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# The effect of ionic strength and surfactant on the dynamic quenching of 6-methoxyquinoline by halides

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**Abstract**

Steady state and time resolved fluorescence measurements have been performed to assess the role of ionic strength and presence of surfactants in the dynamic quenching of 6-methoxyquinoline by halides. The results of quenching by potassium chloride are mainly presented in this paper. The pH dependence of the Stern–Volmer constant has been rationalized in the light of the difference in ionic strengths. The concentration dependent quenching/enhancement of fluorescence by the surfactant, sodium dodecyl sulphate has been observed and explained by considering an equilibrium between free and bound fluorophores and modification of critical micellar concentration of SDS by salts. The concentration of the surfactant has been found to affect the quenching/enhancement of fluorescence by chlorides. Thus, ionic strength and the presence of surfactants are found to be two factors which can affect the sensitivity of the fluorescent probe towards chlorides.


*Keywords:* Fluorescence; Organized assemblies; Chloride sensor

## 1. Introduction

The study of quenching of luminescence has been an active area of research for the last few decades. Quenching can take place through different mechanisms and finds extensive application in the study of biophysical systems. Hence, this much explored area continues to attract considerable interest even today. A wide variety of substances can act as quenchers. Some examples are molecular oxygen [1,2] chlorinated hydrocarbons [3] aliphatic and aromatic amines [4–6], aromatic nitro compounds, [7] metal ions [8,9] and halides [10–17]. Fluorescence quenching is widely used in the investigation of phenomena like partitioning of fluorophores into membranes [18,19] and dynamics in proteins [20,21]. Very recently, the technique has been used elegantly to develop a scale for flexibility of amino acid in peptides [22]. It is often observed that some classes of fluorophores are quenched selectively by certain kinds of quenchers. A fluorophore with such selectivity is often used in qualitative and quantitative analysis of small amounts of its specific quenchers [9,10–13,23–26].

In recent times, there has been a considerable amount of effort in the development of chloride sensing probes. The interest in this field stems from the ubiquitous nature of chloride ions in biological systems [10–17]. A good chloride–sensitive probe would find ample use in the study of important processes like chloride transport across the cell membranes. The mechanism of quenching by chloride ions has been found to be somewhat different from that by compounds containing heavier halides like bromide and iodide, where the predominant mechanism involves intersystem crossing to an excited triplet state, promoted by spin-orbit coupling [27,28]. Fluorescence quenching by chlorides is mainly ascribed to electron transfer and exciplex formation reactions [29,30]. A good starting point for chloride sensing is provided by the knowledge that the fluorescence of quinones is quenched collisionally by chlorides. Compounds containing a quinolium ring are stable and easily loaded in living cells. Therefore, such compounds are an obvious choice for construction of chloride– sensitive probes [14,30]. 6-methoxyquinoline (6-MQ) is one such compound, the photophysics and photochemistry of which probe has been explored extensively

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[14–17]. It has been established that 6-MQ undergoes protonation at pH = 1 whereas it exists as a neutral molecule at pH ∼ 7. A rapid intramolecular charge transfer followed by a nanosecond solvation relaxation process has been reported for 6-MQ and its analogs [14,15]. A red edge excitation shift has been observed for 6-MQ in polymer matrices. This has been explained by the existence of two ground state species of the fluorophore in this environment [16]. Chloride ions have been found to quench the fluorescence of 6-MQ dynamically in aqueous solutions at pH = 1. Hence, it has been proposed that 6-MQ could be a potential chloride sensor [14] An important issue that needs to be addressed at this point is that of factors which might affect the chloride ion sensing ability of this fluorescent probe, especially in complex biological medium. As Jayaraman and Verkman have pointed out, the dynamic quenching of quinolinium containing compounds by chlorides proceeds through a non-emissive excited state charge transfer complex [30]. Hence, the sensitivity of such compounds towards chloride ions in biological media is susceptible to interference from simultaneous quenching by intracellular quenchers. In this report, we attempt to identify some of the factors which might interfere in the chloride sensing ability of 6-MQ. We have concentrated on studies at pH = 7, as opposed to the earlier work at pH = 1, so as to simulate the conditions in biological media to a closer extent.

## 2. Experimental

6-Methoxyquinoline (6-MQ) AR grade has been obtained from Lancaster, UK. All other chemicals (AR grade) are from E-Merck, Mumbai, India. The surfactant sodium dodecyl sulphate (SDS) has been recrystallized from methanol water mixture before use. Doubly distilled water has been used as the solvent. Steady-state absorption and emission spectra have been recorded on JASCO V570 spectrophotometer and Perkin Elmer LS-55 fluorimeter, respectively. The excitation wavelength for the emission studies was 340 nm. An Applied Photophysics nanosecond nitrogen flash lamp based time correlated single photon counting (TCSPC) fluorescence spectrophotometer at SAIF, IIT Bombay has been used for time resolved studies, with excitation wavelength of 337 nm.

## 3. Results and discussion

### 3.1. Quenching studies on 6-MQ in neat aqueous solvents

Chloride ions quench the fluorescence of 6-MQ markedly, as is shown in Fig. 1a. The Stern–Volmer plots for quenching of fluorescence lifetimes of 6-MQ by KCl and KBr at pH = 1 are presented in Fig. 1b. It is apparent from this figure and the data in Table 1 that $Br^-$ is a more efficient quencher than $Cl^-$. This can be rationalized in the line of the earlier work by Jayaraman and Verkman where the quenching efficiency

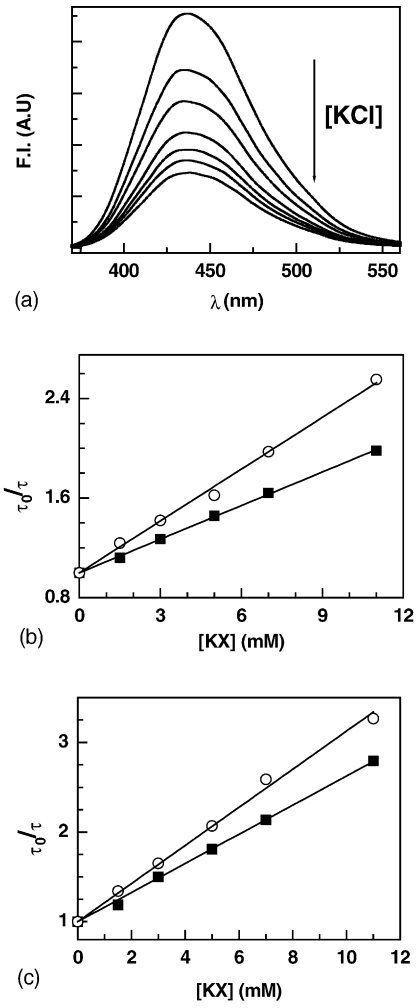


Fig. 1. (a) Fluorescence spectra of 6-MQ in water at pH = 7 with increasing concentration of KCl from 0 to 11 mM. (b) Stern–Volmer plot of quenching of $10^{-5}$ M 6-MQ by KCl (■) and KBr (◯) at pH = 1 using fluorescence lifetimes. (c) Stern–Volmer plots at pH = 7 for addition of KCl (■) and KBr (◯) using fluorescence lifetimes.

has been related to the strength of the quencher as the reducing agent [30]. Similar values of $K_{SV}{}^{Cl^-}$ and $K_{SV}{}^{Br^-}$ are obtained from steady state and time resolved experiments, indicating that the quenching is dynamic in nature. The values of $K_{SV}$ obtained for chloride at pH = 1 are in good agreement with those reported by Mehata and Tripathy [14]. At pH = 7, both the halides are found to be more efficient quenchers than at pH = 1 (Fig. 1c and Table 1). This result might seem

Table 1
The Stern–Volmer constants for quenching of 6-MQ with KCl and KBr in aqueous solutions as a function of pH

| | $K_{SV}$ ($l\,mol^{-1}$) | | | |
|---|---|---|---|---|
| | Steady state | | Time resolved | |
| | pH = 1 | pH = 7 | pH = 1 | pH = 7 |
| KCl | 110 | 190 | 90 | 170 |
| KBr | 150 | 290 | 140 | 240 |

to be somewhat intriguing. In fact, one might expect the order of quenching efficiency to be reverse, as the considerable electrostatic attraction between halides ions and the 6-MQ cations at pH = 1 might as well favour the process of collisions. An electron transfer to a cationic species could also be more favourable than to a neutral species. Overall, the driving force can be expected to be greater when the acceptor is a cation. In order to investigate the reason for the apparent anomaly, the fluorescence quenching of 6-MQ by KCl has been studied at pH = 7, with the ionic strength being maintained as the same value as for a pH = 1 solution, by addition of 0.1N $Na_2SO_4$. It is found that the value $K_{SV}{}^{Cl^-}$ ($90\,l\,mol^{-1}$) obtained is similar to that for pH = 1. The Stern–Volmer plot in this case is much closer to that at pH =1 than that at pH = 7 (Fig. 3). Thus the reduced efficiency of $Cl^-$ as quencher at pH = 1 is rationalized by an increased ionic strength and consequently, reduced activity of $Cl^-$ at pH = 1 as compared to pH = 7. This result serves to identify the ionic strength of the medium to be a possible factor that could interfere with the chloride sensing ability of 6-MQ. It should be mentioned here that the properties of the medium are known to affect excited state electron transfer rates [31]. A similar effect of the ionic strength on the Stern–Volmer constants for dynamic quenching has been predicted by theoretical calculations [32] and has been observed experimentally for the dynamic quenching of fluorescence of quarternised bisacridinium derivatives and lucigenin by halides [33].

At this point, one might ponder whether the difference in the quenching efficiencies at pH = 1 and 7 could be due to an alteration in the acid-base equilibrium of the protonated and deprotonated forms of 6-MQ. We performed a pH titration of 6-MQ in the presence and absence of salts, monitoring the concentrations of the two forms from the absorption spectra. As is shown in Fig. 2a and b, the absorption spectrum of 6-MQ at pH = 1 is distinctly different from that at pH = 9. We fitted all intermediate spectra with a linear sum of the spectra at pH = 1 and 9. The spectrum at pH = 7 is shown in Fig. 2c, along with the fitting curve (Fig. 2d), which yields a value

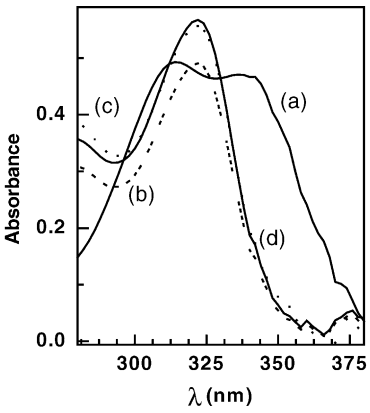


Fig. 2. UV–vis absorption spectra of 6-MQ in water at (a) at pH = 1, (b) at pH = 9, (c) at pH = 7, (d) best fit of (c) as a linear sum of (a) and (b). The relative contribution of (b) is 98%.

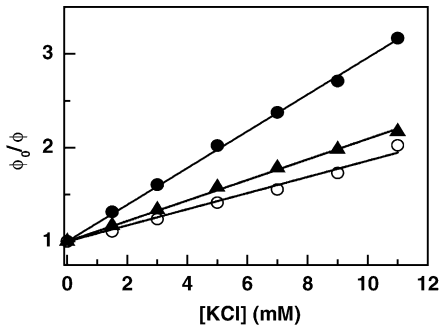


Fig. 3. Stern–Volmer plots for quenching by KCl at (a) pH = 7, without $Na_2SO_4$ (●), with 0.1N $Na_2SO_4$ (◯) and (b) at pH = 1 (▲).

of 98% contribution of the deprotonated form at this pH. In no case did the salts have any effect on the p$K_a$, which were around 4.5. Thus, we can safely say that 6-MQ is always deprotonated at pH = 7, no matter what the concentration of salt in the medium is.

### 3.2. Fluorescence quenching and subsequent enhancement by SDS

The effect of ionic strength on the dynamic quenching of 6-MQ by chloride ions prompted us to explore whether surfactants would interfere with the chloride sensing ability of 6-MQ. Surfactants at low concentration are known to quench the fluorescence of other fluorophores like porphyrin derivatives through formation of electrostatic complexes [34–36]. At high concentrations, on the other hand, surfactants can sequester fluorophores and protect them from being quenched [37–40]. We observe that at low concentration (up to ∼5.5 mM), SDS quenches the fluorescence of 6-MQ. Beyond this concentration, the fluorescence quantum yield as well as lifetime are enhanced considerably (Fig. 4a and b), along with a blue shift of 8–9 nm, indicating a significant change in the polarity in the surroundings of the fluorophore. The plateau region at high SDS concentrations is more obvious in the time resolved study as we are able to work with higher concentrations of the salts as compared to the steady-state experiment. It is also obvious that the maximum value of $\phi_0/\phi$ and that of $\tau_0/\tau$ are not the same. This could be due to the error in the steady-state measurements arising from small but significant changes in absorbance, as the absorbance of 6-MQ at the concentration used has a rather small value to start with. In such cases, the results obtained from the lifetime values are more dependable [40]. It is apparent that SDS acts as quencher when present as a monomer. The situation changes near the critical micellar concentration, where they undergo a reversal of roles and start protecting the 6-MQ molecules from quenching by incorporating them within the sub micellar and consequently, micellar assemblies. This trend is analogous to the observations in the earlier studies of surfactant–porphyrin interaction [34–36]. The increase in fluorescence quantum yield and lifetime can be attributed to a retardation of non-radiative processes in hydrophobic core

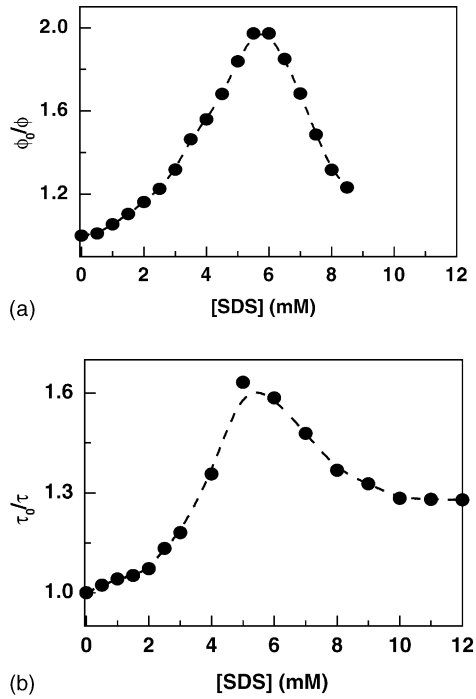


Fig. 4. Stern–Volmer type plots of 6-MQ with addition of SDS using (a) steady-state fluorescence intensity and (b) fluorescence lifetimes. The negative slope beyond 6 mM SDS signifies a protection of 6-MQ from quenching by incorporation in micelles beyond this concentration.

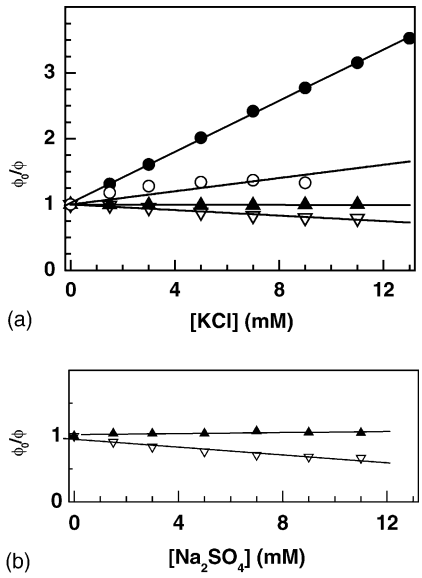


Fig. 5. (a) Quenching/augmentation of fluorescence of 6-MQ by chloride ions in the presence of 2 mM (●), 3 mM (◯), 6 mM (▽) and 24 mM (▲) SDS at pH = 7. Stern–Volmer constants 200, 50, −20 and 0 l $mol^{-1}$, respectively. (a) variation of $\phi_0/\phi$ of 6-MQ with increase in the concentration of $Na_2SO_4$ in the presence of 2 mM (▲) and 6 mM (▽) SDS.

of SDS, as has been proposed in several earlier studies with different fluorophores [37–40]. This argument is substantiated in the subsequent experiment of quenching of 6-MQ by halides in the presence of different concentration of SDS.

### 3.3. Effect of SDS on quenching of 6-MQ by halides

The effect of micelles on the dynamic behavior of fluorescence quenchers has been studied extensively by several groups [37–42]. Several models are used to understand the partitioning of fluorophores in micelles and its consequence on the quenching of fluorescence. In the present study, quenching of fluorescence of 6-MQ by halides is affected significantly by the presence of SDS (Fig. 5a). At a concentration of 2 mM SDS, the quenching constant is similar to that in neat aqueous solution. The quenching efficiency decreases as SDS concentration increases upto 4 mM. At an even higher concentration of 6 mM SDS, the fluorescence intensity actually increases on addition of the halides. One may be tempted to assign this decrease in quenching efficiency at SDS concentrations lower than 4 mM to the increase in the viscosity of the environment, which is expected to hinder dynamic quenching [43,44]. However, the small increase in bulk viscosity on increasing the concentration of SDS from 2 to 3 mM cannot account for the drastic decrease in the $K_{SV}$ accompanying this change in concentration. The increase in fluorescence intensity at the surfactant concentration of 6 mM is another interesting observation that calls for an explanation. Salt effects can rationalize this set of observations. Such salt effects are known to affect emission properties considerably. Bhattacharyya and co-workers have demonstrated that the presence of ions of the salting-in agents in the vicinity of the TNS:cyclodextrin (CD) complexes increase the local polarity and also weaken the binding between the TNS and the CD molecules [45]. In our case, some of the 6-MQ molecules may be associated with premicellar aggregates due to hydrophobic effect, thereby setting up an equilibrium between free and bound forms of the fluorophore. In this situation, the usual dynamic quenching of fluorescence can occur, causing a positive slope in the Stern–Volmer plot. Besides, a salting out effect can operate in this region of concentration of SDS, leading to a greater binding of 6-MQ to the SDS premicellar aggregates. At low surfactant concentration these aggregates do not exist and such salting out may be ruled out. With gradual increase in the concentration of SDS, the salting out effect is expected to predominate at intermediate concentrations of SDS. It should be noted that the Stern–Volmer plot at 4 mM SDS shows a negative deviation, which could be indicative of simultaneous operation of the quenching and salting out effects. At very high concentration of SDS 6-MQ molecules are expected to be fully bound to the micelles any way and so, are expected to be totally passive to the addition to halides.

One should remember that such salt effects could operate not only on the fluorophore, but also on the surfactants. It is well known that the presence of electrolytes can modify the critical micelle concentration considerably. The CMC of SDS has been reported to decrease to 2.5 mM in the presence of 10 mM KCl from the value of 8 mM in the absence of salts [46]. The progressive decrease in CMC with addition of KCl

should cause increased micellization in the range 2–6 mM SDS. These newly formed micelles can incorporate 6-MQ and protect it from further quenching. The effect of KCl on the CMC of SDS should be a more feasible mechanism than that of salting out of 6-MQ. However, both may be operative simultaneously.

Steady-state quenching experiments with $Na_2SO_4$ have been carried out to verify our hypothesis about the salt effect. $Na_2SO_4$ does not quench the fluorescence of 6-MQ in neat aqueous solvents. Nor is there any significant quenching by $Na_2SO_4$ in the presence of 2 mM SDS. However, at an SDS concentration of 6 mM, a slight increase in the fluorescence intensity is observed (Fig. 5b). This indicates that the effect is not specific to KCl, but is observed with other salts as well at intermediate concentration of SDS.

## 4. Conclusion

This study indicates that the fluorescence of 6-MQ is quenched by halides at pH = 7 with a greater quenching efficiency than at pH = 1. The quenching is dynamic in nature. The counter ions have no effect in quenching. There is a marked difference in the quenching constants at the two pH values and this has been rationalized in the light of a difference in ionic strengths, which is found to be an important factor to be considered during the application of this fluorophore in chloride sensing. It has been observed that surfactants at low concentration quench the fluorescence quite efficiently. In a related study in our group, it has been observed that albumins quench the fluorescence of 6-MQ as well (unpublished results). This would severely affect the performance of 6-MQ to act as a chloride sensor in complex biological systems. The fluorescence quenching of 6-MQ by halides in the presence of surfactant aggregates has also been studied. An interesting salt effect has been observed at intermediate surfactant concentrations and this has been rationalized in the light of modification of the CMC of SDS in the presence of salts. To conclude, it can be said that the performance of 6-MQ as a chloride sensor is affected severely by several environmental factors. If one is to use this fluorophore for the purpose, it is imperative that the fluorophore is encapsulated in some kind of a polymer matrix or hydrogel so that it is accessible to chloride ions only and not to the factors, which may interfere with its performance.

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