_0/I = K_{SV}[Q]$. This plot is relative between I_0/I (y-axis) versus $[Q]$ (x-axis) where I_0 is the intensity with absence of quencher molecule; I is the intensity with presence of quencher molecules; and $[Q]$ refers to quencher concentrations. Slope of the linear plot is the Stern-Volmer constant (K_{SV}) which is equal to $k_q \tau_0$ where τ_0 is excited state emission lifetime. The quenching rate constant (k_q) is the rate of reaction. From the results, fluorescence intensities of chlorophyll a decrease with increasing of $\text{Fe}(\text{acac})_3$ concentrations as shown in Figure 5(b). The obtained Stern-Volmer plots in studied solvents exhibit non-linear behavior. The curvatures bend upward to the y-axis (Figure 7). This phenomenon in the present work possibly occurs from two main causes which are ground state complex formation and inner filter effect. Although ground state complex formation can be observed from non-linear Stern-

Volmer plot, but absorbances at the excitation wavelength (430 nm) in the presence of high $\text{Fe}(\text{acac})_3$ concentrations are dramatically increased (Figure 5(a)) without changing in wavelength. This fact is an evidence of inner filter effect more than that of ground state complex formation. In order to prove this assumption, the correction of absorbance and intensity must be taken into account. If the Stern-Volmer plot turns to be linear, the ground state complex formation should not be found.

In principle, the inner filter effect can occur due to high absorption of the sample (primary inner filter effect) and/or the reabsorption of emitted light (secondary inner filter effect). In our experiment, only the primary inner filter effect was observed. Because there exists only the overlapping of absorption spectrum of $[\text{Fe}(\text{acac})_3]$ in the same range of the absorption of chlorophyll a but there is no overlapping of $\text{Fe}(\text{acac})_3$ with emission of chlorophyll a. A competitive excitation among the fluorophore and quencher takes place. The excited fluorescence is quenched by quencher molecules and inner filter effect competitively. The observed fluorescence intensity is less than that of

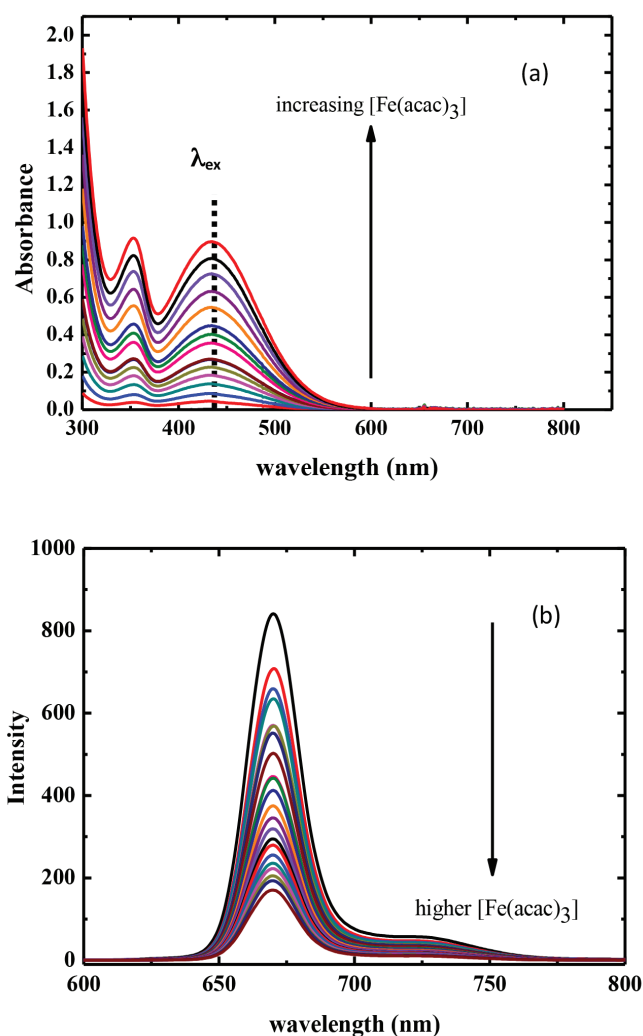


FIGURE 5. (a) Absorption spectra of chlorophyll a in the presence of $\text{Fe}(\text{acac})_3$ in DMF solution and (b) the decreasing of fluorescence intensity of chlorophyll a in the presence of $\text{Fe}(\text{acac})_3$

the usual. The experimental fluorescence intensities from chlorophyll a are low leading to a high ratio between I_0/I . That is the reason why the Stern-Volmer plot bends upward to the y-axis instead of linear. In order to obtain the correct results, the observed intensity must be removed from the $\text{Fe}(\text{acac})_3$ absorption at 430 nm. The correction for the primary inner filter effect can be done by multiplication of the correction factor with each observed intensity based on the idea that the average path length of absorption of the excitation and emission light is $\frac{1}{2}$ of the cuvette length. The detection system collects the fluorescence emitted only from the central part of the exciting beam (Figure 6). If the concentration of the sample is low ($A < 0.02$), the incident light will be slightly attenuated through the cuvette. The correction of inner filter effect can be summarized as shown in (3):

$$I_{0, \text{corr}} = I_0 \cdot 10^{-A_{\text{abs}}/2} \quad (3)$$

The correction factor can be rewritten in term of $10^{-A_{\text{abs}}/2}$ where A_{abs} is the different absorbances of sample absorbance (A_S) and fluorophore (A_F) absorbance. This correction factor was multiplied with the I_0 then it brought the corrected fluorescence intensity, $I_{0, \text{corr}}$ for each quencher concentration.

Then the values of $(I_{0, \text{corr}}/I)$ were plotted with various $[\text{Fe}(\text{acac})_3]$ in each solvent. The correction was compared with the method of correction by Borissevitch (1999) as shown in (4):

$$(I_0/I)\eta = 1 + K_{\text{SV}}[Q], \quad (4)$$

where

$$\eta = \frac{A_{x0}(1-10^{-A_{xi}})}{A_{xi}(1-10^{-A_{x0}})} \quad (5)$$

and A_{x0} is the absorbance of chlorophyll a at the excitation wavelength, A_{xi} is the absorbance of sample ($\text{Chl a} + \text{Fe}(\text{acac})_3$) at the excitation wavelength and the A_{xi} is the absorbance of sample at the emission wavelength.

As previously mention, the Stern-Volmer plot obtained from the experiment without corrected inner filter effect gives the curvature profile bending upward to the y-axis (Figure 7). The K_{SV} of this plot is determined from slope of the linearity part of the curve. In contrast, the corrected Stern-Volmer plots either from our method or Borissevitch method are both linear. Therefore, the ground state complex formation is supposed to be omitted. The K_{SV} values from these two corrections are 4 times less comparing with the uncorrected experimental data. The corrections by our method gives a slightly lower K_{SV} values than that of Borissevitch method but gives a slightly higher deviation of intercept from unity. It is worth to validate our proposed method with Borissevitch method (Table 2), an independent sample t-test was performed (Table 3).

The results indicated that the variances of the intensity ratios corrected by Borissevitch method and those corrected the method proposed in this study are not equal (P -value of 0.03) based on Levene's test. Thus, a t -value with unequal variance was used. A significance level of 0.134 was found for the two-tailed test, which indicates that the intensity ratios corrected by Borissevitch method (mean value = 0.436) and those corrected the method proposed in this study (mean value = 0.384) was not significantly different.

Further validation was performed as shown in Figure 8. The intensity ratios corrected by Borissevitch method and those corrected by the method in this study are compared and illustrated in this figure. The intensity ratios of those corrected by the proposed method are well fitted to those corrected by Borissevitch method whenever the corrected intensity ratio is not greater than 0.5. For the corrected intensity is beyond 0.5, the proposed method

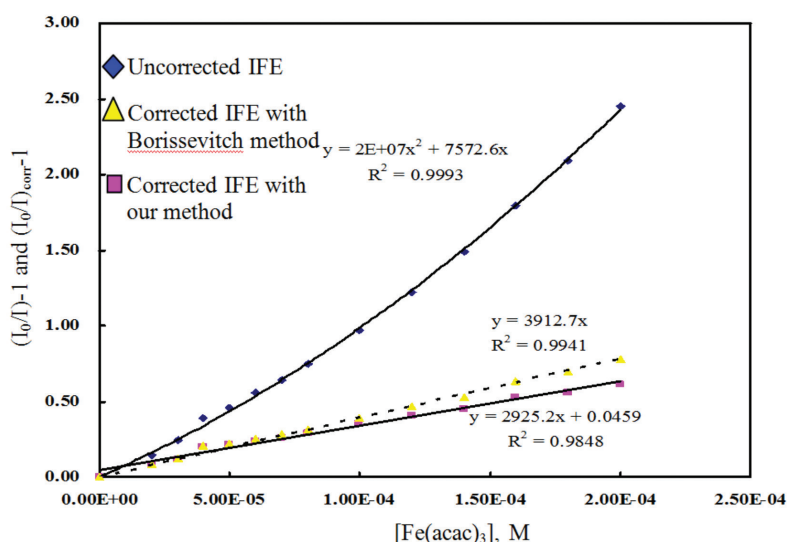


FIGURE 6. Geometry of fluorescence production in a cuvette cell (Leesakul 2007)

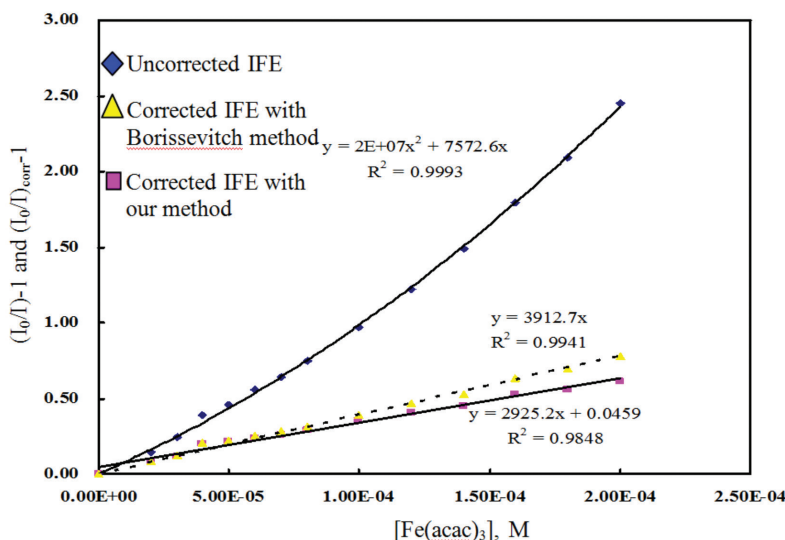


FIGURE 7. Stern-Volmer plots in DMF solution obtained from experiment with uncorrected IFE, corrected IFE with our method and corrected IFE with Borissevitch method

TABLE 2. Quenching rate constants obtained from Stern-Volmer plots of uncorrected and proposed corrected inner filter effect comparison with Borissevitch method in various solvents

Solvents	Viscosity	Rates of quenching $k_q \times 10^{-11}$ ($M^{-1}s^{-1}$)		
		Experimental	IFE correction	Borissevitch method
Acetonitrile	0.352	22.7	3.97	5.69
Methanol	0.544	16.7	5.53	6.70
Toluene	0.560	27.6	5.92	7.10
Benzene	0.604	27.6	6.76	9.49
DMF	0.802	20.2	7.36	9.03
Ethanol	1.074	18.0	4.77	6.38
DMSO	2.140	25.7	6.51	9.50

TABLE 3. Independent samples test to compare means of IFE obtained from proposed method and Borissevitch method

	Levene's test for equality of variances			t-test for equality of means					
	F	Sig.	t	df	Sig.	Mean Diff.	Std. Err. Diff.	95% Confidence Interval	
								Lower	Upper
Equal variances assumed	9.238	.003	1.506	212	.134	5.211×10^{-2}	3.460×10^{-2}	-1.6104×10^{-2}	.1203
Equal variances not assumed			1.506	195.101	.134	5.211×10^{-2}	3.460×10^{-2}	-1.6138×10^{-2}	.1204

yields an underestimation comparing to Borissevitch method. Hence, the reliable concentrations are up to 1.2×10^{-4} M. For higher concentrations, the deviation between Borissevitch method and our proposed method becomes greater. It may arise from the inner filter effect coincide with the ground state complex formation.

The data of K_{sv} and k_q values in various solvents are collected in Table 2. It is clearly seen that without correcting

of inner filter effect, no trend of quenching rate constants comparison with viscosities is observed. Nonetheless, the quenching rate constants show the dependence on solvent viscosities from acetonitrile through-out DMF. Likewise, the quenching rate constants exhibit the trend with solvent viscosities from acetonitrile to Benzene. The viscosity is an important factor for diffusion coefficient in electron transfer system. The viscous solvent can prevent

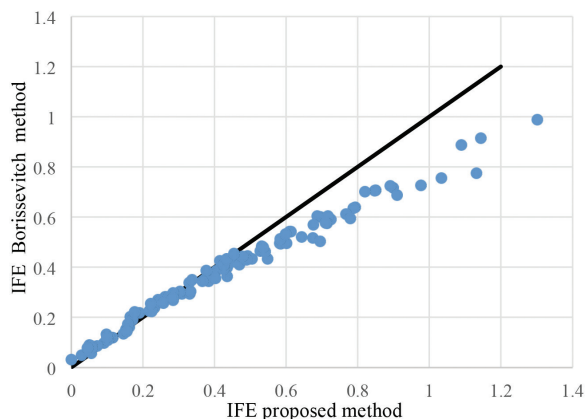


FIGURE 8. Comparison the intensity ratios between those proposed method with Borissevitch method along the 1:1 line

the escape of charge transfer which can bring donor and acceptor molecules closer and solvated around. In another word, the high diffusion rate constant leading to an increasing of electron transfer ability. However, there is the fluctuation of quenching ability in ethanol and dmsolvents which does not come from an artifact because the experiments were carried out for two times. The results were very similar. Besides, an excellent R^2 obtained from fitting the Stern-Volmer was obtained. The result obtained from ethanol and dmsolvents deviate from the trend plotted between the quenching rate constants and the viscosities of all studied solvents as shown in the graph below. However, for ethanol the result obviously showed the dramatically lowest rate constant. The comparison between the quenching rate parameter k_q with solvent viscosity, found that k_q depends on solvent viscosity. High viscous solvent, shows high quenching ability. Nevertheless, for high polarity of solvents like ethanol and dmsolvents, the k_q occurred through the diffusion controlled path. The solutes with high molecular mass has lower diffusion rate constant with high viscosity of solvent at the constant temperature which can be seen from the Smoluchowski and Stokes–Einstein equations (Arik et al. 2005). The decrease in diffusion coefficients may probably cause the reduced quenching efficiency in ethanol and dmsolvents.

CONCLUSION

Photo - physical properties; absorption, fluorescence emission and lifetimes of chlorophyll a and $[\text{Fe}(\text{acac})_3]$ were investigated in various organic solvents. It was clear that higher viscosity and polarity of solvents makes the Soret absorption and emission bands shifted to longer wavelengths. Emission lifetimes of chlorophyll a in various solvents are in the range of 5.5 to 7.5 ns with no trend between the solvent properties and the emission lifetimes either solvent polarity or solvent viscosity. In the different solvents, the emission lifetimes are definitely different because the effects of solvent and environment on energy

relaxation are complex, and are due to several factors for example rate of solvent relaxation, changes in radiative and non-radiative decay rates, rigidity of the local environment and solvent orientation polarizability (Lakowicz 2006). The inner filter effect from high concentration of $\text{Fe}(\text{acac})_3$ affects the non-linear behavior of Stern-Volmer plots. After the real emission intensity is corrected, the Stern-Volmer plots become linear as its usual. Two methods of inner filter effect correction are corresponding up to 0.12 mM higher than that the ground state complex formation is possibly found. The rates of quenching reaction (k_q) are in the range of $10^{11} \text{ M}^{-1}\text{s}^{-1}$ which is high. In addition, the k_q values are higher in more viscous solvents.

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