

ESTIMATION OF EXCITED STATE pK FROM RATES OF PROTON TRANSFER

R. SAKUROVS[†] and K. P. GHIGGINO

Department of Physical Chemistry, University of Melbourne, Parkville, Victoria 3052 (Australia)

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Summary

A method is described for estimating the excited state pK (pK^*) of fluorescent compounds from measurements of the rates of proton transfer between a fluorescent acid or base and proton acceptors or donors. The pK^* values determined for excited state acid-base equilibria in aqueous solutions of tyrosine, β -naphthol, *m*-methoxycinnamic acid and β -carboline are reported.

1. Introduction

Light absorption by chromophores often results in a change in the pK of any associated auxochrome owing to rearrangements in the electronic distribution of the molecule. For example, phenols and pyrroles become stronger acids in the excited state while pyridines and carbonyls become stronger bases [1 - 8]. However, determinations of excited state pK (pK^*) values can be difficult since the two most commonly used methods for estimating pK^* require both acid and base forms of the molecule to be fluorescent. The most widely used method, the Förster cycle [3, 4], requires measurements of the absorption and fluorescence maxima of both the acid and base forms to estimate the difference between the pK of ground and excited states. This method cannot be used if even one of the absorption maxima is not discernible and if either acid or base is non-fluorescent. It is also necessary to know the ground state pK to calculate pK^* . This becomes a problem for very strong acids such as $RCO_2H_2^+$ where the value of the ground state pK may vary by up to 2 or 3 pK units depending on the choice of the scale of acidity [6, 9]. In addition, concern has been expressed that another assumption required by the Förster cycle, that the change in entropy of ionization of ground and excited states is the same, may not

[†]Present address: Department of Chemistry, Monash University, Clayton, Victoria 3168, Australia.

always hold [2, 10]. If rapid equilibration of the excited state acid and base is achieved, the pK^* may also be determined by direct fluorometric titration [2 - 4]. Again, both acid and base forms must be fluorescent for this method to be valid. Thus, there is a need for an independent method to determine pK^* values.

A proton transfer reaction between an acid AH and acceptor B can be described by reaction (I) (charges have been omitted for generality).



For such a reaction the rate constant k_f for proton transfer has been shown to be predominantly determined by the difference in pK (ΔpK) between AH and HB for a wide range of nitrogen and oxygen acids and bases [2, 3, 11, 12]. For the case of a fluorescent acid AH^* whose emission is quenched by a proton acceptor B exclusively via proton transfer, we can obtain k_f from the slope of a Stern-Volmer plot (eqn. (1))

$$\frac{I_0}{I} - 1 = k_f \tau [B] \quad (1)$$

where I_0 and I are the unquenched fluorescence intensity and fluorescence intensity at acceptor concentration $[B]$ respectively and τ is the fluorescence lifetime of AH^* in the absence of quencher. The rate constant for proton transfer between a fluorescent base and proton donor can be obtained by analogous methods.

With the assumption that k_f for the reaction between the excited molecule and quencher is governed mainly by ΔpK , we can obtain pK^* for the fluorophore knowing the pK of the quenching species and interpolating between the rates of proton transfer and ΔpK for a known reference system. In order to assess the general application of this method, we have determined pK^* for several excited state acid-base equilibria using the measured rate constants for proton transfer between a series of carboxylic acids and the acetate ion as the reference system [13] (Table 1).

2. Experimental details

The acid-base equilibria studied are shown in Fig. 1. The compounds investigated were tyrosine (II), β -naphthol (III), *m*-methoxycinnamic acid (IV and V) and β -carboline (VI). Compounds and solvents were the purest available grade and were used without further purification. Fluorescence spectra were recorded on a Perkin-Elmer MPF-44A spectrofluorometer and fluorescence lifetimes were measured on an Applied Photophysics Model SP2X nanosecond spectrometer using the time-correlated single photon counting technique as described previously [1]. Stern-Volmer quenching constants ($K_{SV} = k_f \tau$) were determined by two methods. In one method, increasing concentrations of acid or base were added to the solution of

TABLE 1

Observed rate constants for proton transfer between carboxylic acids and acetate ions (after Ahrens and Maass [13])

Acid	ΔpK	$k_f (\times 10^{-7} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1})$
Dichloroacetic	3.27	1100
Chloroacetic	1.9	210
Formic	1.0	100
Propanoic	-0.12	29
Malonic	-0.95	6

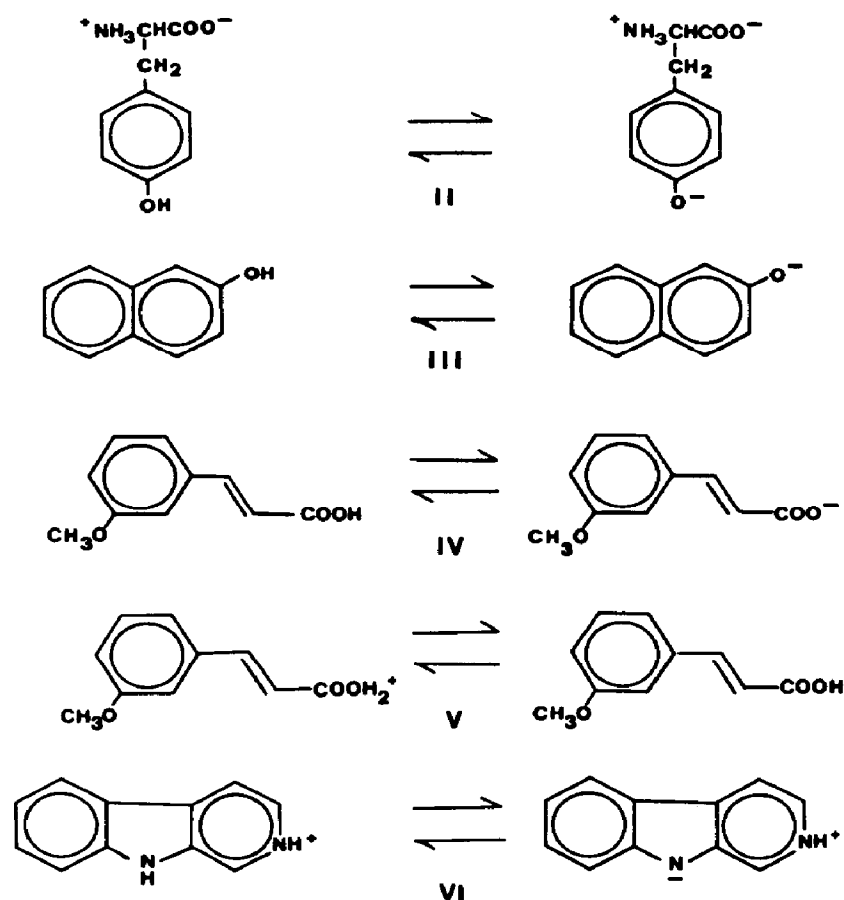


Fig. 1. Acid-base equilibria for compounds studied.

fluorophore and corrections due to ionization of the acid or base were applied. Corrections for changes in ionic strength did not significantly alter K_{SV} . Alternatively, the fluorophore was dissolved in a solution of the acid or base and the relative amounts of ionic species required were varied by the addition of NaOH or HClO₄. In the latter method the reactions between the

fluorescent compound and the acid and base forms of the quencher may be monitored simultaneously.

It has been shown previously by Weller [3] and others [2, 12] that the measured rate of proton transfer between a proton donor and acceptor can be influenced by their mutual diffusion rates, electrostatic interactions and the steric angle of reaction. It is necessary to correct the measured rate for these effects by established methods [3, 12] in order to relate k_f directly to ΔpK . To account for any preferred orientation for reaction with an approaching proton, the fractional steric angle for all interacting species was taken to be $\frac{1}{2}$ except for SO_3^- where a value of 1 was assumed following other workers [3, 12]. The effect of electrostatic interactions of charged species on the reaction rate has also been allowed for assuming proton transfer occurs when the reactants approach within 0.75 nm of each other [3]. Corrections applied for diffusion rates did not significantly affect the precision of the results or the final average result of pK^* .

3. Results and discussion

The pK_A^* values obtained for equilibria II - VI are presented in Table 2. In each case the procedure adopted was to measure the quenching of the fluorophore by a series of proton acceptors or donors. Stern-Volmer plots were constructed by varying the effective quencher concentration and the quenching rate constant for proton transfer was determined. The ΔpK associated with the corrected rate constant for proton transfer was derived by interpolation from the data in Table 1. The pK_A^* for the fluorophore was then obtained from ΔpK and the pK_A of the quenching species. Several quenchers were used with each fluorophore to investigate the influence of specific acid-base interactions on the pK^* values obtained.

The ground state pK_A for tyrosine (II) is 8.4 and an excited state pK_A^* value of 4.2 has been reported using the Förster cycle [14]. Proton transfer to both phosphate ions [15, 16] and carboxylate ions [14] has previously been observed but undissociated carboxylic acids have also been reported to quench tyrosine fluorescence [17]. In order to investigate the mechanism of quenching further, the fluorescence from *o*-methyltyrosine (4-methoxyphenylalanine) was also investigated. The results of quenching of the amino acid zwitterion species by acetic acid/acetate and formic acid/formate are shown in Figs. 2(a) and 2(b). Acetate ions and acetic acid quench tyrosine fluorescence with Stern-Volmer quenching constants of $5.2 \pm 0.5 \text{ dm}^3 \text{ mol}^{-1}$ and $0.6 \text{ dm}^3 \text{ mol}^{-1}$ respectively, in agreement with previous data [17]. It is apparent from Fig. 2 that acetate and formate ions quench the fluorescence from tyrosine but not from *o*-methyltyrosine while undissociated acetic and formic acids can quench the emission from both molecules. Since *o*-methyltyrosine does not have an ionizable phenolic proton it can be concluded that acetate and formate ions quench tyrosine via proton transfer while a different mechanism is involved in quenching by the undissociated

TABLE 2

Quenching rate constants k and estimated pK_A^* values for fluorophores

Fluorophore	Quencher (pK_A)	k ($\times 10^{-7} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$)	pK_A^*
Tyrosine ($\tau = 3.17 \text{ ns}$)	$\text{CH}_3\text{CH}_2\text{CO}_2^-$ (4.87)	195 ± 12	3.1
	CH_3CO_2^- (4.75)	170 ± 7	3.1
	HCO_2^- (3.75)	150 ± 15	2.3
	H_2PO_4^- (2.12)	35 ± 3	2.1
β -Naphthol	$\text{CH}_3\text{CH}_2\text{CO}_2^-$ (4.87)	290^a	2.7
	CH_3CO_2^- (4.75)	290^a	2.6
	HCO_2^- (3.75)	240^a	1.7
	H_2PO_4^- (2.12)	60^a	1.6
<i>m</i> -Methoxycinnamic acid, anion ($\tau = 3.80 \text{ ns}$)	Citrate ²⁻ (6.34)	81 ± 10	8.5
	HPO_4^{2-} (7.21)	124 ± 13	9.1
	Serine (9.21)	15.9 ± 1	8.8
<i>m</i> -Methoxycinnamic acid, neutral form ($\tau = 3.25 \text{ ns}$)	$\text{CCl}_3\text{CO}_2\text{H}$ (0.7)	1600 ± 450	4.3
	HSO_4^- (1.92)	730 ± 100	4.8
	Glycine (2.35)	110 ± 20	3.5
	$\text{ClCH}_2\text{CO}_2\text{H}$ (2.85)	100 ± 20	3.9
	HCO_2H (3.75)	78 ± 6	4.3
β -Carboline, cation ($\tau = 22 \text{ ns}$)	HCO_2^- (3.75)	2.3 ± 0.2	5.4
	CH_3CO_2^- (4.75)	14.7 ± 0.8	5.5
	$\text{CH}_3\text{CH}_2\text{CO}_2^-$ (4.87)	24 ± 1	5.3
	HPO_4^{2-} (7.21)	180 ± 14	6.4
	SO_3^{2-} (6.91)	260 ± 20	6.4

^aTaken from ref. 2.

acids. The pK_A^* values obtained (2.1 - 3.1) are lower than those reported using the Förster cycle (4.2). A possible explanation is that phenols undergo a slightly greater charge redistribution than carboxylic acids on deprotonation [2], increasing the activation energy of proton transfer and reducing the rate compared with the reference system. The pK_A^* values for β -naphthol (1.6 - 2.7) using previously published proton transfer rates [2] are also slightly less than those determined by other methods (2.5 - 3.4) [2], providing further support for the above proposal.

Förster cycle calculations indicate that the pK_A of aromatic carboxylic acids increases by about 5 pK units upon excitation [2]. *m*-Methoxycinnamic acid was selected as an example since we have previously characterized the fluorescence from a range of cinnamic acid derivatives [18] in solution. Quenching of the *m*-methoxycinnamic acid anion by several proton donors (Table 2) yielded an average pK_A^* of 8.8 (IV) in reasonable agreement with a value of 9.9 determined using the Förster cycle. Protonated aromatic carboxylic acids have pK_A values between -4 and -8 [2, 6] and excited state pK_A^* values greater than zero have been reported [4 - 6]. However,

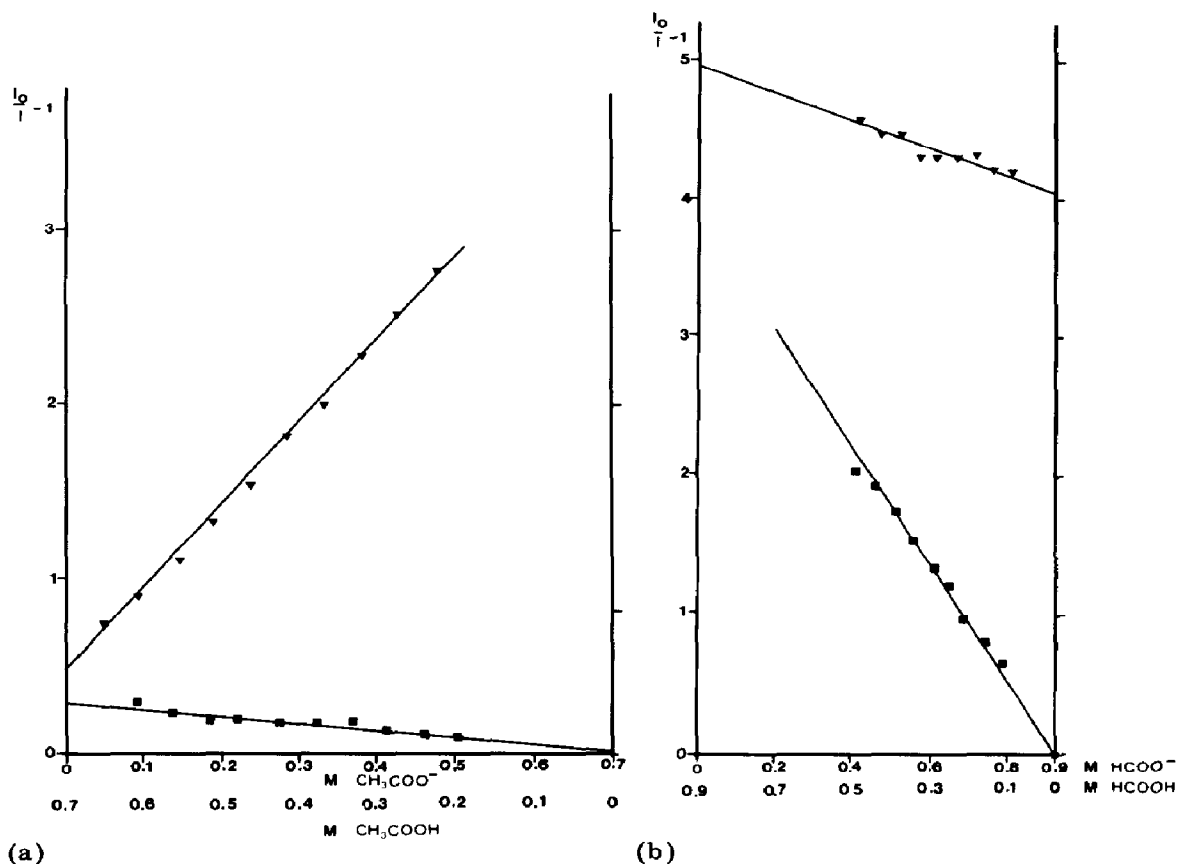


Fig. 2. Stern-Volmer plots for the fluorescence quenching of tyrosine (\blacktriangledown) and *o*-methyltyrosine (\blacksquare) by (a) acetic acid/acetate mixtures and (b) formic acid/formate mixtures.

many protonated carboxylic acids, including *m*-methoxycinnamic acid, are non-fluorescent, precluding use of the Förster cycle or fluorometric titration methods to obtain $\text{p}K_{\text{A}}^*$. The neutral form of *m*-methoxycinnamic acid is quenched by both protons and undissociated acids (Fig. 3). The K_{SV} for quenching by protons is $115 \text{ dm}^3 \text{ mol}^{-1}$ yielding a quenching rate constant of $(3.6 \pm 0.3) \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$, which is comparable with the rate constants for protonation of benzoate ions [11, 12]. Since strong acids dissociate easily, it is necessary to correct the overall quenching of *m*-methoxycinnamic acid for the effect of proton quenching (Fig. 3). From the measured rates of proton transfer, the average $\text{p}K_{\text{A}}^*$ of the protonated acid (V) is 4.2, which is similar to the ground state acidity of the neutral form of the molecule.

The excited state acid-base properties of β -carboline have been reported previously [1]. The excited state cation is quenched rapidly by OH^- ions to produce a zwitterion species. The excited state $\text{p}K_{\text{A}}^*$ for the

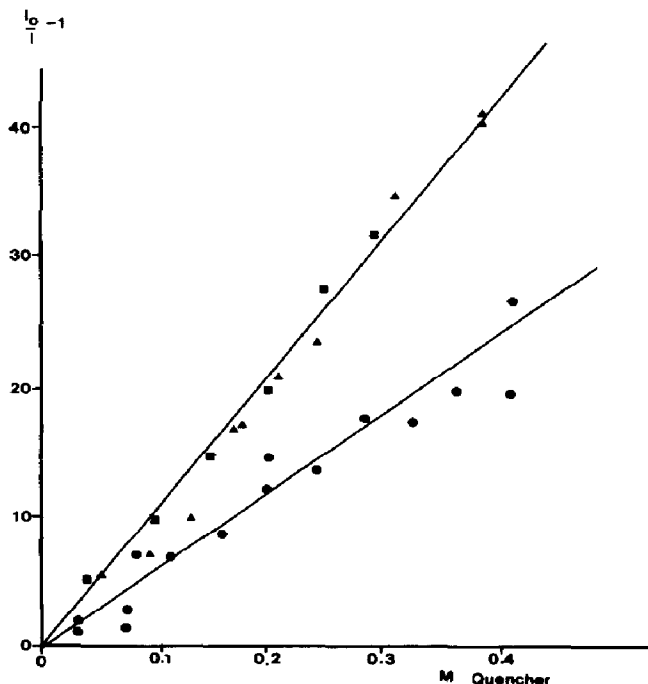


Fig. 3. Stern-Volmer plots for the quenching of fluorescence from *m*-methoxycinnamic acid: H⁺ from HClO₄ (▼); H⁺ from H₂SO₄ (■) (corrected for HSO₄⁻ quenching); chloroacetic acid (●) (corrected for quenching by H⁺ from acid dissociation), y axis × 20.

cation-zwitterion equilibrium (VI) can be determined from the quenching of the fluorescent cation by a range of proton acceptors. The average pK_A^* of the pyrrole ring nitrogen of β -carboline is 5.8 compared with 4.1 obtained from a Förster cycle calculation and a ground state pK of 10.9 [1].

4. Conclusion

Existing methods for determining the pK^* of fluorescent compounds require both acid and base forms of the molecule to be fluorescent and give reliable answers over only a small pK range. The method outlined in this paper allows the estimation of pK^* values between 2 and 12 provided (i) *either* acid or base forms of the molecule is fluorescent, (ii) proton transfer is the only mechanism by which an added acid or base quenches the fluorescent species and (iii) the rate constant for proton transfer is determined primarily by the difference in pK between the excited molecule and the quencher.

Results obtained for any one fluorophore using a number of quenchers with a wide range of quenching rate constants give pK_A^* values which are consistent within about 1 pK unit. Where comparison with other methods is

possible, fair agreement (1 - 2 pK units difference) is obtained. It is likely that the accuracy of the method could be improved with further developments in our understanding of specific interactions between proton acceptors and donors.

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