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A PREVIOUSLY UNREPORTED INTENSE ABSORPTION BAND AND THE pK_A OF PROTONATED TRIPLET METHYLENE BLUE

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ABSTRACT

Excitation by a Q-switched giant ruby laser (1.2 joule output at 694 nm,∿50nsec flash) of 2-10 μM solutions of methylene blue in water, 30% ethanol in water or 50 v/v% water - CH_3CN at pH values in the range 2.0 - 9.3 converted the dye essentially completely to its T_1 state. The absorption spectrum of T_1 dye was measured in different media at pH 2.0 and 8.2 by kinetic spectrophotometry. Previously reported T-T absorption in the violet in acidic and alkaline solutions and in the near infrared in alkaline solution was confirmed. Values found for these absorptions in the present work with 30% ethanol in water as solvent are $\lambda_{max} \sim 370$ nm, $\varepsilon_{max} \sim 13,200\ \underline{M}^{-1}\ cm^{-1}$ at pH 2 and $\lambda_{max} \sim 420$ nm, $\varepsilon_{max} \sim 9,000\ \underline{M}^{-1}\ cm^{-1}$, $\lambda_{max} \sim 840$ nm, $\varepsilon_{max} \sim 20,000\ \underline{M}^{-1}\ cm^{-1}$ at pH 8.2. Long-wavelength T-T absorption in acidic solution is reported here for the first time: $\lambda_{max} \sim 680$nm, $\varepsilon_{max} \sim 19,000\ \underline{M}^{-1}\ cm^{-1}$ in 30% ethanol in water at pH 2. Observation of a pH-independent isobestic point ∿ 720 nm confirms that the long-wavelength absorptions are due to different protonated states of the same species, $MB^+(T_1)$ and $MBH^{2+}(T_1)$. The pK_A of $MBH^{2+}(T_1)$ in water was determined from the dependence on pH of absorption at 700 and 825 nm to be $7.1_4 \pm .1$ and from the kinetics of decay of triplet absorption to be 7.2. The specific rate of protonation of $MB^+(T_1)$ by $H_2PO_4^-$ in water at pH 4.4 was found to be $4.5 \pm .4 \times 10^8\ \underline{M}^{-1}\ sec^{-1}$.

INTRODUCTION

The properties of the triplet state of methylene blue are of considerable interest because of the utility of this state as a sensitizer, e.g., for generation of singlet oxygen (Nillson, Merkel and Kearns, 1972), and as a reversible photooxidant applicable, e.g., to photogalvanic systems (Wildes, Lichtin, Hoffman, Andrews and Linschitz, 1977).

The lowest triplet state of methylene blue is a much stronger base than is the singlet ground state of the dye. Several values of the pK_A of its conjugate acid, $MBH^{2+}(T_1)$, have been reported: 6.7 (Kato, Morita and Koizumi, 1964), 6.8 (Matsumoto, 1964), 7.5 (Faure, Bonneau and Jussot-Dubien (1967), and 7.7 (Roitman and Chibisov, 1971). These values can be compared with pK_A of $MBH^{2+}(S_o)$ = 0.0 (Keene, Land and Swallow, 1965). Many properties of triplet methylene blue depend on its state of protonation, e.g., absorption spectrum (Faure, et al., 1967; Roitman and Chibisov, 1971), intrinsic lifetime (Bonneau, Fornier de Violet and Joussot-Dubien, 1974), reactivity (Bonneau, Fornier de Violet and Joussot-Dubien, 1974; Bonneau, Pottier, Bagno and Joussot-Dubien, 1975) and redox potential (Vogelmann, Schreiner, Rauscher and Kramer, 1976). These differences, together with the proximity of the pK_A of $MBH^{2+}(T_1)$ to the pH of neutral water, increase the value of a precise knowledge of the magnitude of this pK_A.

This paper describes detection and characterization, with the aid of a pulsed (Q-switched) ruby laser, of previously unreported intense ($\epsilon_{max} \geqslant$ 14,500 $\underline{M}^{-1}cm^{-1}$, depending on solvent) absorption by protonated dye, $MBH^{2+}(T_1)$ around 700 nm. It also reports the redetermination of the pK_A of $MBH^{2+}(T_1)$ from the pH-dependences of the absorption of triplet methylene blue at 700 and 825 nm and of the intrinsic rate of decay of the triplet state. Measurement of the previously unreported specific rate of protonation of $MB^+(T_1)$ by $H_2PO_4^-$ ions is also described.

MATERIALS AND METHODS

Materials

Water was either laboratory distilled water which had been further purified by passage through a Millipore deionizer and filter or was Belmont bottled distilled water. All other solvents and reagents were used as supplied. These included Fluka puriss methylene blue chloride, Burdick and Jackson UV grade acetonitrile, U.S.I reagent quality ethanol, Baker's "analyzed" KH_2PO_4, Mallinckrodt analytical grade potassium biphthalate, Fisher Certified ACS grade $Na_2B_2O_7 \cdot 10H_2O$ and hydrochloric acid and Baker's analyzed sulfuric acid.

Aqueous acetonitrile, 50 v/v%, was prepared by mixing equal volumes of water and CH_3CN while 30% ethanol in water was prepared by diluting 30 parts by volume of ethanol to 100 with water.

Water of hydration in the methylene blue chloride was determined by loss of weight to constancy on storage in a vacuum desiccator over Drierite and amounted to 3 molecules of water per molecule of dye (Ray, 1977). The corresponding molecular weight, used in calculating ε values in this work, is 374.

All test solutions were deaerated by purging for 15-20 minutes with deoxygenated nitrogen which had first been bubbled through the same solvent used in the test solution. The nitrogen was deoxygenated by bubbling through a chromous/zinc-amalgam solution.

Apparatus

A Holobeam Series 630 Laser System with a Q-switched ruby laser capable of providing up to 3.6 joules per flash at 694.3 nm with a nominal pulse width of 19 nsec was used. Measured values of the flash width at half-height were ∿28nsec at 1.8J and ∿ 50 nsec at 1.2J. The cross-section of the laser beam was expanded by two cylindrical lenses to illuminate all of the 2.2 cm-long cell. Variations in the intensity of the incident laser pulses were monitored by reflecting a sample of the beam with a microscopy slide onto a Hewlett-Packard hp 2 4203 808 PIN photodiode and measuring the integrated photocurrent using sample and hold electronics similar to those described by Davies and Hodgkinson (1972). This monitoring system was calibrated against a Quantronix 504 Energy-Power Meter.

Samples were monitored optically by a probe-beam oriented perpendicular to the laser beam. The probe-beam was provided by a 100W, 12V Oriel quartz halogen lamp with a 2.3 x 4.2 mm filament in series with a power-dropping resistor and an NJE Corp. model RSE 18 regulated constant-voltage DC power supply. The lamp was operated at 10V and, to increase the light intensity, could be pulsed to 16V by shorting the resistor (Claesson, Finnström and Hunt, 1975). A series of fused-silica lenses was used to collimate the probe-beam, focus it through the cell, recollimate it and focus it on the entrance slit of an Oriel 7240 grating monochromator. One or more cut-off filters were placed between the sample cell and the monochromator to minimize 694.3 nm light and/or between the monitoring lamp and the cell to minimize photolysis. A rotating-sector shutter placed in the probe-beam minimized illumination of the sample. Three micro-switches activated by cams attached to the shutter shaft were used. One assured that the shutter never stopped in a position which would keep the lamp operating at 16V. Another controlled the timing and width of the probe lamp pulse. The third provided a timing signal, when the probe lamp reached maximum intensity, which initiated the time sequence of firing the laser flash lamp, starting the oscilloscope sweep and firing the Pockels cell Q-switch.

Intensity of the monitoring light was measured by a Hamamatsu R955 photomultiplier tube and a Tektronix 466 storage oxcilloscope. In some cases,a fast preamplifier incorporating an Analog Devices 50J FET amplifier was used. Photographs of oscilloscope traces were enlarged by projecting them onto coordinate paper and tracing the image.

Cells were of Pyrex with Pyrex windows attached by a silicone adhesive or were fabricated of quartz without use of adhesives.

Measurements

Transient absorption spectra were measured by kinetic spectrophotometry. Prompt transient changes in absorption were directly measured within 200nsec after the flash. In some cases, prompt transient changes at 650-900 nm were calculated by extrapolation of the first order decay of absorption.

A PHM62 Standard pH meter (RADIOMETER Copenhagen) was used to measure pH.

RESULTS

Since the intrinsic lifetime of the triplet is 4.5 μsec in acid solution and more than an order of magnitude greater at alkaline pH, essentially complete conversion of methylene blue to its triplet state can be inferred from the constancy (±4% for 10 flashes) of prompt changes in absorbance obtained with laser flash-energy in the range 0.45 - 1.42J. With a flash-energy of 0.25J, ΔOD was about 10% smaller. A flash energy of 1.2J was used routinely in this work.

T-T Spectra

Difference spectra at 300 - 900 nm were measured in .01M acid in various media and at pH 8.2 in 30% ethanol in water (borate buffer). Assuming complete conversion of dye to its triplet state, differential absorbances are given by Eq. 1,

$$(\Delta OD)_\lambda = [Dye]_{total} \left\{\varepsilon_\lambda^T - \varepsilon_\lambda^S\right\} \ell \qquad (1)$$

where ε_λ^T and ε_λ^S are the molar absorbancy indices of, respectively, the triplet and singlet dye. In all of the work reported here, ground-state methylene blue was unprotonated MB^+ while the state of protonation of the triplet varied with pH from $^3MBH^{2+}$ to $^3MB^+$. As shown below, under all conditions used to measure triplet spectra, equilibration of protonation of the triplet was essentially complete on the time scale of measurement of prompt signals.

Differential absorbances were observed to decay to zero at first order rates which, at a given pH, were independent of wavelength. Of particular importance was the observation that, in .01 N acid, the rate of decay was the same at 370 nm, in a band previously assigned to $^3MBH_2^+$ (Kato et al., 1964) and at 710 nm, in the band which is reported here for the first time. The observed differential spectra are therefor attributed to methylene blue triplet.

Typical differential spectra in .01 N acid and at pH 8.2 are plotted in Fig. 1. T-T spectra calculated by means of Eq. 1 from differential spectra measured with 5 μM solutions of methylene blue in various media containing .01 N acid are plotted in Fig. 2. S-S spectra in the same media are superimposed to facilitate comparison. A similarly determined T-T spectrum at pH 8.2 in 30% ethanol in water is shown in Fig. 3. Principal spectral features are summarized in Table 1. Wildes et al. (1977) have reported that ε_{370} at λ_{max} of the T-T absorption of methylene blue is 7,000 x 10^4 $\underline{M}^{-1}$ cm^{-1} in .01 $\underline{N}$ aqueous sulfuric acid and 14,000 $\underline{M}^{-1}$ cm^{-1} in .01 $\underline{N}$ H_2SO_4 in 50 v/v% aq. CH_3CN. These values were, however, based on the assumption that the molecular weight of the same batch of dye as was used in the present work was 342 (Wildes, 1979). Correction to the molecular weight used in the present work, 374, gives ε_{370} values of 7,700 in water solution and 15,300 in 50 v/v% aq. CH_3CN. The latter value is the same as that reported here for .01 $\underline{N}$ HCl in 50 v/v% aq. CH_3CN but the 1977 value in .01 $\underline{N}$ aq. H_2SO_4 appears to be anomalous. Several workers have reported λ_{max} = 420 nm for absorption of triplet methylene blue in alkaline solution with ε_{max} = 9,000 $\underline{M}^{-1}$ cm^{-1} in water (Danziger, Bar-Eli and Weiss, 1967), 7,500 $\underline{M}^{-1}$ cm^{-1} in ethanol (Kikuchi, Kokobun and Kikuchi, 1975) and 11,000 in methanol (Kayser and Young, 1976). Previous reports are also in reasonable agreement with the present characterization of the absorption of triplet methylene blue in the near infra-red in alkaline solution: λ_{max} = 840 nm, ε_{max} = 18,000 (Danziger et al., 1967); λ_{max} = 856 nm, ε_{max} = 24,000 $\underline{M}^{-1}$ cm^{-1} in ethanol (Kikuchi et al., 1975); ε_{825} = 18,000 $\underline{M}^{-1}$ cm^{-1} in water (Faure et al., 1967).

pK_A of $^3MBH^{2+}$ in H_2O Solution

The data used to determine the pK_A of $^3MBH^{2+}$ are summarized in Fig. 4. The inflection points of the plots of ΔOD vs pH at 700 and 825 nm give $pK_A = 7.2_2$ and 7.0_6, respectively. The same results are obtained by fitting the data to Eq. 2:

$$\mathrm{pH} = \mathrm{p}K_A - \log \frac{[{}^3\mathrm{MBH}^{2+}]}{[{}^3\mathrm{MB}^{+}]} \qquad (2)$$

Similarly, $\mathrm{p}K_A$ of ${}^3\mathrm{MBH}^{2+}$ can be evaluated from the dependence on pH of k_{decay}, the intrinsic rate of decay of triplet dye as measured at 700 and 720 nm in acid solution, 700, 720 and 825 nm at pH values near 7.2, and at 720 and 825 in alkaline solution. This method depends on equilibration of protonation being much faster than decay. From Eq. 2 it is apparent that $\mathrm{p}K_A = \mathrm{pH}$ when $[{}^3\mathrm{MBH}^{2+}] = [{}^3\mathrm{MB}^{+}]$. At this pH, $k^{obs}_{decay} = (k^{\mathrm{MBH}^{2+}}_{decay} + k^{\mathrm{MB}^{+}}_{decay})/2 = 1.25 \times 10^5\ \mathrm{sec}^{-1}$, according to data presented in Fig. 4. This analysis gives $\mathrm{p}K_A = 7.2$. The mean value obtained from the three measurements is 7.16 with a standard deviation of .087.

The dependence of the pseudo first-order rate of protonation of ${}^3\mathrm{MB}^{+}$, monitored at 810 nm, upon the concentration of $\mathrm{KH_2PO_4}$ is shown in Fig. 5. These data correspond to Eq. 3:

$$k_{exp} = k_{\mathrm{H}^+}[\mathrm{H}^+] + k_{\mathrm{H_2PO_4}^-}[\mathrm{H_2PO_4}^-] \,. \qquad (3)$$

They yield $k_{\mathrm{H}^+} = 7.5 \times 10^9\ \underline{M}^{-1}\ \mathrm{sec}^{-1}$, essentially the same as has been reported for thionine triplet, $7.4 \times 10^9\ \underline{M}^{-1}\ \mathrm{sec}^{-1}$ (Bonneau and Stevens, 1972), and $k_{\mathrm{H_2PO_4}^-} = 4.5 \pm .4 \times 10^8\ \underline{M}^{-1}\ \mathrm{sec}^{-1}$. Under conditions similar to those of Fig. 3, the pseudo first order rate of protonation of ${}^3\mathrm{MB}^{+}$ at pH = 7.2 = $\mathrm{p}K_A$ of $\mathrm{H_2PO_4}^-$ ion, can be estimated easily by recognizing that $[\mathrm{H_2PO_4}^-] = [\mathrm{KH_2PO_4}]_{stoich}/2$. With $[\mathrm{KH_2PO_4}]_{stoich} = .025\ \underline{M}$ at pH 7.2, the calculated rate of protonation is $5.6 \times 10^6\ \mathrm{sec}^{-1}$, 45 times the measured rate of decay of triplet dye at this pH. No significant error in determination of the $\mathrm{p}K_A$ of ${}^3\mathrm{MBH}^{2+}$ from the pH-dependence of the rate of decay of the triplet can, therefor, be ascribed to deviation from protonic equilibrium around pH 7.2.

The value of the pK_A of $^3MBH^+$ reported here falls very nearly at the midpoint of the four literature values, 6.7 (Kato et al., 1964); 6.8 (Matsumoto, 1964); 7.5 (Faure et al., 1967) and 7.7 (Roitman and Chibisov, 1971). The high value reported by Roitman and Chibisov may be in error because they measured absorbance at 825 nm 110 μsec after the beginning of a 40 μs flash. Some of the observed decrease of absorbance at 825 nm with decreasing pH around neutrality was due to rapid decay of $^3MBH^{2+}$ (τ = 4.5 μsec) and associated rapid conversion of $^3MB^+$ to $^3MBH^{2+}$. This decay would be expected to shift the midpoint of the observed plot of absorbance vs pH to a higher value of the pH and result in an erroneously high pK_A. The measurements of Faure et al. are somewhat subject to the same criticism since they measured absorbance 10 μsec after the beginning of a 2.5 μsec flash. The relatively low value of Kato et al. may have been influenced by the use of acetate buffers. Around pH 7 only about 2% of the buffer is present as HOAc. Even if this reacts at a diffusion-controlled rate, the compositions measured in Kato's steady-state measurements probably contained a larger proportion of $^3MB^+$ than they would at equilibrium. The measurements of Matsumoto (1964) did not involve obvious errors due to rapid decay of $^3MBH^{2+}$ or to slow protonation However, the value, pK_A = 6.8, which he reports was estimated from minimal observations. Other possible sources of divergence among pK_A values include differences in temperature and ionic strength.

DISCUSSION

It is worth noting that $^3MBH^{2+}$ and $MB^+(S_0)$ both absorb strongly in the same wavelength region with the integrated intensity of the band of $^3MBH^+ \sim$ 20% of the ground-state absorption. This coincidence, taken together with the 4.5 μsec lifetime of $^3MBH^+$, greatly increases the possibility of 2-photon excitation of methylene blue when its solutions are illuminated in the 665 nm band at pH $\leqslant$ 7.2. Obviously,such 2-photon excitation will be particularly prominent when extensive population inversion is effected, as with the 694.3 nm ruby laser. One possible consequence is photodecomposition of the dye. We have, in fact, observed 7% decomposition of dye in acid solutions in water and 30% ethanol in water after 30 - 1.2J flashes.

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We wish to express our appreciation of the helpful advice provided by Professor Henry Linschitz of Brandeis University while the laser flash apparatus described in this paper was being assembled. This work was sponsored by the U.S. Department of Energy under contract number EY-76-S-02-2889.

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TABLE 1

Principal Features of Absorption Spectra of T_1 and S_1 States of Methylene Blue[a]

Medium	T-T Absorption		S-S Absorption	
	λ_{max}, nm	ε_{max}, $M^{-1}cm^{-1}$	λ_{max}, nm	ε_{max}, $M^{-1}cm^{-1}$
.01 *N* aq. H_2SO_4[b]	690	14,500	665	74,000
	370	12,500		
.01 *N* H_2SO_4, 30% EtOH in water[b]	703	18,700	665	79,000
	680	19,000		
	370	13,200		
.01 *N* HCl, 50 v/v% aq. CH_3CN[b]	670-710	≥16,200	662	91,000
	370	15,300		
pH 8.2, Borate Buffer, 30% EtOH in water[c]	840	20,000	665	82,000
	420	9,000		

a. Values of ε based on weights of solute and mol. wt. of 374 ($MB^+Cl^-\cdot 3H_2O$). Values are uncorrected for dimerization.

b. [Methylene Blue] ∿ 5 μ*M*.

c. [Methylene Blue] ∿ 2 μ*M*.

LEGENDS TO FIGURES

1. Triplet-Singlet difference spectra resulting from illumination of 5 μM methylene blue chloride with a 694.3 nm laser flash: ● in .01 N aq. H_2SO_4; Δ in 30% aq. ethanol buffered at pH 8.2 with borate.

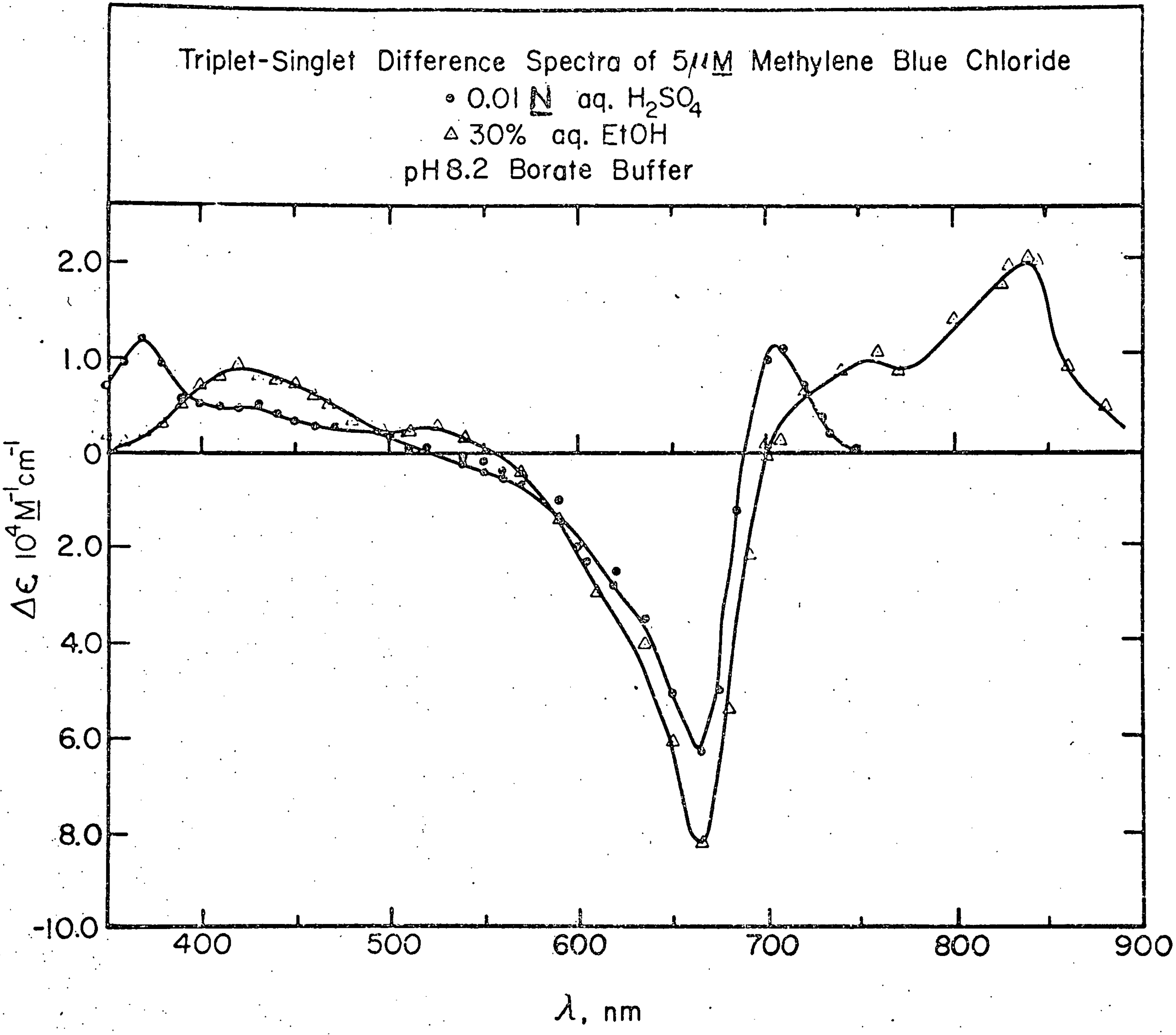
2. S-S, ——, and T-T, ···, absorption spectra of 5 μM methylene blue chloride in .01 N Acid: A) .01 N aq. H_2SO_4; B) .01 N solution of H_2SO_4 in 30% aq. ethanol; C) .01 N solution of HCl in 50 v/v% aq. CH_3CN.

3. S-S, ——, and T-T, ···, absorption spectra of 2 μM methylene blue chloride buffered at pH 8.2 in 30% aq. ethanol by borate.

4. Determinations of pK_A of 5.4 μM triplet methylene blue in aqueous solution. By pH-dependence of T-T absorbance at Δ, 700 nm; ●, 720 nm (isobestic point), ○, 825 nm. By pH-dependence of rate of intrinsic decay of the triplet. Buffers: pH 4, .025 M phthalate; pH 5.8-8.0, .025 M phosphate; pH 8.2-9.3, borate.

5. Dependence of rate of protonation of 10 μM $^3MB^+$ on $[KH_2PO_4]$ in aqueous solution at pH 4.4.

Ohno, Osif & Lichtin, "A Previously Unreported.." FIG. 1



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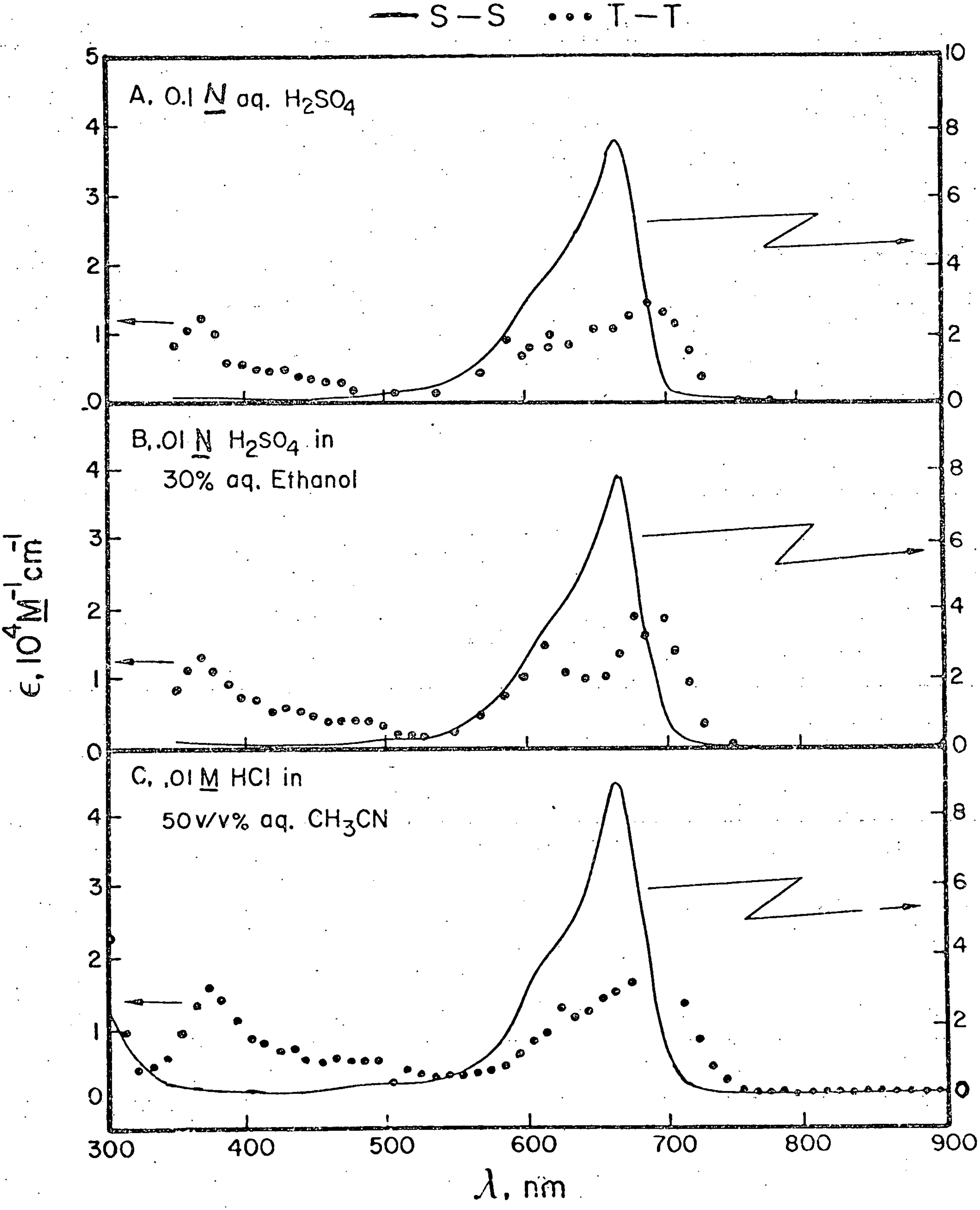
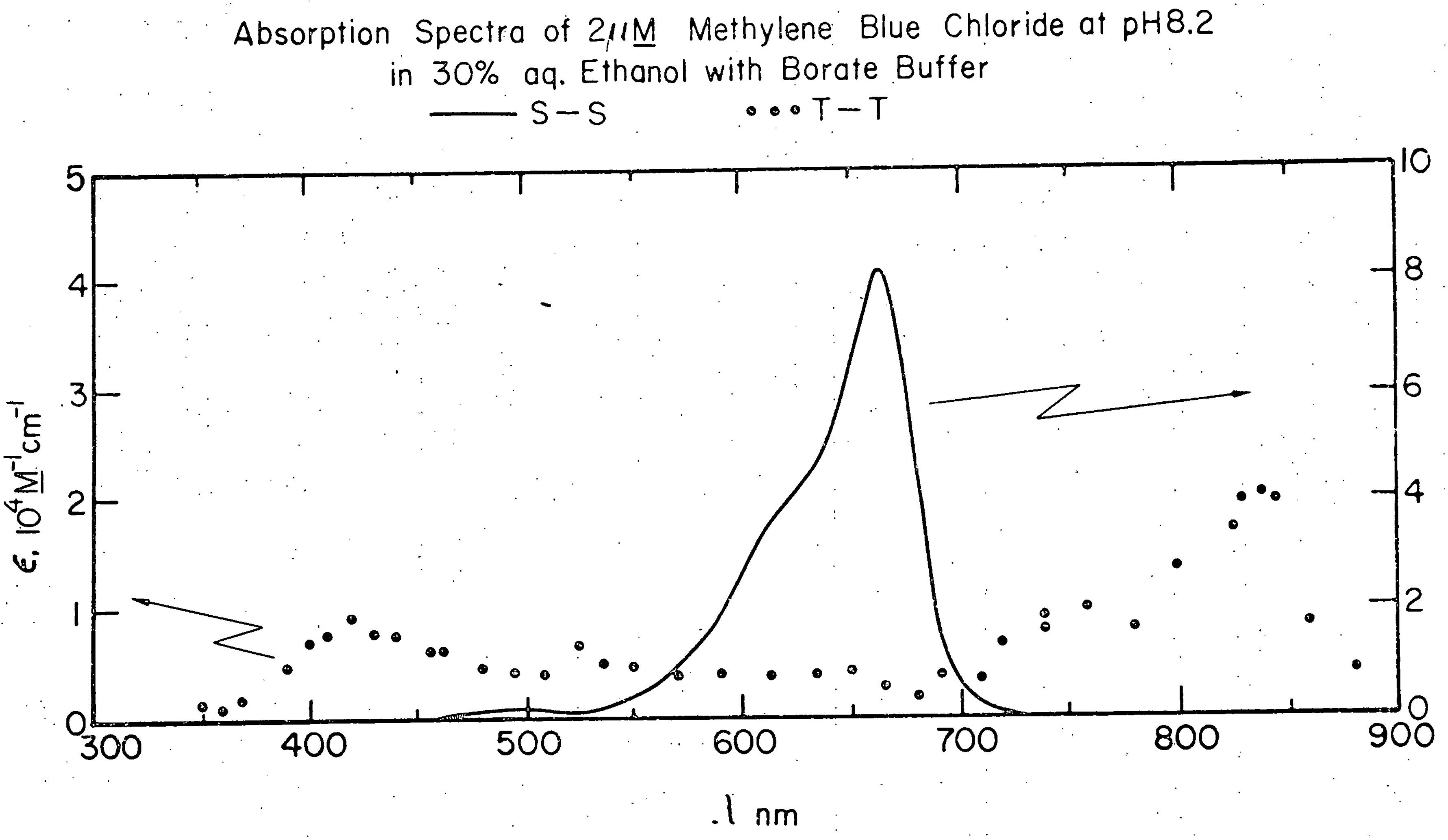
Previously Unreported.."
FIGURE 2

Absorption Spectra of 5 μM Methylene Blue Chloride in .01 N Acid



Ohno et al., "A
Previously Unre-
ported.." FIG. 3



Ohno, et al., "A
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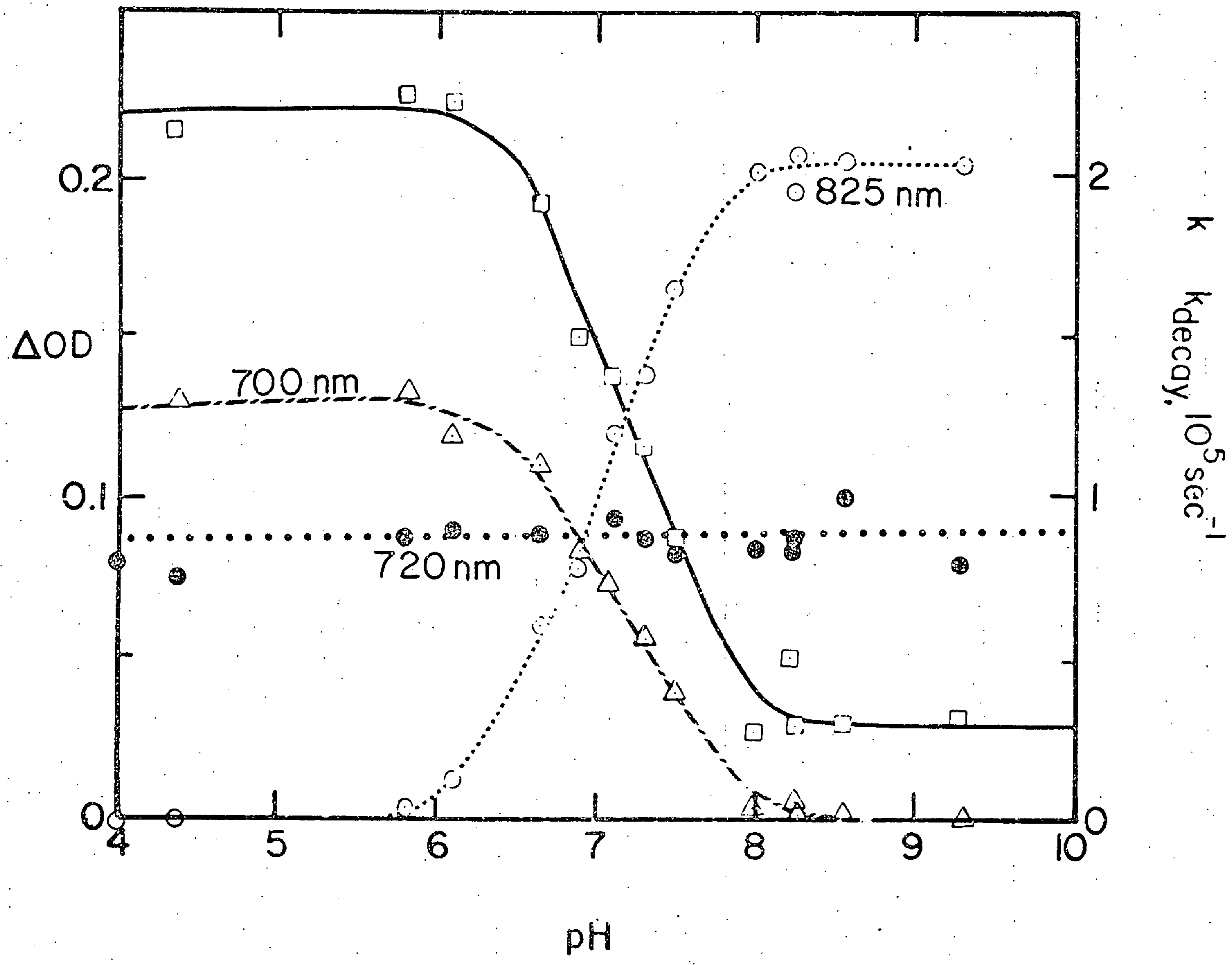
ported.." FIG. 4

Determination of pK_A of 5.4 μM Methylene Blue from pH-Dependence of T-T Absorbance and Rate of Intrinsic Decay



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proted.." FIG. 5

Dependence of Rate of Protonation of 10 $\mu\underline{M}$ $^3MB^+$ on $[KH_2PO_4]$ in Aqueous Solution at pH 4.4