KINETIC STUDY OF EXCITED STATE PROTOLYTIC REACTION IN FLUORESCEIN CATION

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ABSTRACT

The excited state proton transfer rate for fluorescein cation is much faster than the lifetime, hence nanosecond pulse fluorometry shows very little change in the decay curves in aqueous solutions of fluorescein cation. However, in glycerol: water mixtures the proton transfer can be conclusively be proved to follow a two state reversible excited state reaction scheme. The rate constants have been determined.

INTRODUCTION

THE acid base property of fluorescein cation in the excited state differs significantly from that in the ground state. Rozwadwski[1] noted that on changing the acidity of the solvent, the change in the emission spectrum of the cation does not occur simultaneously with the change in the absorption spectrum. Later studies have also shown that in the acid range 3 M to 1 M the protonated molecule undergoes an excited state proton transfer reaction with emission peaks at $\lambda_{max} = 480$ m corresponding to cation and $\lambda_{max} = 515$ nm corresponding to neutral species[2], and there is no change in the absorption spectrum in this range of acidity. However, the kinetics and the rate constants of the excited state protolytic reactions with a phase fluorometre[3] do not seem to give an unambiguous answer regarding the reaction mechanism. Our recent study of 2 Naphythylamine[4] with a single proton counting subnano second technique suggested undertaking of a fresh investigation for the problem. This was further necessasitated by the anomaly in literature regarding the lifetimes of the cation which are reported as 4.2 and 3.4 ns respectively.

EXPERIMENTAL

Chromatographed fluorescein was obtained from E. Merck and was used as such. Sulphuric acid and perchloric acid (analytical grade) were used for changing acidity. Triply-distilled water was used for dilution. Glycerol (G.R. Grade) used in these studies was vacuum-distilled and was fluorescence-free.

The absorption spectra were taken with a Beckman DK-2A spectrophotometer. The fluorescence spectra were recorded with spex fluorolog 1902 under steady state conditions and fluorescence lifetimes were measured with a nanosecond fluorimeter (Edinburgh Instruments 199) using single photon time correlation techniques. A thyratron-controlled nanosecond flash lamp (hydrogen filled) fitted with a monochromator, was used for repetitive exitation of the samples. The emission monochromator attached with a XP2020Q fast photomultiplier and multichannel pulse height analyser (Inotech-5300) form the main measuring part of the instrument. The decay curves were analysed with a PDP 11/2 computer by iterative reconvolution of the instrumental function by one or more exponential decays with a weighted nonlinear least square deconvolution technique until the best fit is obtained with the experimental data as defined by minimum χ^2 value and residuals.
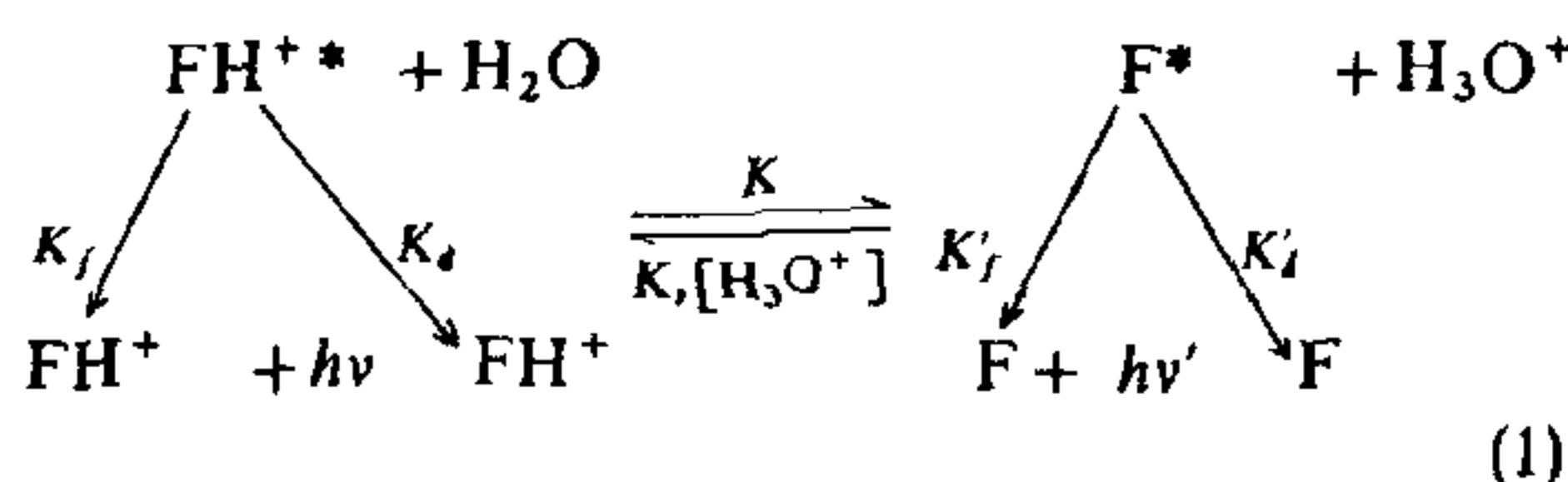
In all the experiments signals were attenuated such that the data were obtained under essentially single photon conditions to avoid pile-up errors. Since the sulphuric acid can exist in various ionic species e.g. HSO_4^-, $H_2S_4O_7^-$, SO_4^{--} etc, all the experiments were repeated with perchloric acid and no discrepancy was found between the results in the two acids in the range studied. The lifetimes of fluorescein cation were measured at 3 M acid when deprotonation just starts and separately at 10 M acid. Both the decay constants had the same value indicating no quenching by hydrogen ion concentration $[H^+]$.

The decay constant for the neutral species was measured at various acid concentrations at long wavelength region of emission (540 nm) and for cation at short wavelength region of emission (450 nm) to avoid the overlap of emissions. No distinct difference was found in the lifetimes when the protonated species gave rise to neutral emission on proton transfer in acid water system from 3 M to 1 M acid.

This anomaly appeared to be due to the larger magnitude of proton transfer rate compared to the decay rate of the fluorescein cation. Separate experiments were performed for the decay rates with varying glycerol and water composition in the solvent keeping hydrogen ion concentration $[H^+]$ constant at 1 M.

RESULTS AND DISCUSSION

The emission spectra given in figure 1 show that at 3 M acid only cation emission is present whereas at 1 M acid neutral emission dominates. The following excited state reaction scheme is assumed to hold

$$FH^{+*} + H_2O \qquad\qquad F^* \qquad + H_3O^+$$

$$K_f\diagup \diagdown K_d \quad \xrightleftharpoons[K,[H_3O^+]]{K} \quad K_f'\diagup \diagdown K_d'$$

$$FH^+ \quad + h\nu \quad \diagdown FH^+ \qquad F + h\nu' \quad \diagdown F \tag{1}$$

Here K and K_r are the rate constants for the forward and reverse reactions respectively and K_f, K_d and K_f', K_d' are the rates of fluorescence and nonradiative deactivation of FH^+ and F^* species which are related to the lifetimes of FH^{+*} (τ) and F^* (τ') species as

$$1/\tau = K_f + K_d \quad \text{and} \quad 1/\tau' = K_f' + K_d'.$$

The rate equations describing the above kinetic scheme

Figure 1. Emission spectra of fluorescein in the aqueous solution at acid concentration.

are given by

$$-\frac{d}{dt}[FH^{+*}] = (K_f + K_d + K)[FH^{+*}]$$
$$- K_r[F^*][H_3O^+], \tag{2}$$

$$-\frac{d}{dt}[F^*] = (K_f' + K_d' + K_r[H_3O^+])[F^*]$$
$$- K[FH^{+*}]. \tag{3}$$

A kinetic treatment of the diffusion-controlled excited state proton transfer reaction under photostationary state conditions gives[5]

$$\frac{1}{[(\eta_0/\eta)-1]} = \frac{1}{K\tau} + \frac{K_r\tau'[H_3O^+]}{K\tau}, \tag{4}$$

where η and η_0 are the quantum yields of FH^{+*} with deprotonation reaction and without.

The value $1/[(\eta_0/\eta)-1]$ is in linear relation with $[H_3O^+]$; therefore, K and K_r values can be calculated from the intercept and slope of eq. (4).

The solution of (2) and (3) for transient conditions on application of initial condition $[FH^{+*}] = [FH^{+*}]_0$ and $[F^*] = 0$ at $T = 0$, gives[6,7]

$$FH^{+*}(t) = \frac{[FH^{+*}]_0}{\lambda_2 - \lambda_1}[(\lambda_2 - X)\exp(-\lambda_1 t)$$
$$+ (X - \lambda_1)\exp(-\lambda_2 t)], \tag{5}$$

$$F^*(t) = \frac{K[FH^{+*}]_0}{\lambda_2 - \lambda_1}[\exp(-\lambda_1 y) - \exp(-\lambda_2 t)], \tag{6}$$

where
$$X = K_f + K_d + K \tag{7}$$

$$Y = K_f' + K_d' + K_r[H_3O^+], \tag{8}$$

and $\lambda_1, \lambda_2 = \tau_1^{-1}, \tau_2^{-1} = \frac{1}{2}[(X+Y) \pm \{(Y-X)^2$
$$+ 4K K_r[H_3O^+]\}^{1/2}]. \tag{9}$$

Figure 2 shows the decay curves for fluorescein cation at 3 M, 1 M acid (curves 1 & 2) and of neutral molecule at 1 M acid in water (curves 3). As will be seen very small changes are noticed in the decay curves 1 and 2 and no change in 2 and 3. All the decays appear single exponential. Table 1 lists the decay constants for both the emissions for various acid concentrations. These data do not fit the two state reversible excited state reaction described by (5) and (6).

Obviously, such a situation does not arise on account of overlap of emissions of cation and neutral species. Equation (9) shows that τ_1 will be very small provided K and $K_r \gg (K_f + K_d)$ and $(K_f' + K_d')$ and both cation and neutral emission will apparently
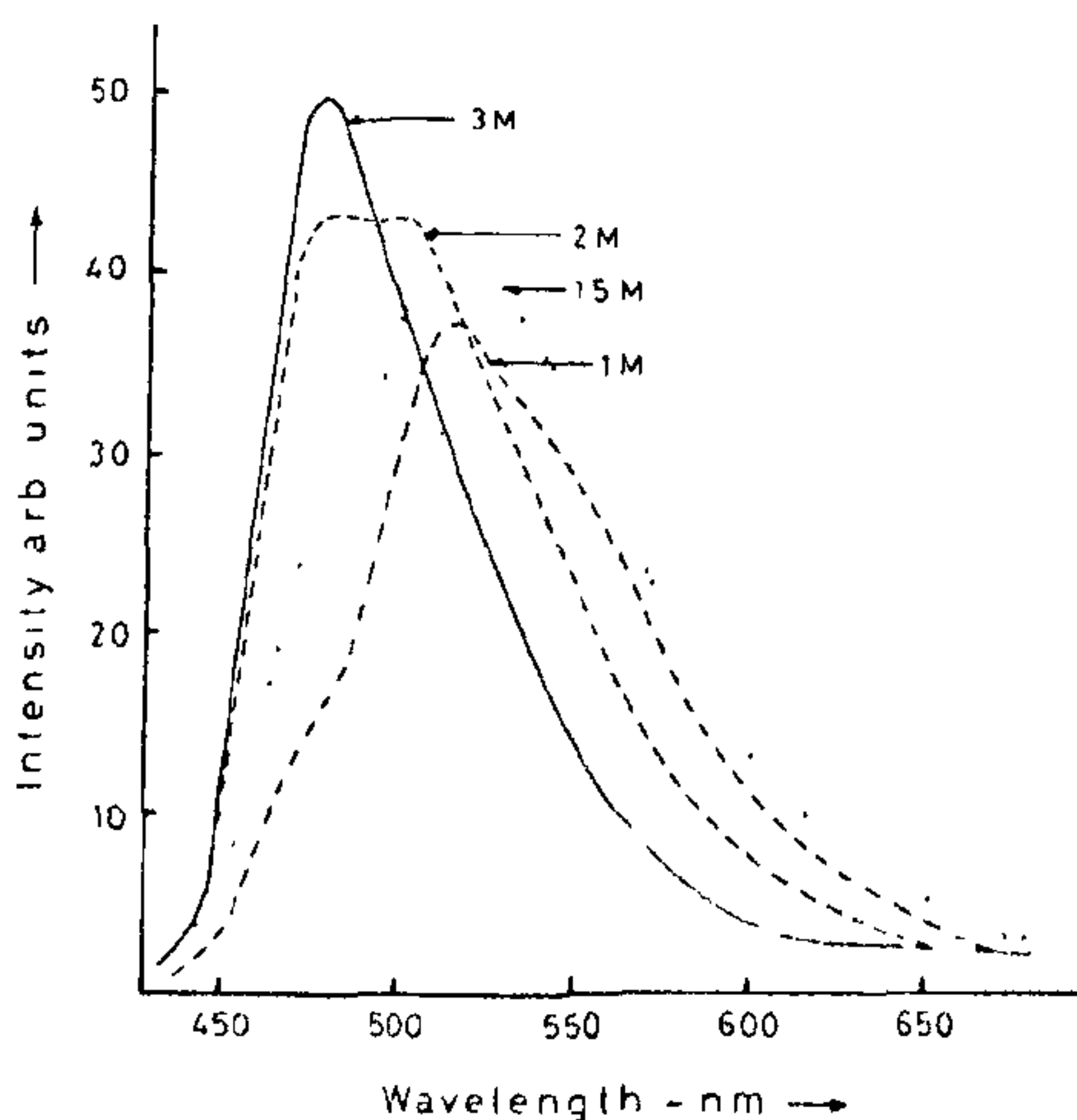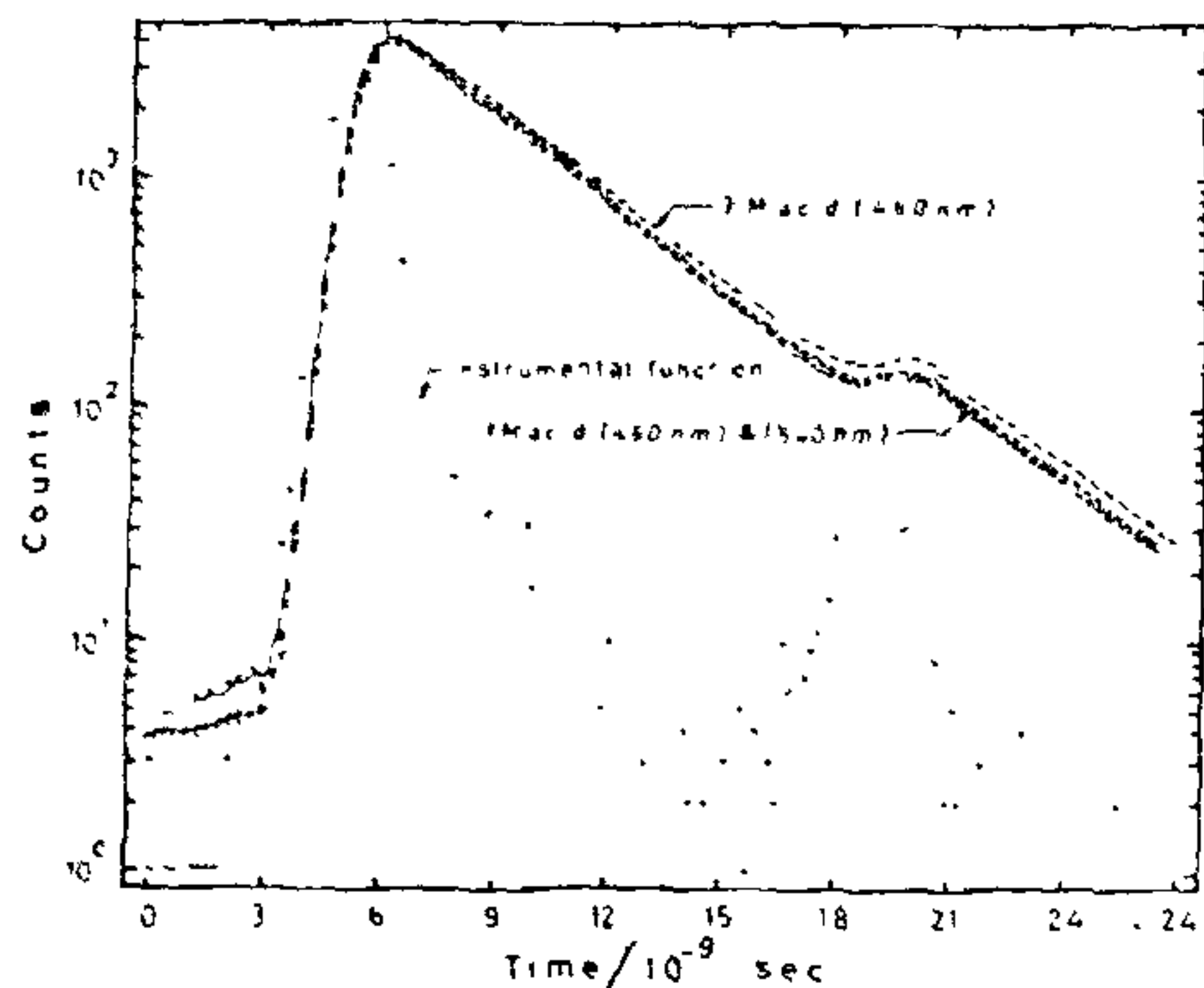
Figure 2. Decay curves of fluorescein in the aqueous solution at acid concentration.

Table 1 *Apparent decay times of fluorescein in acid water solution at various acid concentration.*

Acid conc. (M)	Lifetime (nsec) at	
	450 nm	540 nm
3	3 40	
2.5	3.40	3.40
2	3.36	3 33
1.5	3 29	3 27
1	3.14	3.10

follow single exponential decays. To confirm that the mechanism of proton transfer in fluorescein cation is also a two-state reversible reaction, K and K_r were reduced by performing the experiments in 1 M acid solution of glycerol and water mixtures. The emission spectra for different glycerol concentrations are given in figure 3. As expected, in pure glycerol only the FH^+ emission is observed and in 60% glycerol neutral emission dominates. The lifteime of cation in 3 M acid glycerol solution worked out to be 3.42 nsec which compares favourably with the lifetime of cation in acid-water system (3.4 nsec) shows that glycerol does not affect $(K_f + K_d)$. However, in 1 M acid solution of glycerol, a double exponential decay for both the emissions is observed (figure 4) and the decay constants vary with the glycerol concentration. The decay function for cation now follows (5) and the decay for neutral emission obeys (6) indicating the build-up of the neutral emission from zero concentration at zero
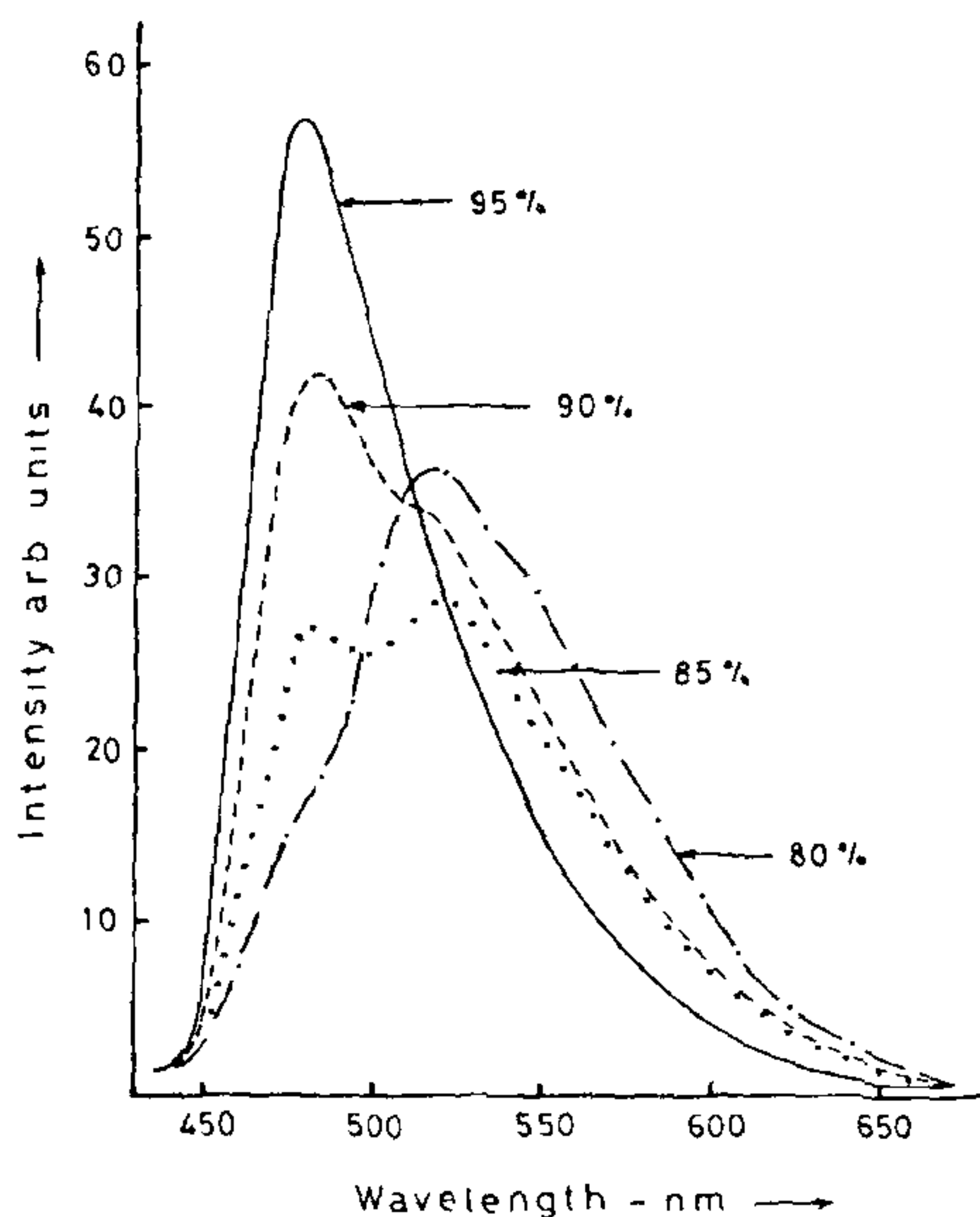

Figure 3. Emission spectra of fluorescein in 1M and, glycerol–water solution at glycerol concentration.
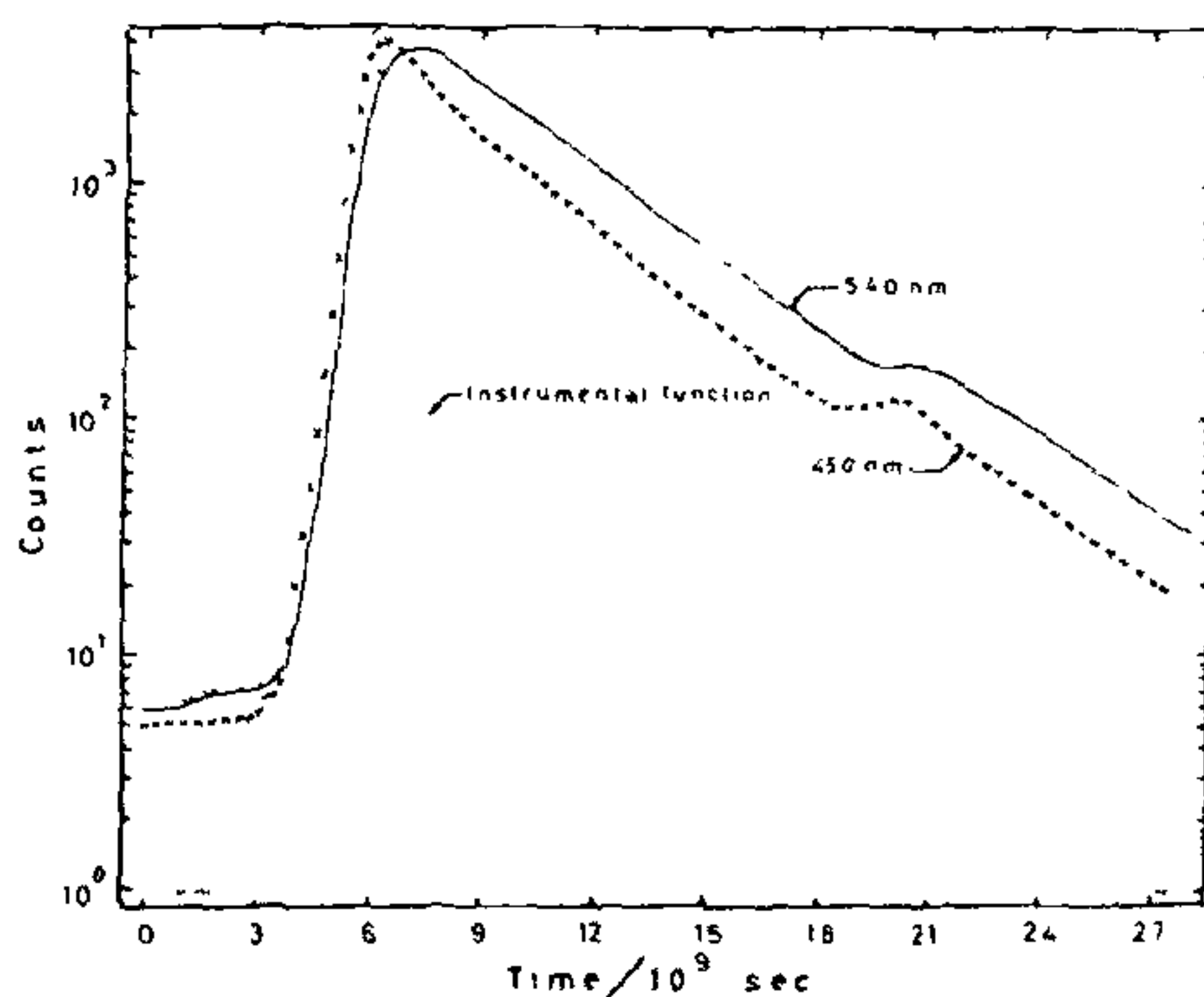

Figure 4. Decay curves in 1 M acid glycerol-water solution at λ em.

time. The value of τ_1 is now measurable and τ_1 and τ_2 are nearly the same for cation and neutral emissions separately as expected. At 60% glycerol where neutral emission is dominant the decay function still follows (6) with $\tau_1 = 300$ psec and $\tau_2 = 3.2$ nsec indicating

that neutral formation takes place only in the excited state. From (9)

$$\lambda_1 \cdot \lambda_2 = XY - K\,K_r\,H_3O^+, \tag{10}$$

$$\lambda_1 + \lambda_2 = X + Y = X + (K'_f + K'_d) + K_r[H_3O^+]. \tag{11}$$

On simplifying these equations, we get an equation independent of $[H_3O^+]$ and K_r

$$\lambda_1 + \lambda_2 = (K'_f + K'_d) + \frac{\lambda_1 \cdot \lambda_2}{(K_f + K_d)}$$
$$+ \left[1 - \frac{(K'_f + K'_d)}{(K_f + K_d)} \right] X. \tag{12}$$

Substituting the values of λ_1, λ_2, $(K_f + K_d)$ and $(K'_f + K'_d)$ for different glycerol concentrations, the values of K and K_r were calculated (table 2) and plotted against the viscosity of the solution (figure 5) and the extrapolation of this curve upto $n = 1.18$ cp (viscosity of water) gives a value of 3.5×10^{10} sec^{-1} for K and 9×10^9 M^{-1} sec^{-1} for K_r in water.

The steady state measurements do not give reliable transfer rates because the cation and neutral emission are not well resolved, yet using Gaussian analysis we have calculated the forward and reverse proton transfer rates by (4) and found a value of 3×10^{10} sec^{-1} for K and 1.06×10^{10} M^{-1} sec^{-1} for K_r. These values agree fairly well with the rate constants calculated by nanosecond pulse fluorometry. Thus, an analysis of the two-state reversible reaction can give rise to anomalous situations if the forward and reverse rates are much higher than the fluorescence decay rates. By slowing down the proton transfer rates, the two-state reversible proton transfer kinetics can be applied satisfactorily.

The value of pK^* deduced from these rate constants according to the following equation

$$pK^* = -\log(K/K_r)$$

Table 2 Fluorescence lifetimes and the values of forward and reverse proton transfer rates in 1 M acid solution of glycerol water.

Viscosity cp	τ_1(nsec)	τ_2(nsec)	K(sec^{-1})[a]	K_r (M^{-1}sec^{-1})[b]
800	1 53	3.17	4.06×10^8	4×10^7
450	1.096	3.16	7.46×10^8	1.2×10^8
280	0.77	3 13	1.36×10^9	3.4×10^8
210	0.42	3 13	2.82×10^9	7.2×10^8

a, extrapolated value $K = 3.5 \times 10^{10}$ sec^{-1}; b, Extrapolated value $K_r = 9 \times 10^9$ M^{-1} sec^{-1}
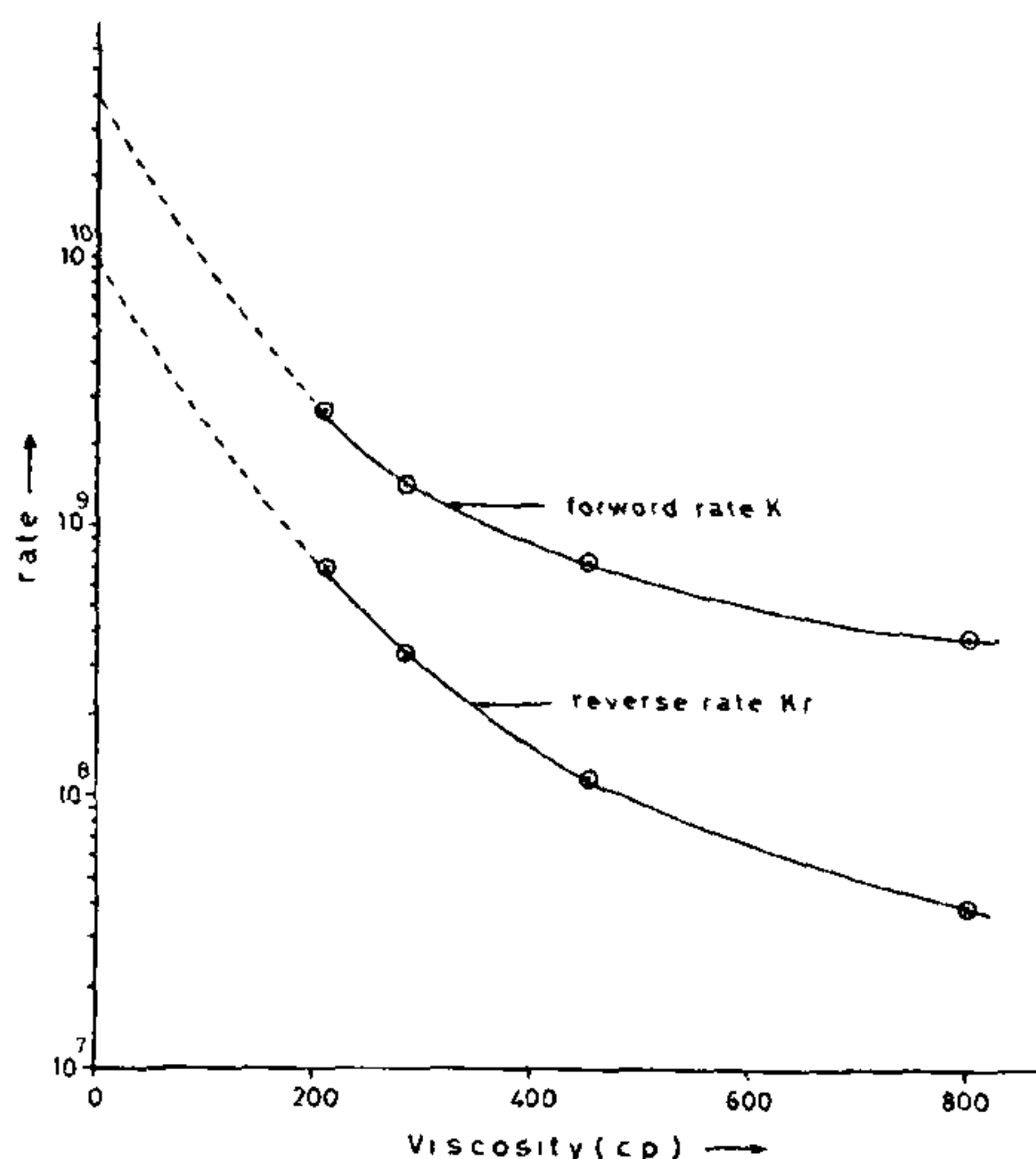

Figure 5. Proton transfer rate vs. viscosity plot.

comes out to be -0.6 as against -1.3 value calculated from Forster cycle.

ACKNOWLEDGEMENT

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