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Research Article

Chemistry**Viscosity effect on absorption and fluorescence spectra of a new functionalized amphiphilic analogue styryl pyridinium dye**

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KEY WORDS**ABSTRACT**Molecular rotor,
Viscosity effect,
Temperature
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Hoffmann
equation,
Fluorescence
quantum yield.

The effect of temperature and viscosity on the absorption and fluorescence spectra of a new synthesized amphiphilic analogue styryl pyridinium dye namely 1-cetyl-4-[4-chloro-styryl]-pyridinium iodide (CCSP) in glycerol has been studied. There is no observable change in its absorption and fluorescence wavelength maximum, λ_{max} , as the temperature changes between 7-65°C. The intensity of the absorption band or in other words the change in the value of molar extinction coefficient is related to the solution temperature. In the excited state, change of the solution temperature affects the viscosity of the medium. Change of solution viscosity has a great effect on CCSP fluorescence intensity. Decrease of the glycerol solution viscosity induced by heating was accompanied by decrease in the fluorescence quantum yield. The relationship between the fluorescence intensity CCSP and the viscosity of the medium was determined by the Förster–Hoffmann equation to measure the sensitivity of the fluorescent probe towards the viscous medium, it is easy to consider CCSP to measure the sensitivity of the fluorescent probe towards the viscosity.

Introduction

It is known that the spectroscopic properties or the photophysical processes are influenced by the type of the solvent used in which they are carried out. The most important property in this regard is the factor of the viscosity which can change the spectroscopic character in case of position of the absorption and emission bands. Viscosity plays an important role in controlling the physical or chemical behaviors of local molecules, which determinate the physical or chemical behaviors of surrounding molecules. Also, solvent viscosity and temperature affect the ability of the electron transition into the excited state which consequently reflects on its photophysical properties by solving a solute molecule or any other molecular species introduced into the solvent matrix. Styryl pyridinium dyes are known in the literature because of their wide range of applications. They were applied in preparation of flexible zinc oxide solar cells showed the best performance as the sensitizer (**Bruce et al., 1994**). Also, for the development of biocompatible dyes for biomedical applications, their spectroscopic and cell labelling properties were studied (**Patsenker et al., 2008**). Molecular Tuning of styryl dyes lead to versatile and efficient plasma membrane probes for cell and tissue imaging (**Grummt et al., 1996**). Gangopadhyay and et al. (**Sreejaya et al., 2024**) have applied some fluorophores coumarin, BODIPY, xanthene, and rhodamine in the development of viscosity-dependent fluorescent probes. Fluorescence, which is directly affected by the high-viscosity heterogeneous microenvironment are explained on intramolecular rotations like twisted

intramolecular charge transfer (TICT), aggregation-induced emission (AIE), and through-bond energy transfer (TBET). This information is useful in the field of diabetes treatment and fluorescent probes and provides the plan for futuristic clinical translation of fluorescent probes for the early-stage diagnosis and therapy of diabetes. These viscosity-sensitive probes show significant use in the early detection of various detrimental diseases like diabetes, cancer, and inflammation. Therefore, researchers have focused on formulating fluorescent probes that can show a prominent change in their fluorescent properties in terms of the large Stoke's shift, significant rise in intensity, etc. upon change in molecular rotation. Development of fluorescence molecular rotors of including push-pull type p-conjugated chromophores, cyanine derivative and porphyrin derivatives is viscosity sensors, and their biological and microfluidic applications are also discussed (**Lee et al., 2018**). Viscosity is an important quality parameter for fluid drinks, which can serve as an indicator for the extent of food spoilage, since the viscosity of fluid drinks varies during the spoilage process. Tetranitrile-anthracene is used as a probe for fluorescence detection of viscosity in fluid drinks via aggregation-induced emission. The approach for viscosity determination could enable on-site direct detection with convenient operation in food safety inspection applications. The results demonstrate that the probe can be applied to detect viscosity changes in fluid drinks through fluorescent response and subsequently monitor the spoilage of fluid drinks. The viscosity of the

biological environment is considered vital for the correct cellular function. Any changes in these parameters are known to underly a few pathologies such as diabetes, malaria, cancer and neurodegenerative diseases, and others. Fluorescent molecular rotors are confirmed to be extremely useful for exploring viscosity, crowding, and underlying molecular interactions in biological processes. Also, fluorescence could be used as sensors for lipid order, protein crowding and conformation, temperature and non-canonical nucleic acid structures in live cells and other relevant biological settings (Pae - Perez and Kuimova, 2024). In different environments synthesis their optical properties were studied (Xiao et al., 1994) and in solar energy conversion they could be used as Photosensitizers (Prasad and Williams, 1991). They already found broad applications in organic materials science, in particular dye sensitized solar cells or in nonlinear optical materials (Ratner, 2002). Because of their solid state and/or solution luminescence, they also became particularly interesting for biological imaging. It has been studied as organic semiconductors in transistors and solar cells. Chemical modifications of these dyes are introduced to develop new molecules with interesting photophysical properties. Although such kinds of dyes are synthesized by different methods that include traditional heating and microwave (Williamson and Schlegel, 1994; Würthner and Meerholz, 2010; Arjona-Esteban et al., 2021).

The aim of this work is focused on a new synthesized amphiphilic analogue styryl pyridinium dye namely 1-cetyl-4-[4'-chloro-styryl]-pyridinium iodide (CCSP) to study the effect of glycerol as a viscous solvent on electronic absorption and photophysical properties.

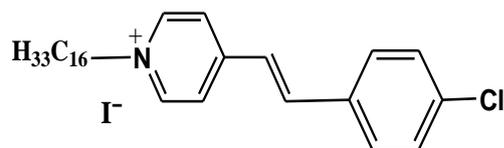


Fig. (1): Structure of CCSP dye

Experimental

Synthesis of 1-cetyl-4-[4'-chloro-styryl]-pyridinium iodide (CCSP)

The CCSP was synthesized according to procedures described in the literature (Binnemans, 2000; Facchetti, 2007; Arunadevi et al., 2020). The purity was checked by thin-layer chromatography and elemental analysis. All solvents and chemicals were acquired from Sigma Aldrich and spectroscopic grade and used without further purification.

Absorption and Fluorescence measurements

Absorption spectrum was measured by using Shimadzu UV3101PC scanning spectrophotometer with 1.0-cm matched quartz cells with a cuvette holder connected to a thermostat. Fluorescence spectra were recorded using a Jasco FP-8200 spectrofluorometer. Fluorescence intensities were measured at right angle to the exciting light where the excitation bandwidth 2.5 nm and emission bandwidth 10 nm.

Fluorescence Quantum Yield

Fluorescence quantum yield (ϕ_f) was determined by the comparative method, using quinine sulphate in 1 N H₂SO₄ as a spectral and quantum yield standard ($\phi_f = 0.55$) at the excitation wavelength ($\lambda_{exc.} = 366$ nm). ϕ_r was determined by using the comparative method, Eq. (1).

$$\varphi_s = \varphi_r \left(\frac{F_s A_r n_s^2}{F_r A_s n_r^2} \right) \dots\dots\dots (1)$$

where φ_s and φ_r are the areas under the emission curves of the sample and reference, respectively. A_s and A_r are the absorbances of the sample and standard, respectively. n_s and n_r are the refractive indices of the solvents used for sample and standard, respectively (Melhuish, 1961).

Results and Discussion

Fluorescence band of CCSP in glycerol and methanol

Looking at the fluorescence band of CCSP in glycerol and methanol in Fig. (2), one can notice that the position of the two bands is the same, 428 nm. On the other side, the fluorescence intensity of glycerol is higher than in methanol which gives us an indication that the property of viscosity may be the main reason for the difference in fluorescence intensity between them.

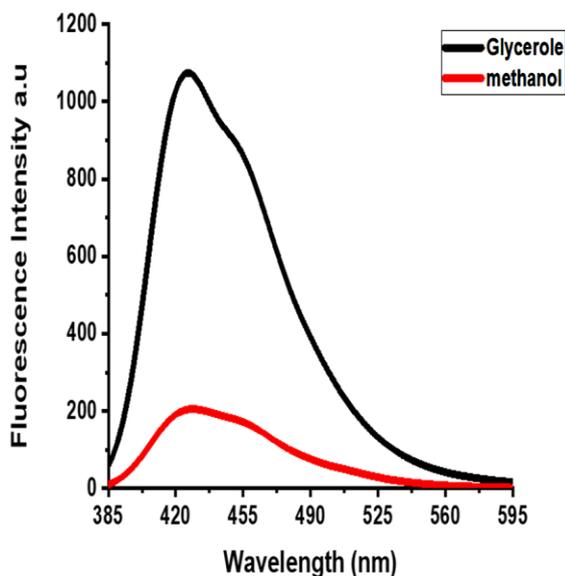


Fig. (2): Fluorescence spectra of 2×10^{-5} M CCSP in glycerol and methanol.

Effect of temperature on the absorption and fluorescence intensity of CCSP in glycerol

The characteristics of liquid flow depend on temperature and viscosity, the absorption and fluorescence spectra were studied at different temperatures ranging between 7 and 65°C, as shown in Figs. (3, 4). Absorption spectrum of CCSP in glycerol exhibits the longest absorption band at 350 nm with molar absorption coefficient, ϵ_{\max} of $29.4 \times 10^3 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ and another band at shorter wavelength 276 nm with ϵ_{\max} of $12.5 \times 10^3 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$

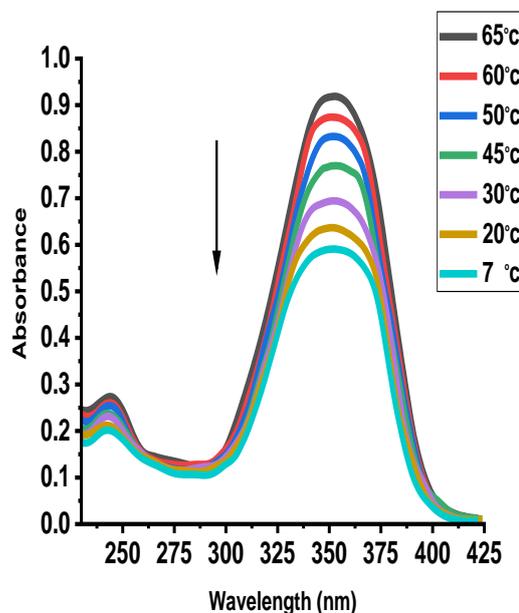


Fig. (3): Absorption spectra of 2×10^{-5} M CCSP in glycerol at different temperatures.

There is no observable change in its absorption wavelength maximum, λ_{\max} , as the temperature changes between 7-65°C Fig. (3), while its absorbance gradually changes. On the other hand, Fig. (4) shows the fluorescence band of CCSP at different temperatures. The fluorescence band maximum, λ_{\max} is at 428 nm and there is no shift as the temperature increases while its intensity decreases gradually.

The calculated molar extinction coefficient and fluorescence quantum yield value were determined at different temperatures and all the data listed in Table (1).

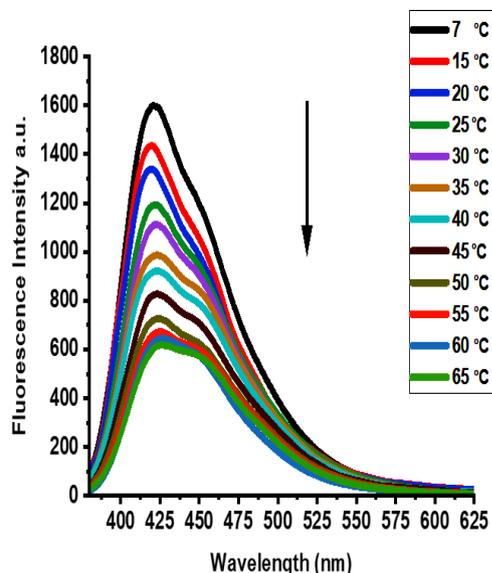


Fig. (4): Fluorescence spectra of 2×10^{-5} M CCSP in glycerol at different temperatures, $\lambda_{ex} = 365$ nm.

One can notice that as the temperature increases the molar extinction coefficient value increases, so good linear correlation between them as shown in Fig. (5).

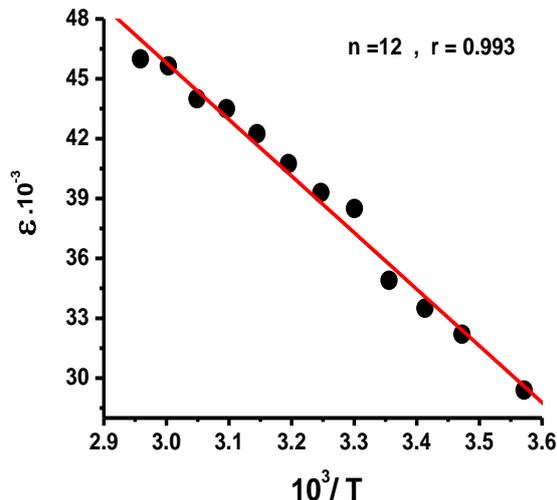


Fig. (5): Linear correlation between the extinction coefficient values of CCSP and the temperature.

The regression equation for the obtained linear correlation is $\epsilon \times 10^{-3} = 130.85 - 28.35 \cdot 10^3 / T$; $n = 12$, $r = 0.993$ ----- (2)

Table (1) shows the dependence of the molar extinction coefficient value of CCSP on the temperature value.

Table (1): Effect of temperature on the molar excitation coefficient and fluorescence quantum yield of CCSP in glycerol.

^a t °C	^b $\epsilon \times 10^{-3}$	^c $\Phi_f \times 10^3$	^d η
7	29.40	20.5	5897
15	32.20	17.10	2700
20	33.50	15.80	1410
25	34.90	14.44	1000
30	38.50	13.33	612
35	39.30	11.50	500
40	40.75	10.90	284
45	42.25	9.52	173
50	43.50	8.34	142
55	44.00	7.46	93
60	45.65	6.60	81.3
65	46.00	6.00	67

- a temperature degree Celsius
- b Extinction coefficient values $M^{-1}cm^{-1}$
- c Fluorescence quantum yield values, $\lambda_{exc}=365$ nm
- d Glycerol viscosity (cp) (Kubin & Fletcher, 1982)

Table (1) also shows dependence of fluorescence quantum yield on the temperature. As the temperature increases, Φ_f value decreases, giving excellent linear correlation as shown in Fig. (6)

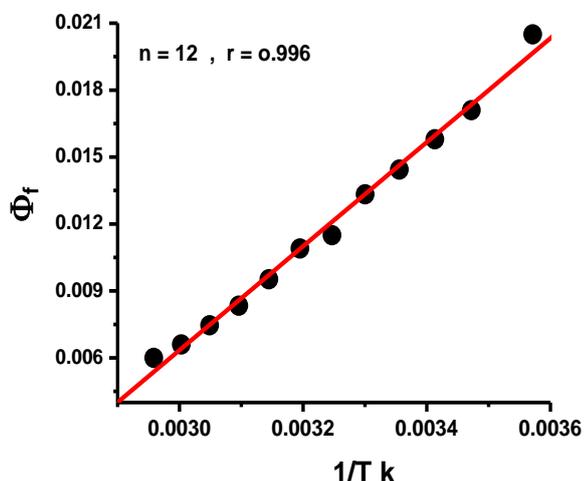


Fig. (6): linear correlation between fluorescence quantum yield, Φ_f and temperature.

The regression equation for the obtained linear correlation is:

$$\varphi_f \cdot 10^3 = -63.58 + 23.31 \cdot 10^3 / T; \quad r = 0.996, n = 12 \text{ -----} \quad (3)$$

Effect of viscosity on the molar extinction coefficient and fluorescence quantum yield values of CCSP

It is clear from the last data that change the solution temperature led to change the molar extinction coefficient ε and φ_f values. Now it is important to say that change the viscosity of the medium may consequently be responsible for ε and φ_f values changes. It is known that resistance of a liquid against gradients in flow plays a key role in processes in the ground and excited states. To prove the importance of the viscosity factor, the molar extinction coefficient, ε and φ_f values were measured at different viscous glycerol solutions.

Table (1) shows that as the viscosity value increases the molar extinction coefficient, ε value decreases. Fig. (7) shows that there is a good linear correlation between them.

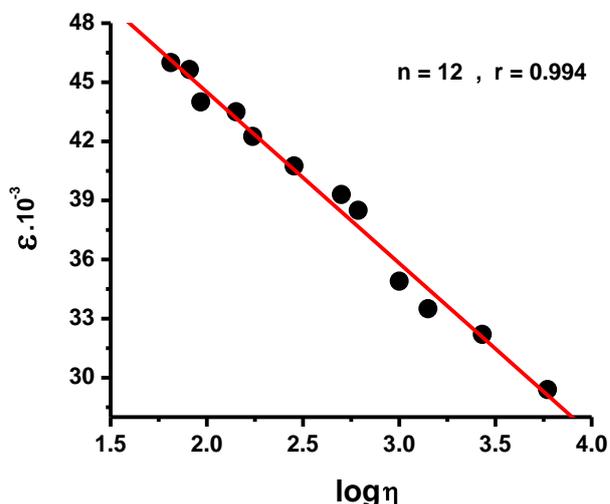


Fig. (7): plot of linear correlation of excitation coefficient, ε , with logarithmic value of viscosity function, $\log \eta$ (cP).

The regression equation for the obtained linear correlation is:

$$\varepsilon \times 10^{-3} = 61.95 - 8.71 \times \log \eta; \quad n = 12, \quad r = 0.994 \text{ -----} \quad (4)$$

As shown in the data that as the viscosity of the medium increases, fluorescence intensity also increases. The relationship between the fluorescence intensity of a probe and the viscosity of the medium was determined by the Förster–Hoffmann equation as following ($I_f \propto \eta^x$) or $\log I = C + x \log \eta$, where η represents the viscosity, I represents the fluorescence intensity of the probe, x represents the sensitivity of the fluorescent probe towards the viscosity and C is a constant. It is easy to consider Φ_f of CCSP to measure the sensitivity of the fluorescent probe towards the viscosity. Fig. (8) shows the plot of

Förster - Hoffmann equation for CCSP. The plot represents that there is a linear correlation between $\log \phi_f$ versus $\log \eta$ with good regression coefficient value.

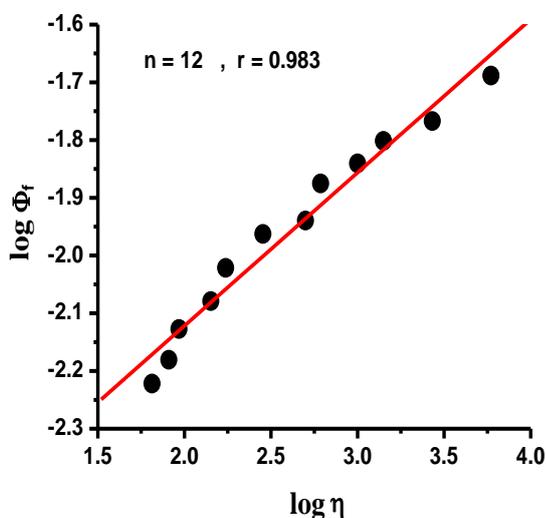


Fig. (8): plot of logarithmic value of fluorescence quantum yield, ϕ_f with logarithmic value of viscosity function, $\log \eta$ (cP).

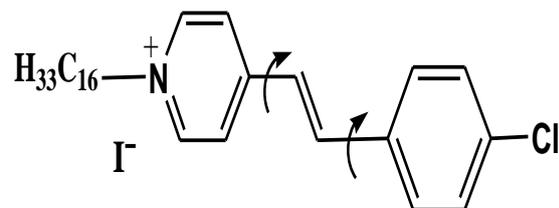
The linear correlation equation is:

$$\log \Phi_f = -2.65 + 0.27 \log \eta ; \quad n = 12 , \quad r = 0.983 \text{-----} \quad (5)$$

In low-viscosity solution the molecules rotate freely leading to fluorescence quenching, while in high viscosity solution where the intramolecular rotation is constrained and the collision decreases leading to an increase in fluorescence intensity (**Karpenko et al., 2016; Loutfy and Arnold, 1982; Mandel et al., 2022**).

The probe can be considered as molecular rotor compound since it is related to a large torsional motion followed by an internal conversion process to the ground state. The rate of the deactivation process may be restricted by assuming that the rotational diffusion obeys the Debye-Stokes-Einstein (DSE) equation (**Stsiapura et al., 2008; Förster and Hoffmann, 1971**) probe can be considered as a molecular rotor compound since it demonstrates its useful

as fluorescent molecules sensitive to the viscosity of the medium.



The sensitivity of such fluorophores is typically controlled by the rate of twisted intramolecular charge transfer TICT between chlorine and pyridinium moiety. In a viscous environment, the TICT is slowed down, and the non-radiative decay of a molecular rotor consequently decreases, while the fluorescence intensity and quantum yield in this case can be correlated to the medium viscosity. In high viscosity solution, the intramolecular rotation is constrained, and the collision decreases leading to an increase in fluorescence intensity as nonexponential fluorescence decay while in low-viscosity solution the molecules rotate freely leading to fluorescence quenching.

Conclusion

This paper demonstrates that the probe is a roto molecule; it is useful as fluorescent molecules sensitive to the viscosity of the medium. In high viscosity solution, the intramolecular rotation is constrained, and the collision decreases leading to an increase in fluorescence intensity as nonexponential fluorescence decay while in low-viscosity solution the molecules rotate freely leading to fluorescence quenching.

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تأثير اللزوجة على طيف الامتصاص وطيف الانبعاث الفلوروسين لصبغة ستايريل بيريدينيوم الجديدة ذات النشاط السطحي

أ.د/ شاكر طه العربي عبد الحليم، أ.د/ سمير سعد احمد قنديل، د/ عبدالباسط مرسى شكر ، * نورهان خلاف مصطفي خلاف

قسم الكيمياء كلية العلوم جامعة طنطا

تمت دراسة تأثير درجة الحرارة واللزوجة على طيف الامتصاص وطيف الانبعاث الفلوروسيني لصبغة جديدة من ١- سيتيل-٤-[-٤-كلورو-ستيريل]-بيريدينيوم أيوديد ذات النشاط السطحي وتعرف اختصارا (CCSP) في مذيب الجلوسرين.

أثبتت النتائج الى عدم وجود أي تغيير ملحوظ في الطول الموجي لطيف الامتصاص الإلكتروني وطيف الانبعاث الفلوروسيني مع تغير درجة الحرارة بين ٧-٦٥ درجة مئوية. ولكن عمليا وجود ارتباط خطي بين شدة طيف الامتصاص للمحلول أي معامل التميز المولاري للمادة مع درجات الحرارة المطبقة. وأيضا في الحالة المثارة تم استنباط ارتباط خطي للحصيلة الكمية الفلوروسينية مع درجات الحرارة للمحلول. وكذلك تم الحصول على علاقات ارتباطات خطية للمادة في الحالة الهامدة بين لزوجة محلول الجلوسرين المقاسة عند درجات الحرارة المختلفة مع كل من معامل التميز المولاري وايضا للحالة المثارة تم تطبيق معادلة فورستر-هومان بالحصول على علاقة ارتباط خطي بين الحصيلة الكمية للانبعاث الفلوروسيني ولزوجة الجلوسرين عند درجات الحرارة المختلفة. من النتائج السابقة يمكن اعتبار واستعمال هذا المركب كنموذج عملي كمجس لمعرفة مقدار لزوجة التجمعات الجزيئية داخل الأوساط المختلفة.