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Pyrene fluorescence in the presence of nonquenching and dynamic quenching salting-out agents

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Abstract

A new, simple, and rapid method to determine Setschenow (i.e., salting-out) constants for luminescent organic compounds in aqueous solutions has been developed using steady-state and time-resolved fluorescence measurements. Application of the new method was demonstrated using pyrene in the presence of nonquenching (K^+) and dynamically quenching (Cs^+) akali metal chloride salt solutions at room temperature. For these two model systems, Setschenow constants for pyrene salting-out by KCl and CsCl were determined to be 0.211 and 0.355 M^{-1}, respectively. We expect that the methodology reported in this paper can be applied equally well to interactions between a variety of salting-out agents and fluorescent biological and/or environmental molecules in aqueous solutions.

Keywords: Fluorescence; Quenching; Metals; Salting-out agents; Setschenow constant

1. Introduction

Photochemical and photophysical interactions between various metal ions and luminescent biological, clinical, and/or environmental analytes have been widely studied in aqueous solutions [1–4]. Proposed luminescence quenching mechanisms in such systems are determined by the chemical natures of the respective metal ions and luminophors used. For example, it is well known that electron transfer, energy transfer, and/or paramagnetic interactions can lead to luminescence quenching by heavy metals [5].

The aqueous solubilities of many organic compounds (e.g., polycyclic aromatic hydrocarbons (PAHs), proteins) decrease in the presence of increasing concentrations of simple electrolytes (e.g., alkali metal salts) because these so-called salting-out agents decrease the water-solute interfacial area necessary to effect solute dissolution [6–11]. These results imply, then, that the emission intensity of a luminescent analyte will be smaller in the presence versus absence of a nonquenching salting-out agent if that analyte is at its solubility limit. Likewise, if a dynamic luminescence quencher is also acting as a salting-out agent, it is possible that the bimolecular quenching constant could be overestimated if the decrease in analyte solubility is not taken into account.

Xie et al. [8] have reviewed the various methods commonly used to determine aqueous solubilities of various organic compounds in the presence of salting-out agents such as inorganic salts and ionic species. Unfortunately, however, these common methods (e.g., generation of saturated aqueous solutions using shake flask or generation column techniques followed by quantification via light absorption, molecular fluorescence, or gas/liquid chromatography detection) to determine solubilities of organic compounds in aqueous solutions containing salting-out agents are generally slow and subject to experimental complications [8]. Here, we report a new, simple, and rapid method to

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determine salting-out (i.e., Setschenow) constants using steady-state and time-resolved fluorescence measurements of pyrene in the absence and presence of nonquenching (K^+) and dynamically quenching (Cs^+) akali metal chloride salts.

2. Experimental

2.1. Chemicals

KCl (99.0%), CsCl (99.0%), pyrene (99.0%), and HPLC-grade ethanol (denatured with 5% isopropanol and 5% methanol) were purchased from Aldrich and used without further purification. Distilled, deionized water (Super-Q™ Plus, Millipore) having a resistivity greater than 18.0 MΩ-cm was used to prepare all aqueous solutions.

2.2. General procedure

KCl, CsCl and pyrene stock solutions were prepared at room temperature (23 ± 2 °C) and stored in dark containers to minimize photoreactions. Samples for absorption and fluorescence measurements were prepared in an anaerobic chamber (95% N_2, 5% H_2; Coy Laboratory Products Inc.) using deoxygenated solvents and then sealed in 1 cm screw cap anaerobic fluorescence cells (Starna) to exclude oxygen.

Ultraviolet–visible (UV–vis) absorption spectra (resolution of 0.05 nm) were measured with a double-beam, double-monochromator spectrophotometer (Shimadzu 2501PC) using appropriate background solutions as references. Steady-state fluorescence spectra were recorded with a PTI QuantaMaster™ spectrometer (Photon Technology International Inc.) equipped with a 75 W xenon arc lamp. The primary excitation wavelength used for pyrene was 334 nm, and fluorescence emission was monitored over a wavelength range of 360–600 nm. Fluorescence lifetimes and time-resolved fluorescence spectra were obtained with a PTI TimeMaster™ spectrometer using a nitrogen-filled nanosecond flashlamp and a stroboscopic optical boxcar detection methodology. Additional details on the absorption and fluorescence methodologies utilized here have been previously reported [3,4].

3. Results and discussion

As shown in Fig. 1(a), the emission spectrum of 0.6 μM pyrene is different in the absence versus presence of K^+. The broad, low-energy, excimer-like emission observed in the latter case likely results from interactions between excited singlet pyrene molecules (Py*) and micro-crystallized pyrene (Py_c) formed due to salting-out effects. Consistent with Fig. 1(a and b) demonstrates that the pyrene monomer fluorescence intensity at the 0–0 band (373 nm) decreases with increasing KCl concentration even though K^+ is not a static or dynamic quencher of pyrene fluorescence. For example, the results shown in Fig. 1 contrast with those observed using a lower concentration of pyrene (0.2 μM, which is well below its solubility limit in water [11]). For the lower pyrene concentration, addition of 2.0 M KCl did not result in a similar excimer-like pyrene emission, but instead

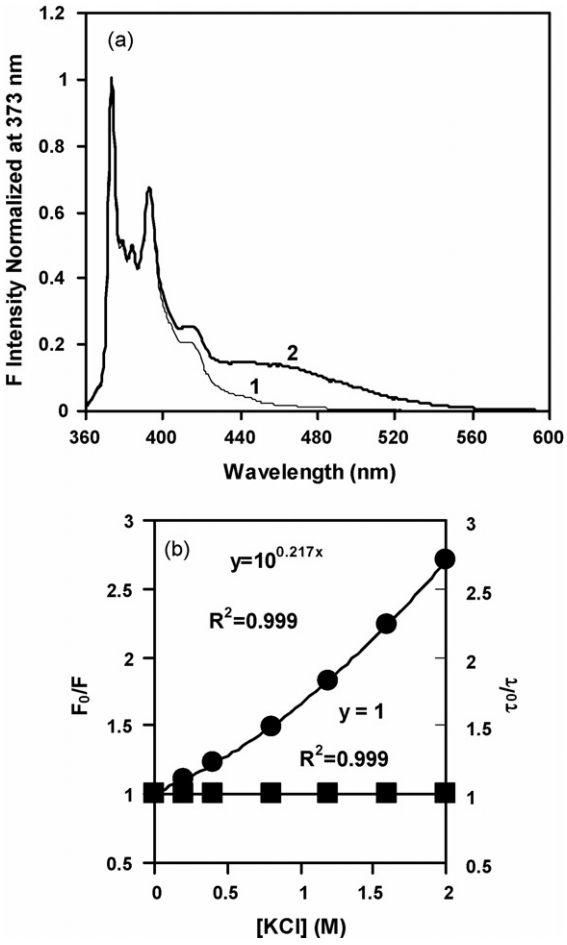


Fig. 1. (a) Pyrene emission spectra normalized at 373 nm in the absence (1) and presence (2) of K^+ (2.0 M). (b) Effects of K^+ concentration on the steady-state (●) and time-resolved (■) pyrene fluorescence (λ_{ex} = 334 nm, λ_{em} = 373 nm). For all measurements, [pyrene] = 0.6 μM (i.e., its solubility limit in pure water at room temperature).

resulted in an emission spectrum that completely overlapped the one obtained in the absence of K^+ ions (data not shown). Similarly, the lower concentration of pyrene led to the observation of $F_0/F = \tau_0/\tau = 1$ for KCl concentrations from 0 to 2 M because K^+ is not a dynamic or static fluorescence quencher under our experimental conditions. Therefore, as shown in Fig. 1, the only role of K^+ under these experimental conditions is to decrease the solubility of pyrene via the Setschenow/salting-out effect.

The steady-state F_0/F ratios presented in Fig. 1(b) showed excellent agreement with an equation derived from the empirical Setschenow equation [11]:

$$\log\left(\frac{\gamma}{\gamma_0}\right) = \log\left(\frac{S_0}{S}\right) = \log\left(\frac{F_0}{F}\right) = K_{set}C_{set} \quad (1)$$

$$\frac{F_0}{F} = 10^{K_{set}C_{set}} \quad (2)$$

where γ_0 (or S_0 or F_0) and γ (or S or F) are the aqueous pyrene activity coefficients (or solubilities or fluorescence intensities) in the absence and presence of K^+, respectively, K_{set} the Setschenow/salting-out constant, and C_{set} is the molar concentration of the aqueous salt (KCl) solution. Using Eq. (2), a value

of $K_{set} = 0.217\ (\pm 0.002)\ M^{-1}$ at room temperature ($23 \pm 2\ ^{\circ}C$) was determined from the data shown in Fig. 1(b). This value is consistent with one ($0.187 \pm 0.009\ M^{-1}$) determined by direct pyrene solubility measurements at 20 °C [11]. The ~15% larger value determined here can be attributed to the slightly higher temperature. Using Eq. (1) and our measured K_{set} value, therefore, the aqueous activity coefficient and/or solubility of pyrene can be predicted for any KCl concentration.

It is well known that Cs^+ is a dynamic, but not static, quencher of pyrene fluorescence [12,13]. Overlap in the pyrene absorption spectra in the absence and presence (1.0 M) of CsCl confirmed the absence of static quenching by Cs^+ ions in our systems (data not shown). For a high concentration of pyrene, the resulting excimer-like emission observed in the prescence of 0.4 M CsCl demonstrates that, like K^+, Cs^+ is an aqueous salting-out agent for pyrene (Fig. 2(a)). The combined effects of dynamic quenching and salting-out can be observed in the steady-state measurements shown in Fig. 2(b). Because the time-resolved measurements only reflect the dynamic quenching of pyrene fluorescence, the steady-state and time-resolved measurements do not overlap.

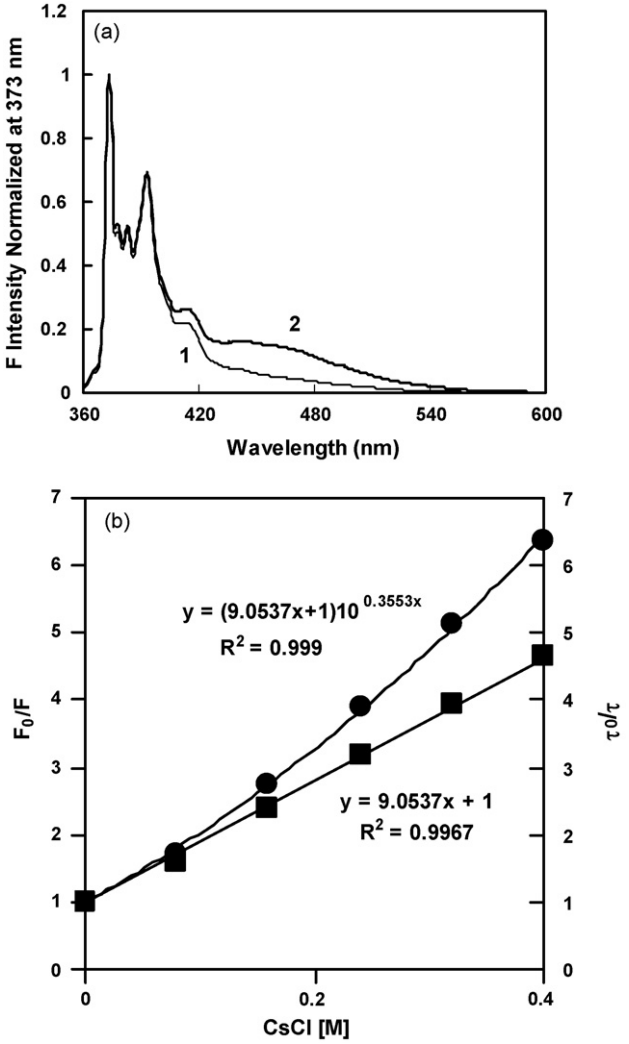
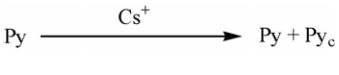
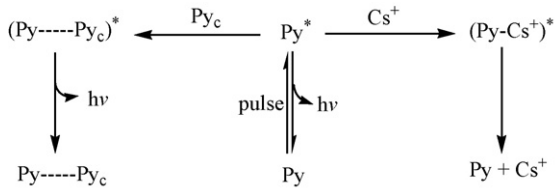


Fig. 2. (a) Pyrene emission spectra normalized at 373 nm in the absence (1) and presence (2) of Cs^+ (0.4 M). (b) Effects of Cs^+ concentration on the steady-state (●) and time-resolved (■) pyrene fluorescence ($\lambda_{ex} = 334$ nm, $\lambda_{em} = 373$ nm). For all measurements, [pyrene] = 0.6 μM (i.e., its solubility limit in pure water at room temperature).



Scheme 1.



Scheme 2.

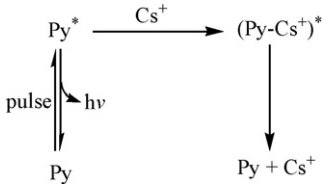
To fit the curve for steady-state quenching of pyrene fluorescence shown in Fig. 2(b), a new equation was derived by combining the Stern–Volmer equation (Eq. (3)) for bimolecular quenching with the empirical Setschenow equation (Eq. (2)) for salting-out effects:

$$\frac{\tau_0}{\tau} = K_D[C_{set}] + 1 \tag{3}$$

$$\frac{F_0}{F} = (K_D[C_{set}] + 1)10^{K_{set}C_{set}} \tag{4}$$

where τ_0 and τ are the pyrene fluorescence lifetimes in aqueous solution in the absence and presence of Cs^+, respectively, K_D the dynamic Stern–Volmer quenching constant, and $[C_{set}]$ is the molar concentration of CsCl. As shown in Fig. 2(b), Eqs. (3) and (4) provide very good fits to the time-resolved and steady-state pyrene fluorescence measurements, respectively. To conduct the model fit to the steady-state fluorescence data, the value for K_D ($9.05 \pm 0.07\ M^{-1}$) first was selected by fitting Eq. (3) to the lifetime data. Then, the best-fit value for K_{set} ($0.355 \pm 0.011\ M^{-1}$) was determined for Cs^+ and pyrene based on Eq. (4). Note that it is possible to fit both K_D and K_{set} simultaneously to the steady-state data using Eq. (4) directly, thereby eliminating the need to collect time-resolved fluorescence measurements. For example, using this one-step approach resulted in similar values to those above for K_D and K_{set} (6.68 ± 0.63 and $0.395 \pm 0.068\ M^{-1}$, respectively). However, the ability to forgo making time-resolved fluorescence measurements does have a tradeoff, namely the higher associated uncertainties (i.e., less precision) in the values of the two fitted parameters.

Schemes 1 and 2 show the likely pathways leading to observed pyrene emission when Cs^+ acts both as a dynamic quencher and salting-out agent in aqueous solution. Due to the decrease of pyrene (Py) solubility in aqueous solutions con-



Scheme 3.

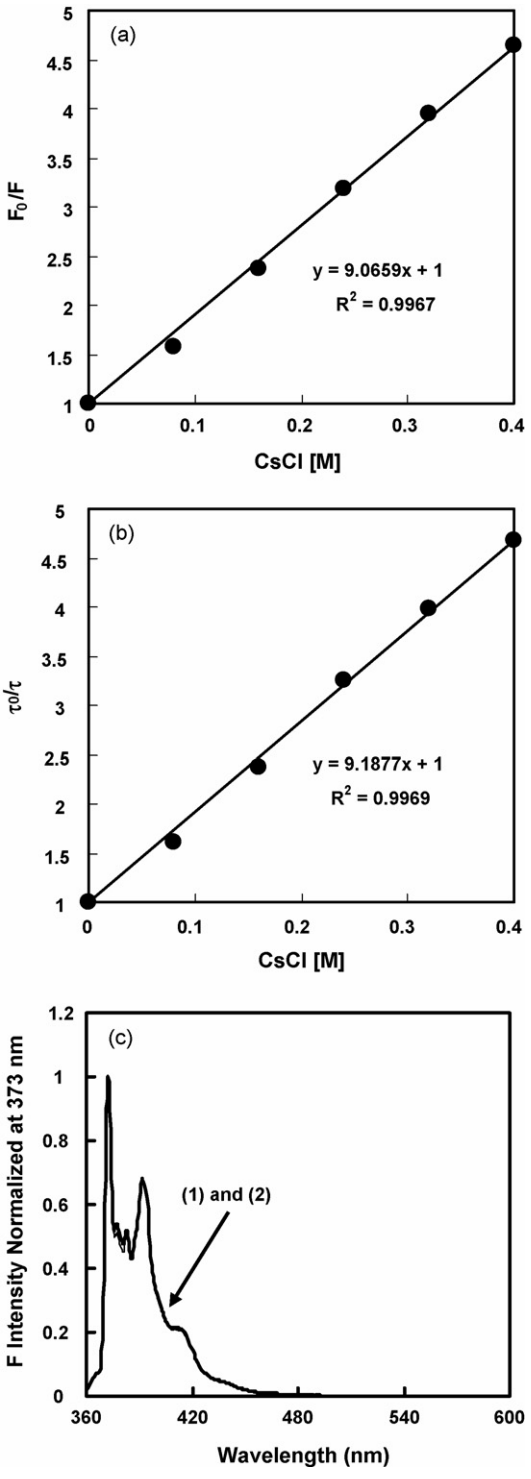


Fig. 3. Effects of Cs^+ on the steady-state and time-resolved fluorescence (λ_{ex} = 334 nm, λ_{em} = 373 nm) of pyrene (0.40 μM) at room temperature. (a) F_0/F determined from steady-state pyrene fluorescence. (b) τ_0/τ determined from time-resolved pyrene fluorescence. (c) Pyrene emission spectra normalized at 373 nm in the absence (1) and presence (2) of Cs^+ (0.4 M). The pyrene concentration (0.40 μM) used in these experiments was lower than its solubility limit in the presence of Cs^+ (0.4 M).

taining Cs^+, micro-crystallized pyrene (Py_c) will be formed as shown in Scheme 1. Then, as shown in Scheme 2, monomer fluorescence of excited singlet state pyrene molecules (Py^*) in saturated pyrene solutions will be lower in the presence versus absence of Cs^+ because Py* molecules interact both with Py_c and Cs^+ while emitting monomer fluorescence.

From the results above, the solubility of pyrene in an aqueous solution containing 0.4 M CsCl was estimated to be ~0.43 μM based on Eq. (1) and the experimentally determined value of K_{set}. Therefore, at pyrene concentrations lower than this value, macroscopic salting-out effects will not occur as shown in Scheme 3. This was verified by repeating the steady-state and time-resolved emission measurements for a lower concentration of pyrene (0.40 μM). As shown in Fig. 3(a) and (b), for example, all values of F_0/F are equivalent to τ_0/τ for CsCl concentrations up to 0.4 M. In other words, the dynamic Stern–Volmer quenching constant, K_D, calculated in Fig. 3(a) was equal to that obtained in Fig. 3(b) within the limits of experimental uncertainty because pyrene was not being salted-out of solution under these conditions. Thus, both the relative emission intensity and fluorescence lifetime for pyrene in the presence of 0.4 M CsCl were 4.67 (±0.07) times smaller than their respective values in the absence of Cs^+ ions (Fig. 3(a) and (b)). At this lower pyrene concentration, Fig. 3(c) shows that the normalized emission spectra in the absence and presence of Cs^+ completely overlapped, and no excimer-like emission was observed.

4. Conclusions

Using steady-state and time-resolved fluorescence measurements, we have developed a simple and rapid method to determine Setschenow constants for fluorescent organic compounds in the presence of aqueous salting-out agents. In particular, we have demonstrated that the steady-state fluorescence emission of PAHs such as pyrene in the presence of a dynamic quenching salting-out agent such as Cs^+ should be interpreted using our new Eq. (4), which was derived from the Stern–Volmer equation for bimolecular quenching and the empirical Setschenow equation for salting-out effects. Chemical researchers in a wide variety of fields (e.g., biological, environmental, marine, medicinal chemistry) are interested in the interactions between metals and organic compounds in aqueous solution under controlled ionic strength and pH conditions. In general, many salts selected to adjust ionic strength (e.g., NaCl, KCl) and pH (e.g., K_2HPO_4, KH_2PO_4) are salting-out agents. By using the simple techniques introduced here, chemists will be better able to account for salting-out effects in their systems and will be able to collect more accurate and precise experimental data. We expect that the methodology reported in this paper can be applied equally well to interactions between fluorescent biological molecules and salting-out agents in aqueous solutions. For example, the concentration of buffer solutions used in separation and/or reaction channels of capillary electrophoresis systems (e.g., lab-on-a-chip) with fluorescence detection may be an important factor to quantify biological molecules.

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References

[1] D. Gao, Y. Tian, S. Bi, Y. Chen, A. Yu, H. Zhang, Spectrochim. Acta A 62 (2005) 1203.
[2] J. Dessingou, R. Joseph, C.P. Rao, Tetrahedron Lett. 46 (2005) 7967.
[3] J.H. Lee, E.R. Carraway, M.A. Schlautman, S. Yim, B.E. Herbert, J. Photochem. Photobiol. A: Chem. 167 (2004) 141.
[4] J.H. Lee, M.A. Schlautman, E.R. Carraway, S. Yim, B.E. Herbert, J. Photochem. Photobiol. A: Chem. 163 (2004) 165.
[5] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, 2nd ed., Kluwer Academy/Plenum Publisher, New York, 1999.
[6] R. Breslow, Accounts Chem. Res. 24 (1991) 159.
[7] E.B. Abuin, E.A. Lissi, J. Photochem. Photobiol. 71 (1993) 263.
[8] W.H. Xie, W.Y. Shiu, D. Mackay, Mar. Environ. Res. 44 (1997) 429.
[9] A. Turner, M. Martino, S.M.N. le Roux, Environ. Sci. Technol. 36 (2002) 4578.
[10] N. Ni, S.H. Yalkowsky, Int. J. Pharm. 254 (2003) 167.
[11] M.A. Schlautman, S. Yim, E.R. Carraway, J.H. Lee, B.E. Herbert, Water Res. 38 (2004) 3331.
[12] L.K. Patterson, S.J. Rzad, Chem. Phys. Lett. 31 (1975) 254.
[13] I.R. Gould, P.-L. Koo, N.J. Turro, J. Phys. Chem. 89 (1985) 3030.