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The Quenching of the Fluorescence of the Eosin Ion By the Iodide Ion

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THE QUENCHING OF THE FLUORESCENCE OF THE EOSIN ION BY THE IODIDE ION

by
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TABLE OF CONTENTS

	Page
THEORY	1
Self Quenching	1
Kinetic Quenching	3
Static Quenching	7
Quenching Relationships	8
The Effects of Ionic Strength	13
The Influence of Dielectric Constant	19
The Temperature Coefficient of Quenching and the Effect of Viscosity	20
EXPERIMENTAL	35
Materials	35
Apparatus and Measurements	36
Preparation of solutions	44
Treatment of Data	45
EXPERIMENTAL RESULTS AND DISCUSSION	48
The Self Quenching of the Fluorescence of the Eosin Ion	52
Iodide Ion Quenching of the Fluorescence of Eosin	59
Comparison of Theoretical and Observed Quenching Constants for the Iodide Quenching of Eosin	63
The Influence of Potassium Iodide Concentration on the Quenching Constant	69
The Influence of Viscosity on Quenching as a Function of Dielectric Constant	71

Discussion of Dielectric Effects..... 76
Temperature Coefficients of Quenching..... 88
SUMMARY..... 97
REFERENCES..... 100

THEORY

A great many molecules of various types have the ability to absorb radiant energy, and to emit, after a greater or lesser time interval, light of a longer wave length. If the time interval between absorption and emission of the light is so short that the emission ceases immediately upon removal of the exciting source, the molecule is said to fluoresce.

Fluorescence is a general property, occuring in both organic and inorganic compounds, in liquid, solid, gaseous and solution phases. It is more common in organic materials than in inorganic materials.

The intensity of fluorescence is subject to variation with light intensity, and increases with increasing light intensity. On the other hand, the fluorescent intensity may be either increased or decreased by changes in concentration of the fluorescing molecule; or it may be decreased by the addition of foreign salts or non-electrolytes. The decrease in fluorescent intensity by the addition of other materials is known as quenching. These quenching reactions may be divided into three categories, and a plausible explanation advanced for each type. The three types of quenching have been referred to as self or concentration quenching, kinetic quenching and static quenching.

Self Quenching. The intensity of fluorescence in a solution of a fluorescent dye increases in general with increasing concentration until a maximum is reached, after

which the intensity falls to zero as the concentration is further increased. An aqueous solution of uranin (disodium fluorescein) has been shown[1] to increase rapidly in fluorescent intensity up to a concentration of 0.30 grams/liter, and to fall off more slowly with further increasing concentration. Three theories have been proposed which can in part account for the occurence of this maximum in the fluorescence intensity-concentration curve.

(1) At low concentrations, the number of molecules per unit volume is too small to produce much emission. As the concentration is increased, the number of molecules contributing to the fluorescent effect increases. The fluorescent intensity thus increases to the maximum value. At the maximum value, a collision picture is capable of accounting for many of the facts. This collision picture does not invalidate the previous conception of more molecules per unit volume to emit, but states that at the maximum and beyond, collisions between like molecules become more important. A dye molecule absorbs radiant energy, $h\nu$, and becomes excited. This energy remains in the molecule for a longer or shorter time before it is radiated. If, during this time interval, the activated molecule collides with an inactivated molecule of the same kind, sufficient energy can be transferred to the inactivated molecule so that neither molecule can radiate. Beyond the maximum in the intensity-concentration curve, the concentration is sufficiently great

that there is a high probability of collisions of this type. These collisions are particularly effective in the quenching action due to the near equality of the energy levels in the activated and nonactivated molecules. The energy transfer is facilitated by resonance.

(2) At very high concentrations, where quenching is essentially complete, association between molecules producing non-fluorescent dimers or polymers may cause the quenching. Bowen, Barnes and Holiday[2] have explained the diminution of quenching constants with temperature in the quenching of anthracene by bromobenzene and other halogenated hydrocarbons in various solvents as due to the formation of temporary complexes between the anthracene and the quencher molecule. At the higher temperatures there is a higher degree of dissassociation of these complexes, with resulting increase in fluorescent intensity.

(3) Bowen[3] states that the selfquenching of anthracene is due to the photochemical formation of bianthracene. This same author[4] offers the intriguing theory that since the term quenching can be given to any effect reducing fluorescence efficiencies below unity, it follows that from this point of view, all non-fluorescent colored substances are 100% quenched.

Kinetic Quenching. The addition of foreign substances to fluorescent solutions often causes a lowering of the fluorescent intensity. Potassium iodide, as well as other salts, quenches fluorescein, eosin, quinine sulfate and many

other dyes, and other materials as widely different as aniline and hydroquinone have the same effect to a greater or lesser degree. Jette and West[5] investigated the quenching action of various salts on the fluorescent intensity of quinine bisulfate, disodium fluorescein and uranyl sulfate. Their results showed the quenching effect to be associated practically completely with the anion. Of all the cations investigated, only silver had a noticeable effect. The order of the extinguishing effect found was

$$I^- > CNS^- > Br^- > Cl^- > C_2O_4^= > CH_3COO^- > SO_4^= > NO_3^- > F^-$$

This order was shown by these authors to be identical with the deformability order of these ions as shown by their molar refraction calculated by the Lorenz-Lorentz equation. Since the molar refraction is a measure of the displacement of the electrons under the influence of the electromagnetic wave, it is thus also a measure of the ease with which the electrons are displaced by electrical forces. It was also shown by dispersion measurements on the ionic refractions of the cations investigated that silver ion was also highly deformable compared to the other cations.

For disodium fluorescein, the same general order was found, although in this case the nitrate, sulfate, oxalate and chloride ions had no effect on the fluorescence up to a concentration of 1.00 normal. Silver ion had a larger quenching action up to a concentration of 0.1 normal.

Silver ion concentrations above this value precipitated the fluorescein. These results were interpreted in terms of collisions of the second kind. It was postulated that the energy of the fluorescent ion was transferred to the quencher, whereby no radiation could occur. The efficiency of such a transfer, and thus the quenching efficiency depended upon the deformability of the quencher ion.

These same data of Jette and Best were further treated by Loss[6] on the basis of stationary state kinetics and on Pavilov's[7] adaptation of the Smoluchowski[8] equation. Loss was able to set up tables of efficiencies for the quenching process. For quinine sulfate, these efficiencies were found to range from a maximum of 2.8 for the iodide ion to a minimum of 0.004 for the nitrate ion, while for sodium fluoresceinate the maximum was 0.40 for the silver ion and a minimum of 0.012 for the bromide ion. Iodide ion had a value of 0.232. These data also were interpreted on the basis of collisions of the second kind. For self quenching, Loss postulated the formation of non-fluorescent polymers to account for quinine sulfate and aniline-iodeosine.

Specific quenching action was found to occur when it was discovered that halides had no quenching action on the fluorescence of sodium naphthionate[9] but that nitrate ion is a good quencher for this molecule. Bollefson and Stoughton[10] extended the investigation of this effect, and found that fluorescent materials can be divided into two classes, each of which has a characteristic group of quenchers.

Class I is quenched by

$$I^-,\ Br^-,\ Cl^-,\ CNS^-,\ S_2O_3^=,\ SO_4^=$$

and contains acridone, α and β anthracene sulfonic acid, quinine sulfate, fluorescein, eosin, rhodamine B and rhodamine 6g.

Class II is quenched by

$$IO_3^-,\ BrO_3^-,\ NO_3^-,\ SnO_4^=$$

etc., and contains anthranilic acid, sodium sulfanilate, sodium naphthionate, α -naphthol and 1,4-naphthol sulfonic acid.

Both classes are quenched by

$$Ag^+,\ Cu^{++},\ Fe^{++}$$

The nature of the quenching action is discussed by Rollefson and Stoughton in this same paper. The formation of non-fluorescent addition compounds was ruled out on the basis of two criteria: the absorption spectra in the visible region and the activities of the fluorescent molecules. These two criteria are briefly discussed below.

The visible absorption spectra of the dye was determined in both the presence and absence of the quencher. If a new compound were formed it was expected that the spectrum of the quenched solution would be changed, either in intensity or the position of the absorption maximum, or in both. Their findings, however, were that the two spectra were identical within the limits of experimental error.

Later,[11] feeling that this criterion was not sufficiently sensitive, they determined the relative activities of the fluorescent molecules in the absence and presence of the quencher. The method required a neutral molecule; thus fluorescein and acridone were investigated. The distribution equilibrium of the dye between aqueous solution and benzene was determined in the presence and absence of the quencher. The fluorescent intensities of both quenched and unquenched aqueous solutions were measured and the solutions were extracted with benzene. Reextracting the benzene phases with aqueous potassium hydroxide and measuring the fluorescent intensities of the extracts gave identical values. If an addition complex had been formed, the extent of quenching (in the case of fluorescein) predicted only a ten percent recovery in the quenched system compared to the unquenched system.

Static Quenching. A third type of quenching, of less importance than self or kinetic quenching occurs if the dye molecule is activated in the immediate vicinity of the quencher ion. In this case the dye can be inactivated nearly immediately and the average life is shortened, leading to quenching. Sveshnikoff[12] has concluded that static quenching is relatively unimportant, although Weber[13] has concluded in the quenching of eosin with potassium iodide that some static quenching occurs. This static quenching is slight for the uranin-potassium iodide quenching reaction[14] but according to these authors, is high for the quenching of

uranin with aniline, while the quenching of uranin with pyrogallol is practically all static. Weber assumes that static quenching occurs due to the formation of a temporary complex between the dye and the quencher. He establishes this formation by absorbing the quenched system on paper and comparing its fluorescent intensity with the original solution. If static quenching occurs, the dye-quencher complex will have a different absorbability from the dye absorbability alone, and thus static and kinetic quenching can be differentiated. Thus it would seem that static quenching, in this light, is due to a complex being formed between the dye and quencher, and not due merely to the quencher molecule being within the sphere of action of the dye at the moment of activation.

Weber also found that the quenching of eosin with hydroquinone had a negative temperature coefficient. This is consistent with complex formation, since increased temperature would facilitate dissassociation of the complex, leading to decreased quenching.

Quenching Relationships. Various relationships have been proposed for the dependence of fluorescent intensity on the concentration of the fluorescent material and on the concentration of the quencher. Perrin[15] proposed the equation

$$\frac{I_o}{I} = e^{ks} \qquad (1)$$

for solutions containing fluorescent molecules alone, where I and I_o are the intensities of fluorescence per unit mass

of fluorescent substance at concentrations s and infinite dilution respectively, and k is a constant. If a quencher is present, this equation can be written

$$\frac{I_o}{I} = e^{ks + k'c} \tag{2}$$

where k' is an additional constant and c is the concentration of the quencher.

For the quenching of quinoline red by potassium iodide, Weber and Loker[14] stated the dependence of fluorescence seemed to follow the equation,

$$\log \frac{I_o}{I} = k\sqrt{[KI]} \tag{3}$$

an equation previously given by Banow.[16]

Banow actually proposed two equations for the dependence of fluorescence intensity on electrolyte concentration. His first equation is given in the form

$$\frac{I}{I_o} = e^{-k\sqrt{c}} \tag{4}$$

where I is the fluorescent intensity at an electrolyte concentration c and I_o is the fluorescent intensity at zero concentration of electrolyte. k is a constant. Banow's other equation for lower concentrations of dye is given in the obviously incorrect form

$$-\frac{I}{I_o} = e^{-k\sqrt{c-c_o}} \tag{5}$$

where C_o is the "quenching threshold."

The negative sign before I/I_o in equation (5) is obviously a misprint in the original article since a function of the type e^{-x} can have only positive values for all real values of x.

Bouchard[17] proposed the equation

$$\frac{I}{I_o} = e^{-(kc + hs)} \qquad (6)$$

where c is the concentration of the dye and s is the concentration of the quencher. Bouchard states that h varies with various solvents in the same manner as k.

The most useful equation relating fluorescent intensity and quencher concentration is that given by Stern and Volmer[18] for sensitized fluorescence in the gas phase, which was adapted to solution by Vavilov.[19] This equation is based on the number of collisions between activated and quencher molecules. It can be derived by stationary state kinetics, as adapted below from Moelwyn-Hughes.[20]

The rate of formation of electronically excited molecules A* by the absorption of radiation is proportional to the concentration of unexcited molecules n_A and is given as equal to $k_2 n_A$. These excited molecules may lose energy by the spontaneous emission of light at a rate $k_1 s$, where s is the concentration of the excited molecules and k_1 is the decay constant, equal to the reciprocal of their mean life. These excited molecules may also lose energy by collision with normal molecules or with other molecules in the solution. Then

$$A \underset{k_3}{\overset{k_2}{\rightleftharpoons}} A^* \qquad (7)$$

$$A^* + A \xrightarrow{k_4} 2A + \text{heat} \qquad (8)$$

$$A^* + B \xrightarrow{k_2} A + B + \text{heat} \qquad (9)$$

At a constant level of fluorescent intensity

$$\frac{da}{dt} = k_3 m_A - k_1 a - k_2 m_B a - k_4 m_A a = 0 \qquad (10)$$

from which

$$a = k_3 m_A \Big/ k_1 + k_2 m_B + k_4 m_A \qquad (11)$$

The emission rate of fluorescent light is

$$I = k_1 a = k_1 k_3 m_A \Big/ k_1 + k_2 m_B + k_4 m_A \qquad (12)$$

In the absence of the quencher this expression takes the form

$$I_o = k_1 k_3 m_A \Big/ k_1 + k_4 m_A \qquad (13)$$

and

$$\frac{I_o}{I} = 1 + k_2 m_B \Big/ k_1 + k_4 m_A \qquad (14)$$

The quenching constant k_Q is defined as

$$k_Q = \left(\frac{I_o}{I} - 1 \right) \Big/ m_B = k_2 \Big/ k_1 + k_4 m_A \qquad (15)$$

In the absence of self quenching this takes the form (if $k_1 = 1/\gamma$, where γ is the average life of the activated molecule)

$$K_Q = K_2/K_1 = K_2\tau \qquad (16)$$

which is identical with the Stern-Volmer equation.

Frank and Yavilov[21] later modified the Stern-Volmer equation by the addition of an exponential term to allow for static quenching, giving an equation having the form

$$\frac{I_o}{I} = (1 + Km_B)\, e^{K'm_B} \qquad (17)$$

Rollefson and Dodgen[22] have discussed the applicability of these various equations. At low concentrations any of these equations fit the fluorescence intensity data, but they yield distinctly different results at high concentrations. In spite of the failure of these equations at high concentrations of the quencher, they are all applicable, although the most useful equation is the one of Stern and Volmer.

The failure of the Stern-Volmer equation at high concentrations of quencher led Frank and Yavilov[21] to the addition of the exponential term based on the assumption that the failure was due to static quenching. Sveshnikoff[12] concluded, however, that static quenching was unimportant. The Stern-Volmer equation requires that a plot of I_o/I against the concentrations of the quencher should be a straight line with a slope equal to K_Q. Experimentally, there is a tendency for the quenching constant to increase at high concentrations of the quencher. Rollefson and Voss[23] are inclined to attribute the increase to a third

order process, although they do not rule out the possibility of static quenching. These authors find that the data for the quenching of acridone by potassium iodide fit an equation of the type

$$\frac{I_o}{I} = 1 + k_Q[Q] + K'[Q]^2 \qquad (18)$$

This is equivalent to k_Q being linear in the concentration of the quencher. Umberger and La Mer[24] state that the rate increase is due to the reduction of the repulsion between the dye ion and the iodide ion caused by the screening effect of the potassium ion atmospheres about these two negative ions. However, this cannot account for the increase in the constant for the fluorescein-aniline quenching reaction.[25]

Ionic Strength Effects. The Bronsted treatment of the Debye-Hückel theory for reaction rates to account for the primary salt effect postulates the formation of an intermediate complex which may decompose reversibly to regenerate the reactants or which may decompose irreversibly to yield products. This scheme may be written

$$A + B \rightleftharpoons X \longrightarrow C + D \qquad (19)$$

The equilibrium constant for the formation of x is given by the mass action law by

$$K = a_X / a_A a_B = c_X f_X / c_A f_A c_B f_B \qquad (20)$$

where the a's refer to the activities of the components of the system, the c's to their concentrations and the f's to their activity coefficients. The rate of the reaction is proportional to the concentration of x, so that

$$\text{rate} = k' c_x = k' K c_A c_B f_A f_B / f_x = k_0 c_A c_B f_A f_B / f_x \quad (21)$$

Based on the classical method, one can also state

$$\text{rate} = k c_A c_B \quad (22)$$

$$k = k_0 f_A f_B / f_x \quad (23)$$

where k is the observed rate constant and k_0 the rate constant at zero ionic strength.

An important assumption of the Bronsted theory is that if either reactant is an ion, the charge on the intermediate x is equal to the sum of the charges on A and B.

From the Debye-Huckel-Scatchard theory, the activity coefficient of any ion is given by the expression

$$-\ln f_i = z_i^2 A\sqrt{\mu} / (1 + aB\sqrt{\mu}) \quad (24)$$

Where z_i is the charge on the ion. The quantity A is given by the expression

$$A = \frac{\epsilon^3}{DkT}\sqrt{\frac{2\pi N}{1000DkT}} \quad (25)$$

and the quantity B by the expression

$$B = \sqrt{\frac{8\pi N \epsilon^2}{1000 D k T}} \qquad (26)$$

In these two expressions for A and B, all factors are constant except the dielectric constant D and the temperature T, both of which can be, within limits, varied at will. a is commonly referred to as the distance of closest approach of the reactant ions. The expression for the rate constant in terms of the ionic strength of the solution becomes

$$\ln k = \ln k_0 + 2 z_A z_B A \sqrt{\mu} / (1 + a B \sqrt{\mu}) \qquad (27)$$

at low ionic strengths this expression takes the limiting form

$$\ln k = \ln k_0 + 2 z_A z_B A \sqrt{\mu} \qquad (28)$$

The quenching constants obtained when fluorescent ions are quenched by other ions have been shown to obey these equations.[10, 24]

It has also been shown[24] that self quenching may follow the Debye-Hückel law. The fluorescent intensities of solutions of fluorescein ion were measured as a function of ionic strength at 25°C. Extrapolation of the intensity to zero ionic strength gave I_0, the intensity in the absence of self quenching. The data were evaluated by use of the Stern-Volmer equation, in the form

$$\left(\frac{I_o}{I} - 1\right) \Big/ [F1^-] = k_q^s \qquad (29)$$

The fluorescent ion is here acting as its own quencher, and the concentration of the quencher ion is thus the same as that of the fluorescent ion. The self quenching constants so evaluated were found to fit the expanded Debye-Hückel-Bronsted equation

$$\log k_q^s = \log k_{q(\mu=0)}^s + 4.1\sqrt{\mu}/(1+2.4\sqrt{\mu}) \qquad (30)$$

When the natural logarithms are converted to the base 10 in equation (27), and the equation is then evaluated at 25°C in water, the Debye-Hückel constant A has the value 0.509. Therefore, for kinetic measurements at 25°C in water, equation (27) is written

$$\log k = \log k_o + z_A z_B \sqrt{\mu}/(1+aB\sqrt{\mu}) \qquad (31)$$

Although the data of Umberger and La Mer for the self quenching of fluorescein were obtained at 23°C, no serious error is introduced by use of equation (31), since neither A nor B in equation (27) is seriously affected by slight changes in temperature. Thus, increasing the temperature from 0°C to 50°C changes A only from 0.488 to 0.535 and B from 0.325×10^8 to 0.335×10^8 [26]. If self quenching results from an encounter between an excited molecule and an unexcited molecule, the $z_A z_B$ term in equation (31) will be 4.07. The experimentally determined equation (30) thus

offers evidence that self quenching does result from the encounter between an excited and an unexcited dye molecule.

Under the same conditions the quenching of the fluorescence of uranin by iodide ion was found to follow the equation

$$\log k_q = \log k_{q(\mu=0)} + 2.0\sqrt{\mu}/(1+2.4\sqrt{\mu}) \qquad (32)$$

The same considerations thus indicate that iodide ion quenching results from an encounter between the dye ion and the iodide ion.

Umberger and La Mer[24] found that $\log k_{Q(\mu=0)}$ (and hence $k_{q(\mu=0)}$) was complex, depending on the concentration of the fluorescein ion. The quenching constant at $\sqrt{\mu}=0$ was found to increase with increasing dye concentration. This effect was attributed to the reabsorption of the fluorescent light by the solution. Based on the probability of successive emissions and absorptions of the photon of fluorescent light, Umberger and La Mer were able to derive an equation relating $\log k_Q$ to the concentration of the dye. This equation predicted that $\log k_Q$ concentration curves would be linear, and this prediction was confirmed for low concentrations of fluorescein. The "true" quenching constant $k_{q(\mu=0)}^{(c=0)}$ is obtained by extrapolation to zero concentration of the dye.

This effect of the concentration on fluorescent intensity had previously been predicted by Rollefson and Dodgen.[22] These authors derived an equation having the form

$$I = \frac{\omega I_o' k_1 c e^{-k_3 c l}}{(1+k_2 c)} \int_o^l e^{-(k_1-k_3)cs} ds = \frac{\omega I_o' k_1}{(k_1-k_3)} \left[\frac{e^{-k_3 c l} - e^{-k_1 c l}}{1+k_2 c} \right] \quad (33)$$

I is the intensity of light reaching the observer, I_o' is the intensity of light incident on the cell, k_2 is the self quenching constant defined in the Stern-Volmer equation, k_1 is the absorption coefficient of the fluorescent light, k_3 is the absorption coefficient of the fluorescent light averaged over its spectral band. ω is the fraction of the absorbing molecules which would emit light toward the point of observation, if there were no quenching; this can be considered constant. l is the depth of the cell, ds is the element of thickness emitting an increment of light dI at a distance s from the front surface of the cell. c is the concentration of the dye and $e^{-k_3(l-s)c}$ is the fraction of the fluorescent light reaching the observer.

These authors found $\log \frac{I_o}{I}$ to be linear with the concentration of the dye up to 0.015 molar fluorescein and 0.0075 molar acridone. They summarized the work as follows:

> .... In the absence of a quencher, the intensity of the fluorescence observed is given by a function of the concentration which takes into account the following three effects: (1) the absorption of the exciting light by the solution, (2) the absorption of the fluorescent light, and (3) the quenching by the molecules of the fluorescent substance. No other effects need be considered for the substances studied.

The Influence of the Dielectric Constant on Quenching Constants. The Scatchard equation

$$\log k_0 = \log k_0^\circ + \frac{\epsilon^2 z_A z_B}{2.3\,kT\omega}\left(\frac{1}{D^\circ} + \frac{1}{D}\right) \qquad (34)$$

where D° is the dielectric constant in some reference solvent, relating rate constants to the dielectric constant of the medium predicts that in a reaction between ions of like charge that the rate constant at zero ionic strength will increase with increasing dielectric constant of the medium.

Very little previous work has been done on this effect. Stoughton and Rollefson[2] have investigated the quenching of quinine sulfate by silver nitrate, potassium bromide and potassium chloride, in 45 per cent by weight of dioxane ($D=38.48$). The logarithms of the quenching constants, when plotted as functions of $\sqrt{\mu}$ showed limiting slopes which were in agreement with that predicted by the equation used

$$\frac{2}{|\Delta z^2|}\log\frac{k}{k_0} = \frac{3.0\,\Delta z^2\sqrt{\mu}}{|\Delta z^2|} \qquad (35)$$

where Δz^2 is the difference between the square of the charge of the intermediate complex and the sum of the squares of the charges of the reactant molecules.

The change in the limiting slope with decreasing dielectric constant was as predicted by the Scatchard equation for a bimolecular reaction.

The Temperature Coefficient of Quenching Constants and the Effect of Viscosity. The effect of temperature on reaction rates is ordinarily governed by the Arrhenius equation

$$\frac{d \ln k}{dT} = \frac{\Delta E}{RT^2} \qquad (36)$$

where k is the reaction rate constant, T the absolute temperature, R the gas constant and ΔE the energy of activation. It was expected, in view of ionic strength and dielectric effects that the quenching constant would also follow this equation regarding changes in temperature.

Vavilov,[7] investigating the effect of temperature on the quenching of the fluorescence of quinine bisulfate by potassium iodide, found that the temperature coefficient was best expressed by an equation of the type

$$k_q = f(T/\eta) \qquad (37)$$

rather than the Arrhenius equation. (Equation 36)

The relation given by Vavilov for self quenching is

$$\frac{I_0}{I} - 1 = \frac{4\pi \bar{D} \gamma \sigma N c}{1000} \qquad (38)$$

where I_0 is the fluorescence intensity at vanishingly small concentrations c, I is the fluorescence intensity under the conditions investigated, $\bar{D}$ is the diffusion coefficient, N is the number of molecules in 1 grem of the dissolved material, σ is the actual radius of the molecule, and γ is the average life time of the excited molecules. The factor

ϑ , due to simplifications used in the derivation, was undetermined, but had a range of

$$1 < \vartheta < 2.83 \tag{39}$$

For the elapsed time between excitation and the first collision t , Vavilov applied the Smoluchowski equation for the coagulation of colloids.

The elapsed time for the collision becomes

$$\bar{t} = 1/\gamma 4\pi \bar{D} \sigma N_c \tag{40}$$

where γ is a factor not less than 1. Comparing his expression for $\bar{t}$ with that of Smoluchowski and of Leontowitsch,[28] Vavilov evaluated $\gamma = 4/3$ and $\vartheta = 6/\pi$. Applying these equations to foreign material quenching, making the assumptions that

$$\sigma = \frac{\sigma_1 + \sigma_2}{2} \tag{41}$$

and

$$\bar{D} = \frac{\bar{D}_1 + \bar{D}_2}{2} \tag{42}$$

and evaluating the diffusion coefficient

$$\bar{D} = kT/6\pi\eta\sigma \tag{43}$$

where η is the viscosity, Vavilov was able to obtain the equation

$$\frac{I_0}{I} = 1 + \frac{\gamma N_c kT \alpha}{3\eta} \tag{44}$$

where

$$\alpha = 2 + \sigma_1/\sigma_2 + \sigma_2/\sigma_1 \tag{45}$$

In terms of the Stern-Volmer quenching constant,

$$k_q = \left(\frac{I_o}{I} - 1\right)\Big/ c = \frac{\pi k T N \alpha}{3\eta} \qquad (46)$$

In general, α has a value of about 6. Experimentally, Pavilov found I_o/I to be linear in T/η for the quenching of quinine bisulfate with potassium iodide. A further confirmation of this theory is the fact that activation energies for quenching have been reported low, ranging from 4 to 6 kilocalories per mole.[29, 2] This low "activation energy" is of the same order of magnitude as the energy of viscosity, which can be expressed as[30]

$$\eta = Be^{E_v/RT} \qquad (47)$$

Quenching is thus considered controlled solely by the diffusion of the molecules, and is therefore an inverse function of the viscosity. This same conclusion has been drawn by Svedshnikoff.[12, 31, 32, 33] This theory of the dependence of quenching on the viscosity has been further elaborated by Frank and Pavilov's "sphere of action," the volume into which the quenching molecule can enter.[21] The following derivation is that of these authors.

If ω is the radius of the sphere of action, σ_1 and σ_2 the kinetic radii of the colliding molecules, and ω the volume of the sphere of action,

$$\omega = \frac{4\pi(\sigma_1 + \sigma_2)^3}{3}\left[\frac{(\omega + \sigma_2)^3}{(\sigma_1 + \sigma_2)^3} - 1\right] = \frac{4\pi(\sigma_1 + \sigma_2)^3}{3}(z^3 - 1) \qquad (48)$$

The probability that there aren't any quenching molecules in the volume ω at the beginning of the excitation is

$$e^{-\omega N_c}$$

The assumption is made that within the sphere of action the probability of a collision is unity, and is zero outside ω. The probability that no quenching molecule will invade ω during the time t from the beginning of the excitation is $e^{-\omega N_c \frac{t}{T}}$ where T is the average time between the beginning of the excitation and the first collision.

In addition the probability of spontaneous emission between t and $t+dt$ is $e^{-t/\tau_0} dt$. The total probability of spontaneous emission between t and $t+dt$ becomes

$$e^{-\omega_0 N_c} e^{-t\left(1/T + 1/\tau_0\right)} dt$$

The totality of light is thus

$$J = J_0 e^{-\omega N_c} \int_0^\infty e^{-t\left(1/T + 1/\tau_0\right)} dt = J_0 e^{-\omega N_c \tau_0 T/T+\tau_0} \quad (49)$$

In the absence of quencher this becomes

$$J_0 = J_0 \int_0^\infty e^{-t/\tau_0} dt = J_0 \tau_0 \quad (50)$$

Thus

$$J_0/J = e^{\omega N_c \left(1 + \frac{\tau_0}{T}\right)} \quad (51)$$

According to diffusion theory

$$T = 1/8\pi \bar{D} \sigma N_c \quad (52)$$

and here

$$\sigma = \frac{\omega + \sigma_2}{2} \tag{53}$$

Frank and Vavilov give also the diffusion coefficient as

$$\overline{D} = \frac{\overline{D_1} + \overline{D_2}}{2} = \frac{kT}{12\pi\eta}\left(\frac{1}{\sigma_1} + \frac{1}{\sigma_2}\right) \tag{54}$$

Then it follows that

$$\frac{J_0}{J} = e^{\omega N_c}\left[1 + \frac{\gamma_0 N_c kT z (\sigma_1 + \sigma_2)^2}{3\eta\sigma_1\sigma_2}\right] \tag{55}$$

where

$$z = \frac{\sigma_1 + \omega}{\sigma_1 + \sigma_2} \tag{56}$$

for small concentrations of quencher thus becomes

$$\frac{J_0}{J} = 1 + \frac{\gamma_0 N_c kT z (\sigma_1 + \sigma_2)^2}{3\eta\sigma_1\sigma_2} + \omega N_c \tag{57}$$

Thus the quenching constant k_q is a function of T/η as is I_0/I. It is, however, not necessarily a linear function in T/η due to the added term. Frank and Vavilov applied the data of Jette and Best[5] to this theory and found excellent agreement. The curve was not linear, implying that in the system under investigation $\omega \neq 0$.

This effect of diffusion is the principal difference between fluorescence quenching and ordinary bimolecular reactions in solution. The Stern-Volmer equation gives

$$k_q = k_2 \gamma_0 \tag{16}$$

Since τ_0 is the order of 10^{-8} seconds, and k_q usually varies between 1 and 100, the bimolecular reaction rate constant, k_2 is of the order of 10^9. Hoelwyn-Hughes[20] (p. 259) points out that this magnitude is considerably less than the collision frequency afforded by the gas formula and that therefore the deactivation process is either inefficient or that the frequency of collision is slow and due to the process of diffusion.

Umberger and La Mer[24] have pointed out that the Bronsted primary salt effect can be as easily explained by collision controlled processes as by the formation of an activated complex, and thus that the existence of a primary salt effect in quenching constants does not preclude reactions as diffusion controlled. The Svashnikoff and Vavilov application of the Smoluchowski theory to fluorescence quenching included the time dependent effect, but not charge effects. Debye[34] has applied Smoluchowski's theory to include ionic reactions by considering the electrostatic interactions arising from the ions present, but did not include transient state diffusion. Umberger and La Mer[24] have applied both time dependent as well as charge effects.

As indicated, the fundamental assumption from which the Vavilov equation was derived as valid only in a restricted sense. The number of collisions is not necessarily simply proportional to the velocity of diffusion and inversely proportional to the viscosity. In a condensed medium such as a solution, a pair of molecule diffuses through the solution as

a result of thermal motion and finally collide. Due to the cage of surrounding molecules, an individual quenching molecule has an increased probability of repeated collisions with the excited molecule rather than further diffusion to collide with a second excited molecule. This coordination of collisions has been termed an encounter by Fowler.[35]

If the solution is ideal, the product of the number of vibrations per encounter and the encounter frequency is approximately equal to the gaseous collision frequency. If the reaction is activation controlled, this product is the frequency factor. However, since quenching reactions have been assumed to have little or no energy of activation, and every collision has therefore led to reaction, the encounter rate should equal the reaction rate. It is therefore necessary to devise an expression for the encounter rate between ions A^a and B^b in solutions. The following treatment is that of Umberger and Le Roy.[24]

Based on the Smoluchowski equation, an expression for k was obtained. In a solution of bulk concentrations n_a^o and n_b^o of particles A and B per cc, A is held fixed. B then approaches A by a spherically symmetrical thermal motion. At a distance r near the surface of A, the concentration of B is zero due to the reaction, and at large distances it is n_b^o. At intermediate distances from A the concentration of B is n_b, with the exception that at time zero the concentration of B is uniformly n_b^o. The flow of B particles toward A under this concentration gradient is

controlled by the following forces:

(1) Diffusion or thermal force: $-\frac{kT}{n_b}\left(\frac{\partial n_b}{\partial x}\right)_{t,T}$

this is proportional to the concentration gradient.

(2) Friction force: $-\rho_b\left(\frac{dx}{dt}\right)$

this is the viscous drag of the solvent. ρ_b is the coefficient of friction of in the solvent and equals $6\pi\eta a_b$ if Stoke's law holds.

(3) Electrical force: $-\left(\frac{\partial U}{\partial x}\right)_{t,T}$

where is the mutual electrical potential energy between A and B. Neglecting dynamical reaction $(-ma \sim 10^{-22})$

the sum of these forces is

$$-\frac{kT}{n_b}\left(\frac{\partial n_b}{\partial x}\right)_{t,T} - \rho_b\left(\frac{dx}{dt}\right) - \left(\frac{\partial U}{\partial x}\right)_{t,T} = ma = 0 \qquad (51)$$

Flow, the negative of the number of particles flowing per second toward A through an area of one square centimeter at right angles to the direction between and is given by

$$\text{Flux} = n_b\left(\frac{dx}{dt}\right) = -\left[\frac{kT}{\rho_b}\left(\frac{\partial n_b}{\partial x}\right)_{t,T} + \frac{n_b}{\rho_b}\left(\frac{\partial U}{\partial x}\right)_{t,T}\right] \qquad (52)$$

which holds only if the position of A is fixed. Generalizing for the motion of

$$n_b\left(\frac{dx}{dt}\right) = -\left[kT\left(\frac{1}{a} + \frac{1}{\rho_b}\right)\left(\frac{\partial n_b}{\partial x}\right)_{t,T} + \left(\frac{1}{\rho_a} + \frac{1}{\rho_b}\right) n_b\left(\frac{\partial U}{\partial x}\right)_{t,T}\right] \qquad (53)$$

Changing the variables by introducing $a = m_b e^{U/kT}$ where a is the thermodynamic activity at position ω at time t

$(a = m_b$ when $U = 0)$

the flow expression simplifies to

$$m_D \frac{dr}{dt} = -(\bar{D}_a + \bar{D}_b)\, \frac{m_b}{a} \left(\frac{\partial a}{\partial \omega}\right)_{t,T} \tag{61}$$

where $\bar{D}_a = kT/\beta_a$ and $\bar{D}_b = kT/\beta_b$. The divergence of the flow (the source strength of ions B at position ω at time t) equals the negative of the rate of change of B concentration with time.

$$\left(\frac{\partial m_b}{\partial t}\right)_{\omega,T} = \bar{D} \nabla \cdot \left[\frac{m_b}{a}\left(\frac{\partial a}{\partial \omega}\right)_{t,T}\right] \tag{62}$$

which can be simplified to

$$\frac{\partial a}{\partial t} = \bar{D}\left[\frac{\partial^2 a}{\partial \omega^2} + \frac{\partial a}{\partial \omega}\left(\frac{2}{\omega} + \frac{1}{kT}\left(\frac{\partial U}{\partial \omega}\right)\right)\right] \tag{63}$$

This is given as Fick's equation for the spherical interdiffusion of ions with an interaction energy U . The expression $-\frac{1}{kT}\left(\frac{\partial U}{\partial \omega}\right)$ equals L/ω^2 where L is the distance between A and B at which the thermal energy kT equals the coulombic energy of interaction of the net charges $z_a \epsilon$ and $z_b \epsilon$, of A and B. In terms of new independent variables $\chi = r/L$ and $\tau = t/L^2$ equation (63) takes the form

$$\frac{\partial a}{\partial \tau} = \bar{D}\left[\frac{\partial^2 a}{\partial \chi^2} + \frac{\partial a}{\partial \chi}\left(\frac{2}{\chi} + \frac{1}{\chi^2}\right)\right] \tag{64}$$

solution for this general equation has not yet been obtained. Debye solved the special case corresponding to $\frac{\partial a}{\partial t} = 0 = \frac{\partial m_b}{\partial t}$. Smoluchowski and Lattey solved only the special case where $2/x \gg 1/x^2$. Their solution is

$$\omega = m_b^\circ \left[1 - \frac{R}{\omega} + \frac{R}{\omega}\frac{2}{\sqrt{\pi}} \int_0^{\frac{\omega - R}{2\sqrt{Dt}}} e^{-z^2} dz \right] \tag{65}$$

$$m_b = m_b^\circ e^{-U/kT} \left[1 - \frac{R}{\omega} + \frac{R}{\omega}\frac{2}{\sqrt{\pi}} erf\left(\frac{\omega - R}{2\sqrt{Dt}}\right)\right] \tag{66}$$

which is the final approximate form of the dependence on position and time of the average concentration of species around A as A moves about in solution.

The number of encounters between one central body A and all bodies per second is I, the flow toward A through a spherical area of $4\pi R^2$ square cm.

$$I = 4\pi R^2 \bar{D} e^{-U/kT} \frac{\partial a}{\partial \omega}\Big]_{\omega = R} = 4\pi \bar{D} R m_b^\circ \left[1 + \frac{R}{\sqrt{\pi \bar{D} t}}\right] \Big/ e^{U/kT} \tag{67}$$

Smoluchowski and Lattey found that equation (67) was improved by making the substitution

$$f = R \int_R^\infty \frac{e^{U/kT}\, d\omega}{\omega^2} \quad \text{for} \quad e^{U/kT} \tag{68}$$

Since there are also m_a° bodies of A species per cc., the number of encounters per cc. per second is given by the encounter frequency

$$K_D = 4\pi \bar{D} R m_a^\circ m_b^\circ / f \tag{69}$$

For reactions involving little chemical activation energy, every encounter with the proper orientation is effective. This then requires a probability factor P, and if due to some energy of activation, the concentration of B on the surface of A is not zero, but some fraction α of the bulk concentration, then the rate equation must also be corrected by a factor $(1-\alpha)$. Assuming Stoke's law for the calculation of the diffusion constant as equal to

$$\overline{D} = \frac{kT}{2\pi\eta}\left(\frac{1}{u_a} + \frac{1}{u_b}\right) \tag{70}$$

the molar rate constant becomes:

$$k_m = \frac{P(1-\alpha)}{f}\left(\frac{2 + \frac{u_a}{u_b} + \frac{u_b}{u_a}}{4}\right)\frac{8\pi N kT}{3000\,\eta} \tag{71}$$

The Sveshnikoff and Vavilov[33] form of the Stern-Volmer equation can be given in the form,

$$\frac{I_0}{I} - 1 = \beta\tau_0\left[1 + R/\sqrt{\tau_0 D}\right] \tag{72}$$

and the general theoretical equation first given by Umberger and La Mer takes the form

$$k_q = \frac{8\pi N kT\tau_0}{3000\,\eta}\left(\frac{2 + \frac{u_a}{u_b} + \frac{u_b}{u_a}}{4}\right)\left(1 + R/\sqrt{\tau_0 D}\right)\Big/ R\int_R^\infty \frac{e^{v/kT}\,du}{u^2} \tag{73}$$

Thus the quenching constant is the product of the encounter frequency k_D, the average life of the unquenched molecule τ_0, and probability factors.

$$k_q = \tau_0 P(1-\alpha)\,k_D \tag{74}$$

In later papers, La Mer and coworkers,[25, 36] following a suggestion of Kimball's, abandoned the transient state term $(1+R/\sqrt{\pi D})$, pointing out that essentially the same numerical results resulted when the stationary state solution is used as when the "quasi-crystalline" formula is used.

Thus in these later papers by La Mer and coworkers[25, 36] the theoretical molar encounter rate k_D is given by the expression

$$k_D = 4\pi D R N / 1000 f \tag{75}$$

which in terms of the quenching constant becomes

$$k_q = 4\pi D R N \gamma_b / 1000 f \tag{76}$$

This method of approach has made possible the evaluation of activation energies of quenching, which cannot be determined by the more conventional method of Arrhenius due to the diffusion effects. The energy term in a quenching reaction consists of a diffusion energy component and an activation energy component. The separation of these two energy components in the Arrhenius treatment is difficult, if not impossible. A treatment of the probability of either reaction or of diffusion[25] has made possible the separation of activation and diffusion energy. Let w be the probability of a reaction when reactants A and B collide and x be the probability of the encounter being broken by diffusion between collisions of A and B. The total probability of reaction per encounter is expressed by

$p = \omega + (1-\varkappa)(1-\omega)p$ (77) If $w\varkappa \ll \varkappa$ or w $p = \omega/(\varkappa+\omega)$ (78)

The general equation for k, the reaction rate constant in solution (or where reactions occur in sets) is given by

$$k = k_D [\omega/(\varkappa+\omega)] \tag{79}$$

or the reaction rate equals the encounter rate multiplied by the probability of reaction. Compare Equation (74).

The probability of reaction per collision can be expressed by

$$\omega = Pe^{-E_a/RT} \tag{80}$$

where P is the orientation factor, and Ea is chemical energy of activation. The probability of diffusional escape between collisions of A and B can be expressed by a similar equation

$$\varkappa = Ce^{-E_v/RT} \tag{81}$$

where C is mainly an orientation factor and E_v is the energy of activation of diffusion.

Equation (78) can be written in an equivalent form

$$\log(1/p-1) = \log \varkappa - \log \omega \tag{82}$$

and making the proper substitutions of Equations (80) and (81) gives

$$\log(1/p-1) = \log C/P + (E_a - E_v)/2.3RT \tag{83}$$

A plot of log $(1/p-1)$ against $1/T$ has a positive slope if $E_a > E_v$, a negative slope if $E_a < E_v$ and zero slope if $E_a = E_v$. The constant C was shown to be approximately equal to unity, and for nearly spherical molecules, evaluation of c/P will lead to the value of the orientation factor P.

Since the parameter τ_0 occurs in both[37, 24] treatments of viscosity influence, the treatment of quenching data by these techniques is simplified if τ_0 is a constant. The Perrin[15] equation relating degree of polarization to viscosity treats τ_0 as a constant. If τ_0 is a function of viscosity, the Perrin equation cannot be applied to polarization data.[37] (Pringsheim p. 371). The data of Gaviola[33] for the average life time of fluorescein show that τ_0 is independent of viscosity, although Vavilov[19] shows in general an increasing τ_0 with increasing viscosity. Gaviola's data show τ_0 for eosin to vary with viscosity, since in water $\tau_0 = 1.9 \times 10^{-9}$ seconds and in methanol $\tau_0 = 3.4 \times 10^{-9}$ seconds. In this case, τ_0 decreases with increasing viscosity. Unfortunately, however, the data for the average life of fluorescence of eosin in various solvents are scarce. In general, it may be assumed that τ_0 values are functions of the viscosities of the solvents. Pringsheim[37] (p. 373) states

> .... The values of τ_0 cannot be assumed in general to be the same in solutions of different viscosities....

It is possible to arrive at estimates of τ_0 from quenching efficiencies. From the Stern-Volmer equation it can be shown that

$$\frac{\tau}{\tau_0} = \frac{I}{I_0} = Q \qquad (34)$$

Q is the quantum efficiency. $1/\tau_0 = \alpha$ is the probability of a radiating transition and I_0 the fluorescence intensity corresponding to a yield of 100 per cent. This value of I_0 cannot

be found, in general, due to the existence of internal conversion. However, if the transition probability or the intensity of the absorption band remains unchanged, Equation (84) can be replaced by

$$\frac{\tau_1}{\tau_2} = \frac{I_1}{I_2} = Q_{rel} \qquad (85)$$

where τ_1, τ_2, I_1, I_2 are the fluorescence intensities and lifetimes under two different conditions and Q_{rel} is the relative efficiency. For eosin, the Q value in water, ethanol and glycerin is 15, 40 and 60 per cent,[38] while τ_0 in water is 1.9×10^{-9} seconds. Thus values of τ_0 for ethanol and glycerin may be calculated and are 5.1×10^{-9} seconds and 7.8×10^{-9} seconds respectively.

Since the observed viscosities in this study are, in general, of the same order of magnitude as of pure water, the most probable value to use for τ_0 would be 1.9×10^{-9} seconds. This value will be used throughout this paper, although it is realized that in some solutions it may lead to slight error.

In the same manner, a question is raised regarding the diffusion coefficient. If the diffusion coefficient is obtained from the Stokes-Einstein equation

$$\bar{D} = kT/6\pi\eta\omega \qquad (43)$$

the pertinent question is the proper value for the diffusion radius ω. A consideration of the data of Perrenseco[39] for the eosin molecule and of Hodges and La Mer[36] for the uranin ion shows that a highly probable value for the diffusion radius of eosin is 7.0×10^{-8} cm.

EXPERIMENTAL

Materials

Eosin. National Aniline Eosin (disodium tetra bromofluorescein) was recrystallized three times from normal butyl alcohol until free of inorganic chlorides.

Potassium Nitrate and Potassium Iodide. Mallinckrodt C. P. products were used without further purification.

Methyl Alcohol. Methyl alcohol was purified by the method of Bjerrum and Zechmeister.[40] $n_D^{20(obs.)}$ 1.3290; $n_D^{20(lit.)}$ 1.3288.

Ethyl Alcohol. Ethyl alcohol was purified by the method of Fieser[41] $n_D^{20(obs.)}$ 1.3614; $n_D^{20(lit.)}$ 1.3610.

Dioxane. Dioxane was purified by the method of Fieser[42] $n_D^{20(obs.)}$ 1.4221; $n_D^{20(lit.)}$ 1.4221.

Glycerin. 2 liters of Baker C. P. glycerin were treated with calcium carbonate and distilled under vacuum, collecting the middle portion. Boiling point 134° at 1.2 mm. $n_D^{20(obs.)}$ 1.4735; $n_D^{20(lit.)}$ 1.4729.

Acetone. Acetone was purified by the method of Baldwin and Svirbely[43] $n_D^{20(obs.)}$ 1.3601; $n_D^{20(lit.)}$ 1.3591.

Ethylene Glycol. Ethylene glycol was purified by the method of Baldwin and Svirbely[43] $n_D^{20(obs.)}$ 1.4320; $n_D^{20(lit.)}$ 1.4318.

Apparatus. The basic theory and application of the balanced type amplifying circuit used has been given in the literature.[24] An amplifier of the type described by these authors was constructed, but was found to lack stability, and in spite of extensive experimentation and rebuilding with the best components available, was never sufficiently stable for use. A new modification, using electrometer tubes (Victoreen 41B) was constructed and found to be completely stable.

The entire apparatus is shown schematically in Fig. 1. From left to right the components are: K Leeds and Northrup type K potentiometer used to return the grid potential to balance; G, G' control grids of amplifier tubes (Victoreen 41B); P, P' photocells, RCA 926; C, C' glass absorption cells, 75 ml. capacity, 6 x 6 x 2 cm, drilled in the face with a 1 inch diameter hole over which was cemented the isolating filter (Corning 586-738 combination); S ultraviolet light source, General Electric AH4 100 watt mercury vapor lamp, operating off a Raytheon VR-4 voltage regulator; D, D' iris diaphragms; L, L' collimating lens, focal length 2 inches; B potential source, 90 V.D.C., cathode to ground, anode to photocells and amplifier. The leads from the photocell cathodes to the photoload resistors were of coaxial cable. All other external leads (potentiometer, galvanometer) were of shielded wire.

The amplifying and photocell circuit is shown in Fig. 2. This was housed in a grounded all metal box, rendered light tight since the amplifying tubes are photosensitive. A is a 2 volt storage cell; B, B' are 10 megohm resistors. These resistors were matched, although this is not considered necessary;[24] C, C' are stabilizing condensers (0.03 microfarad) required since the ultra-violet source was operated on alternating current, emitting an intermittent beam of a frequency of 60 cycles per second; D, D' are amplifying tubes (Victoreen 41B). The amplifier tubes were connected in parallel and were supplied with current from a 2 volt air cell, adjusted through a wire-wound variable resistor (R) to give a current through each tube of 10 milliamperes. E is a 2 volt Eveready air cell to heat the amplifier filaments. This cell is nominally rated at 2 volts, and is constant under steady drain for long periods of time at low current requirements. F is a 1.5 volt "C" battery, positive to amplifier filaments, negative to negative of potentiometer cell. G, G' are 75000 ohm wire wound resistors. H is a 5000 ohm wire wound potentiometer, and is used to adjust the plates of the amplifying tubes to the same potential. J is a 90 Volt, D. C. source, (2 heavy duty B batteries in series), negative pole to ground and positive pole to photocell anodes and amplifier. I is a Leeds and Northrup type E reflecting galvanometer. This was mounted on a sponge rubber pad to minimize the transmission of mechanical vibrations. P, P' are photocells, (R.C.A. 926.)

FIGURE 1

Schematic Diagram of Apparatus

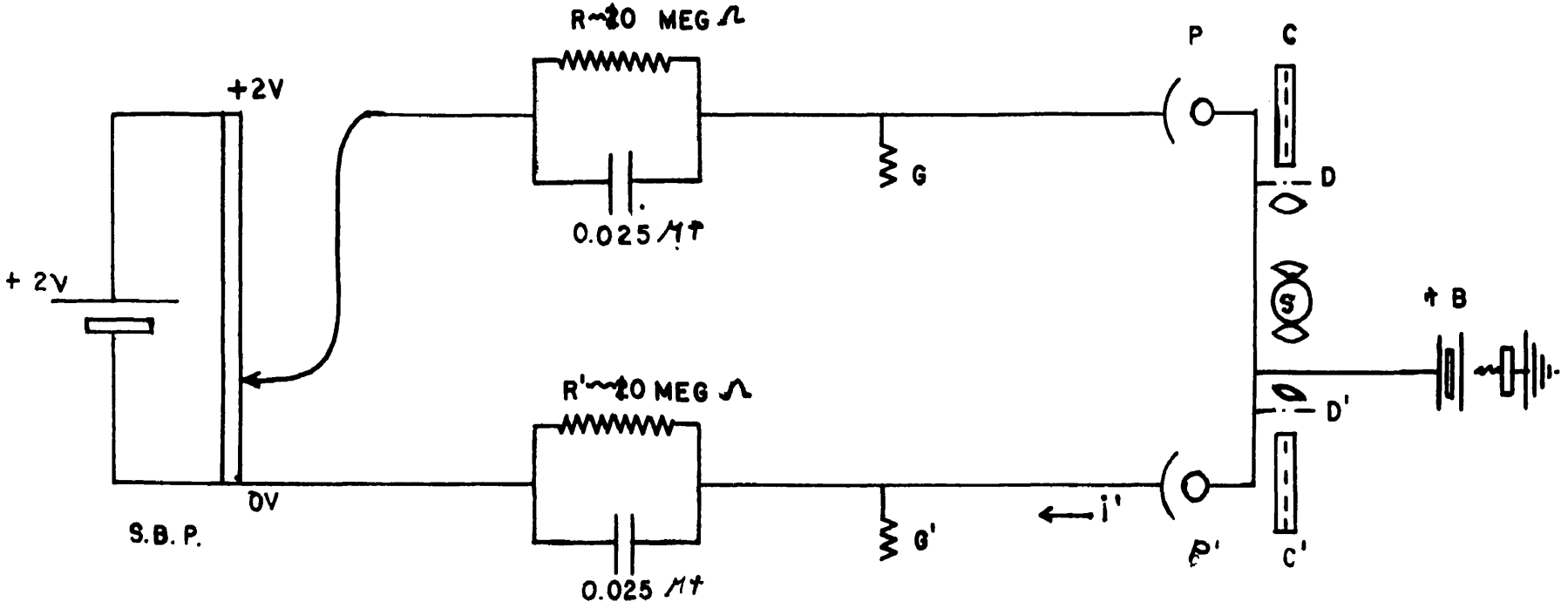
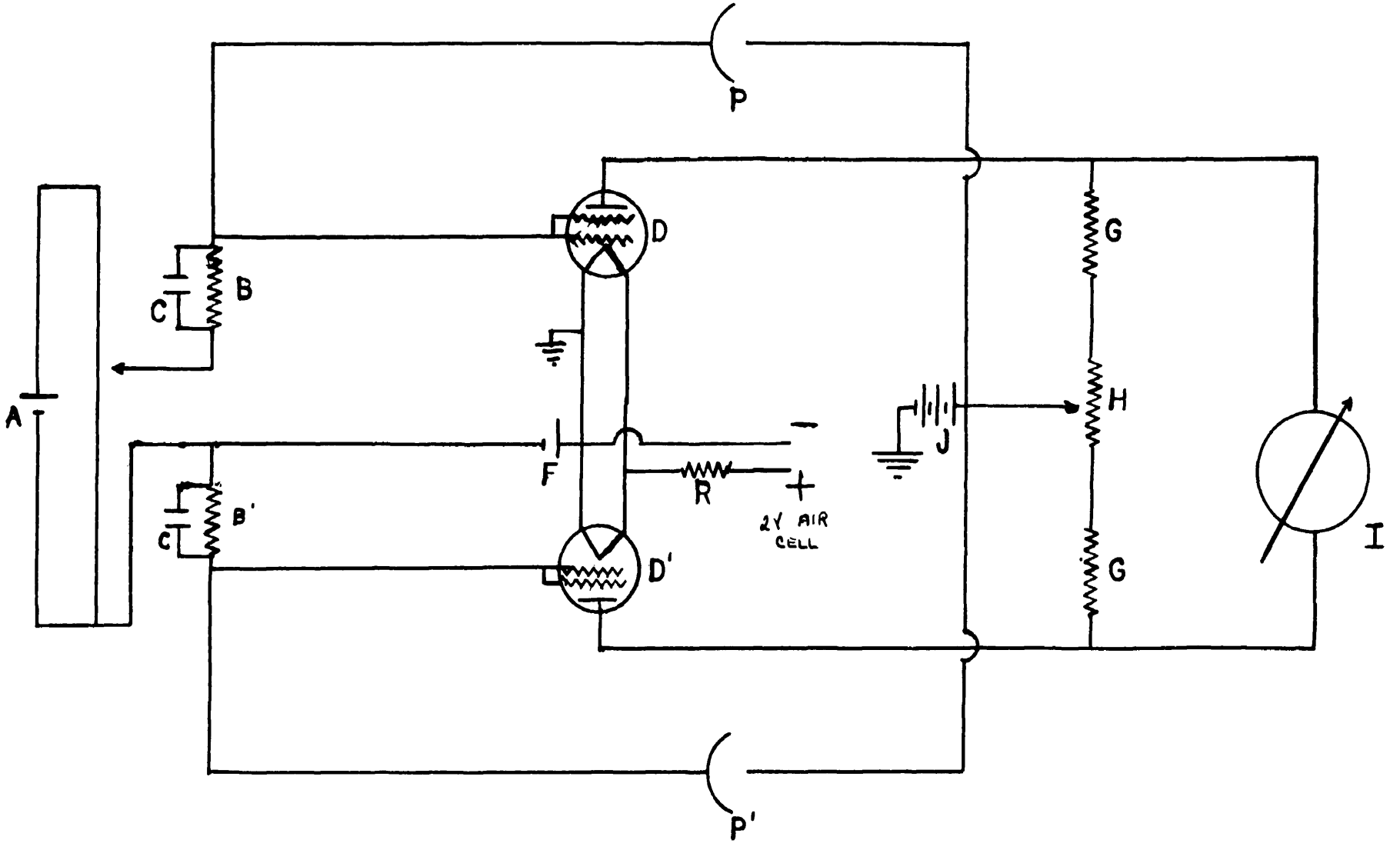


FIGURE 2

Amplifying Circuit



In addition, the amplifying housing was mounted on 3/4 inches of packed cotton backed by 1 1/2 inches of layers of paper to minimize mechanical shock to the filaments of the amplifying tubes.

The optical bench consisted of a thirty inch length of channel iron slotted to accept the ultra-violet light mounting, the photocell-cuvette housings and the lens-diaphragm assemblies. The slot was counter sunk to receive steel bushings fastened to the support rods of the units for greater stability. The support rods were threaded and tightened to the beam with wing nuts. This arrangements gave a high degree of variability in the positions of the units, combined with maximum rigidity of the optical system.

Each cell was housed in the larger chamber of a double unit brass box; the smaller contained the photocell. A hole was drilled in the wall opposite the photocell, and a yellow filter (Corning No. 038) was mounted over the hole to retain any scattered ultra violet light. The cuvette was mounted on a small bracket flush against the filter opening with a 1 1/2 inch tube leading from the ultra violet light source to the isolating filter cemented on the cell. Water at constant temperature could then be circulated around the cuvette. The joints between the filters and the box were sealed with litharge-glycerin cement. Litharge-glycerin cement was finally settled on since tests showed that the great majority of common laboratory cements (glyptal, "Duco" and waterproofing ters) as well as many waterproofing paints were all highly fluorescent.

Water from a constant temperature bath at the required temperature was circulated through these chambers with a small pump. After thermal equilibrium, no difference could be detected in the temperature of the solutions in the cells and the bulk of the water in the constant temperature bath. The thermometers were calibrated against a standard thermometer certified by the Bureau of Standards. For the runs made at 25°C, the temperatures of the systems were constant to ± 0.05 degree. At 35°C and 45°C, due to more rapid heat loss from the bath and lag in the thermoregulator, the temperatures were constant to ± 0.10 and ± 0.20 degrees respectively.

Since the entire instrument was somewhat sensitive to stray electrical effects, all secondary electrical equipment (transformers and the voltage regulator) were shielded with grounded wire cages.

Measurements in this apparatus were taken in the same manner as in that of Umberger and La Mer.[24] (1) With no light on the photocells, the galvanometer was brought to zero with the amplifier rheostat. At this point the plates of the two amplifier tubes are at the same potential. (2) Both absorption cells were filled with identical unquenched solutions of the fluorescent dye and excited. If the two halves of the apparatus are identical, the potentials of the grids increased by the same amount, and there is no deflection of the galvanometer. Any asymmetry in the apparatus (light paths, photocells or amplification) is removed by adjusting the iris diaphragms. (3) The fluorescent solution in the

lower cell is then replaced by an identical solution plus quenching salts. The difference in fluorescent intensity unbalances the system, and the galvanometer is thrown off balance. (4) The potential on the lower control grid is brought back to balance by the potentiometer. This reading, ΔV is proportional to the difference in fluorescent intensity of the two solutions.

$$\Delta V = C(I_o - I_Q] \tag{36}$$

where C is the apparatus constant, I_o is the intensity of the unquenched solution and I_Q is the intensity of the quenched solution. (5) The light is blocked off the lower photocell, throwing the galvanometer off balance again. The galvanometer is again brought to zero with the potentiometer, giving an unquenched reading

$$V_o = CI_o \tag{37}$$

The quantity desired is obtained from the relation

$$\frac{\Delta V}{V_o - \Delta V} = \frac{I_o}{I_Q} - 1 \tag{38}$$

The quenching constant

$$k_Q = \left(\frac{I_o}{I_Q} - 1\right) \Big/ [KI] \tag{15}$$

To check the performance of the apparatus, a run was made using 1×10^{-4} molar sodium fluorescein solution, and the results compared with the data of Umberger and La Mer.[24] The results are shown in Table I.

TABLE I

Quenching Constants of Fluorescein, Compared with Data of Umberger and La Mer.[24]

Concentration of Disodium Fluorescein 10^{-4} Molar

[KI]	$\sqrt{\mu}$	log k_Q This study	log k_Q literature 24
0.100	0.317	1.137 ± .007	1.156
.050	.224	1.078 ± .008	1.083
.025	.160	1.016 ± .004	1.023
.015	.123	0.988 ± .002	0.981
.005	.074	0.923 ± .027	0.926

Preparation of Solutions. To prepare the solutions for the quenching determinations, stock solutions of 10^{-3} molar sodium eosin and 1 molar potassium nitrate and potassium iodide were prepared. Solutions containing potassium iodide were unstable, and prepared fresh for each run. In the aqueous solutions the required volumes of the dye and salts were diluted to volume and the fluorescent measurements were made.

The solutions of the various solvents at the required dielectric were prepared according to the data of Åkerlöf.[44] The dye solutions at definite dielectric constant and ionic strength were prepared by adding to the stock dye and salt solutions sufficient pure solvent to give the required dielectric constant. The whole was then made up to volume with the stock solution of required dielectric constant.

Measurements. Viscosities were determined with an Ostwald viscometer. The viscosities so determined are listed in Table VI and VII, and were in good agreement with those of Hodges and Le Mer[36] and the International Critical Tables.[45] Experimentally, no difference could be detected in the viscosities of solutions containing only organic solvent in aqueous solution when compared with the same solution made up to 0.10 molar with either potassium nitrate of potassium iodide.

Although many fluorescent compounds are quenched by molecular oxygen,[3] eosin is unaffected.[46] Special precautions to eliminate air from the solutions under investigation were therefore considered unnecessary.

The following data are illustrative of a typical run and calculations, together with its check run:

Dielectric constant = 55 (Ethanol) Temperature 25.0°C. Concentration of potassium iodide 0.100 molar in quenched solution; concentration of potassium nitrate 0.100 molar in unquenched solution. Eosin concentration 5×10^{-5} molar.

RUN 1

ΔV	V_o	$V_o - \Delta V$	$\frac{\Delta V}{V_o - \Delta V}$	k_q
131.1	1873.0	1741.9	0.0754	0.75
136.2	1901.0	1764.8	.0772	.77
140.8	1921.5	1780.7	.0791	.79
138.6	1907.7	1769.1	.0784	.78
143.2	1918.6	1773.4	.0819	.82
			Average	.78
			Average deviation	0.02

RUN 2

138.6	1862.3	1723.7	0.0805	0.81
135.5	1872.8	1737.3	.0780	.78
135.9	1871.4	1735.5	.0783	.78
138.9	1881.5	1742.6	.0797	.80
141.9	1891.1	1749.2	.0812	.81
143.1	1891.2	1748.1	.0818	.82
			Average	.80
			Average deviation	.01

Theoretical encounter frequencies were calculated by the use of the following series of equations:

$$k_D = 4\pi \bar{D} R N / 1000 f \qquad (75)$$

f has been evaluated[94]

$$f = R e^{-KL}(e^{L/R}-1)/L \qquad (89)$$

where

$$K = \left(\frac{8\pi N' e'^2}{1000\, DRT}\right)^{1/2} \qquad (90) \text{ and } \qquad L = 2\epsilon^2/DkT \qquad (91)$$

Evaluating constant terms

$$K = 16.08 \times 10^{8}/(DT)^{1/2} \qquad (92) \text{ and } \qquad L = 33.5 \times 10^{4}/DT \qquad (93)$$

The diffusion coefficient $\bar{D}$ has been defined in equation (70) by

$$\bar{D} = \frac{kT}{6\pi\eta}\left(\frac{1}{r_{Co^{2+}}} + \frac{1}{r_{I^-}}\right) \qquad (70)$$

Substituting equations (32) and (71) in equation (75) gives

$$k_D = \frac{3.32 \times 10^{17}\, TL e^{KL}}{\eta(e^{L/R}-1)} \qquad (94)$$

The choice of a value of 7.0×10^{-8} cm. for the diffusion radius of the cobaltammine ion has been discussed previously. The radius of the iodide ion is given as 2.2×10^{-8} cm.[47]

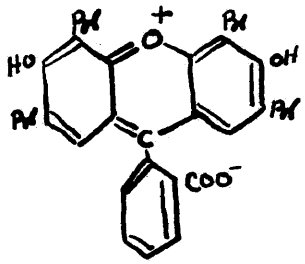
Equation (94) was used to calculate the various theoretical encounter frequencies, and the product of these by the average life of the unquenched cobaltammine ion (1.0×10^{-9} seconds) gives the theoretical quenching constants listed in Tables VI and VII.

The ratio of the observed quenching constant and the theoretical quenching is the probability of an encounter resulting in quenching. These probabilities are listed in Tables VI and VII also. When measured as functions of the temperature, they lead to an expression for the evaluation of the activation energy as discussed previously.

The reciprocal of the probability is, on the average, the number of encounters necessary to bring about one quenching action.

EXPERIMENTAL RESULTS AND DISCUSSION

Eosin, tetrabromofluorescein, has as its most probable structure[48]



The compound forms a disodium salt which has a moderate degree of fluorescence between 5200 and 6000 Å, with a maximum at 5400 Å.[49, 50] Measurements of the fluorescent intensity showed that its fluorescence was, however, only about one-tenth as intense as the equivalent concentration of uranin. This is in agreement with the statement of Pringsheim,[51] that the transition from uranin through eosin, erythrosin (tetraiodofluorescein) to Rose Bengal (tetrachloro-tetraiodofluorescein) causes a diminution of fluorescent power in aqueous solutions. The reason for this lowering of the fluorescent ability with increasing substitution is obscure. Since the halide ions are excellent quenchers for dyes of this type, Lewschin[52] regarded this as internal quenching. This term, however, begs the question, since as Pringsheim points out, the quenching effect is regarded as a chemical reaction involving the excitation energy of the dye and one in which the halide ions are involved. Internal quenching would then require a process wherein the halogens incorporated in the

dye receive the energy from the primarily excited electron, with no explanation as to the ultimate fate of the energy. A more plausible view is that the halogens confer a more strongly acid character to the dye and can thus cause a closer interaction between the dye and the surrounding water molecules, facilitating in some manner the transfer of energy from the dye.

The lower fluorescent intensity of the eosin found in this investigation is in agreement with this view.

The fluorescent intensity of a 10^{-4} molar solution of eosin was found to remain constant when the pH was increased to a maximum of 12.7 with sodium hydroxide, indicating that there was no hydrolysis of the ion to the neutral molecule.

The possibility of the quenching action resulting because of the formation of a non-fluorescent complex between the dye and the quencher was investigated by means of visible absorption spectra measurements. Solutions 2.96×10^{-5} molar eosin were made up to 0.012 molar in either potassium nitrate (a non-quencher) or potassium iodide. Visible absorption spectra of the two solutions were made with a Beckman model DU spectrophotometer over a wavelength range from 400 millimicrons to 590 millimicrons. (Table II), (Fig. 3) Within the limits of experimental error the spectra are identical in regards to both intensity and position of the principal maximum.

TABLE II

Absorption spectra, quenched and unquenched eosin solutions.

Wave length	Optical Densities	
mμ	KNO_3	KI
400	0.030	0.031
410	.032	.033
420	.032	.033
430	.036	.036
440	.043	.043
450	.062	.060
460	.087	.087
470	.139	.137
480	.220	.217
490	.286	.282
495	.320	.315
500	.377	.374
505	.455	.453
510	.560	.561
515	.642	.644
516	.652	.650
518	.659	.660
520	.653	.655
522	.627	.627
524	.589	.588
525	.562	.562
530	.420	.420
535	.276	.276
540	.179	.179
545	.119	.118
550	.079	.078
560	.033	.033
570	.013	.014
580	.007	.007

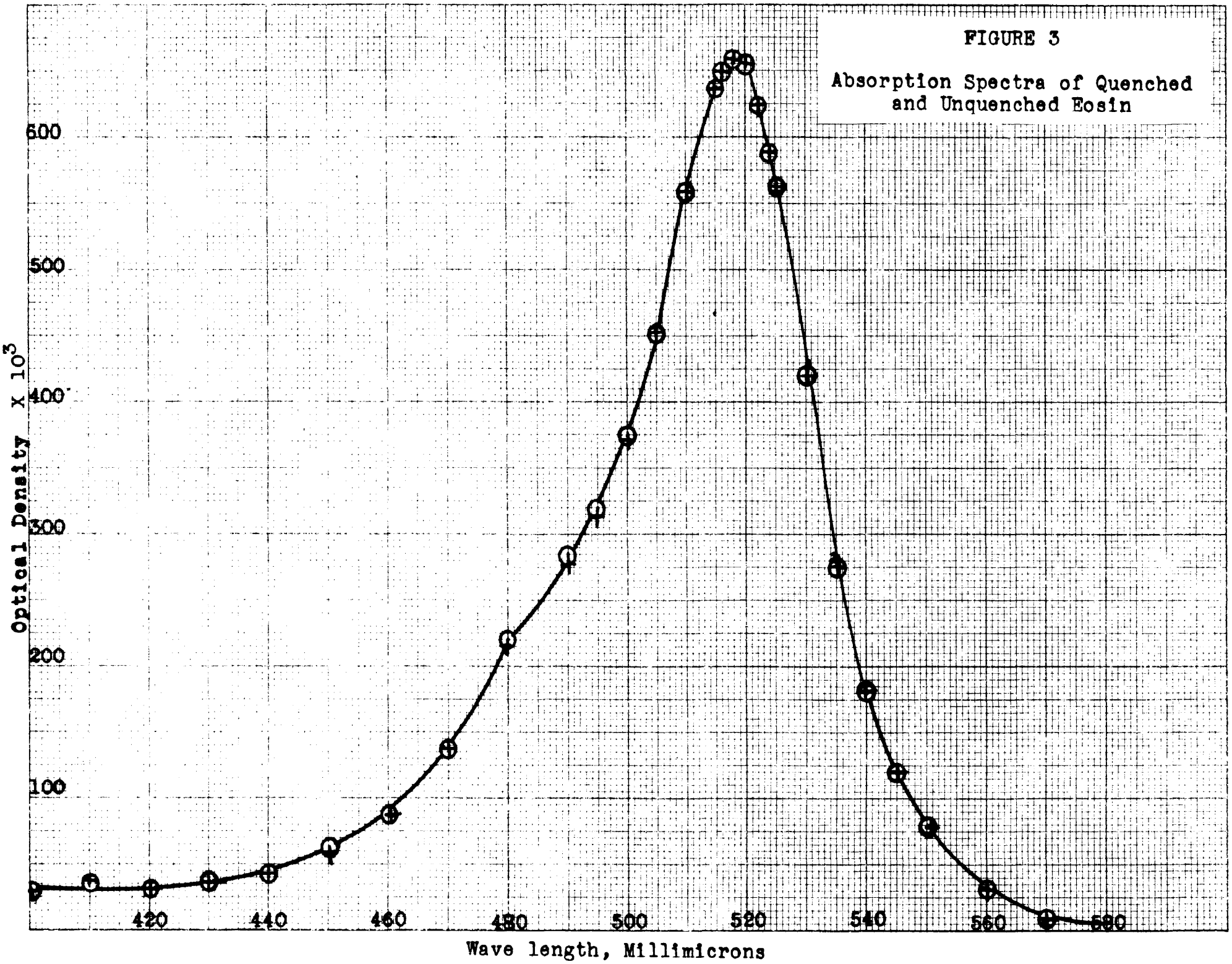


FIGURE 3
Absorption Spectra of Quenched and Unquenched Eosin
Optical Density X 10^3
600
500
400
300
200
100
420
440
460
480
500
520
540
560
580
Wave length, Millimicrons

This criterion of identical spectra has been shown[10] to be a necessary, although not sufficient evidence for the lack of compound formation between dye and quencher. A more sensitive method[11] requires, however, that the fluorescent dye be a neutral molecule.

The Self Quenching of the Fluorescence of the Eosin Ion. Umberger and Le Mer[24] have discussed self quenching in the case of the fluorescein ion. The addition of 0.1 molar potassium nitrate caused a 12 per cent decrease in the fluorescence, but the absorption was unchanged at the wave length of the exciting light (λ = 0.365). The phenomenon was interpreted as a primary salt effect on the frequency of self quenching encounters and not as a salt effect on dimerization. The effect was not due to specific quenching, since it disappeared at low concentrations. It was, therefore, concluded that self quenching resulted from encounters between fluorescein ions. This same effect was investigated in this study for the eosin ion. Due to the low fluorescence intensity of the eosin ion and the small changes in the fluorescent intensity with added potassium nitrate, the results did not lend themselves to a very high degree of precision. The fluorescent intensities were measured as functions of potassium nitrate concentration.

The use of Equation (15) to calculate quenching constants requires the quantity I_o, the intensity of the unquenched solution. In self quenching determinations this quantity is not directly measurable, and must be obtained

by other means. A provisional fluorescent intensity in the absence of self quenching was obtained by the extrapolation of the measured intensity values to zero ionic strength. (Fig. 4) Since the actual shape of the curve in Fig. 4 is unknown at very low ionic strengths, the linear extrapolation gives only the provisional value of I_o. Preliminary calculations were made using the provisional value.

Since this is a reaction between two negative ions, equation (27) should fit the experimental data. Using the provisional value of I_o, self quenching constants were calculated by inserting the fluorescent intensities into the equation

$$\left(\frac{I_o}{I} - 1\right) \Big/ [E_o^-] = k_Q^S \qquad (15)$$

The quenching constants so obtained were found to fit the equation

$$\log k_Q^S = \log k_{Q(\mu=0)}^S + 4.0\sqrt{\mu}/(1+2.5\sqrt{\mu}) \qquad (95)$$

The constant 2.5 was obtained by a trial and error method of substituting various values in Equation (27) until the value 2.5 was obtained which gave constant values of $\log k_Q^S$ ($\mu=0$) at all ionic strengths. Having shown that the data could be fitted by equation (95), improved values of I_o were obtained by combining Equations (15) and (95) in the form

$$\log\left(\frac{I_o}{I} - 1\right) - \log [E_o^-] = \log k_{Q(\mu=0)}^S + 4.0\sqrt{\mu}/(1+2.5\sqrt{\mu}) \qquad (96)$$

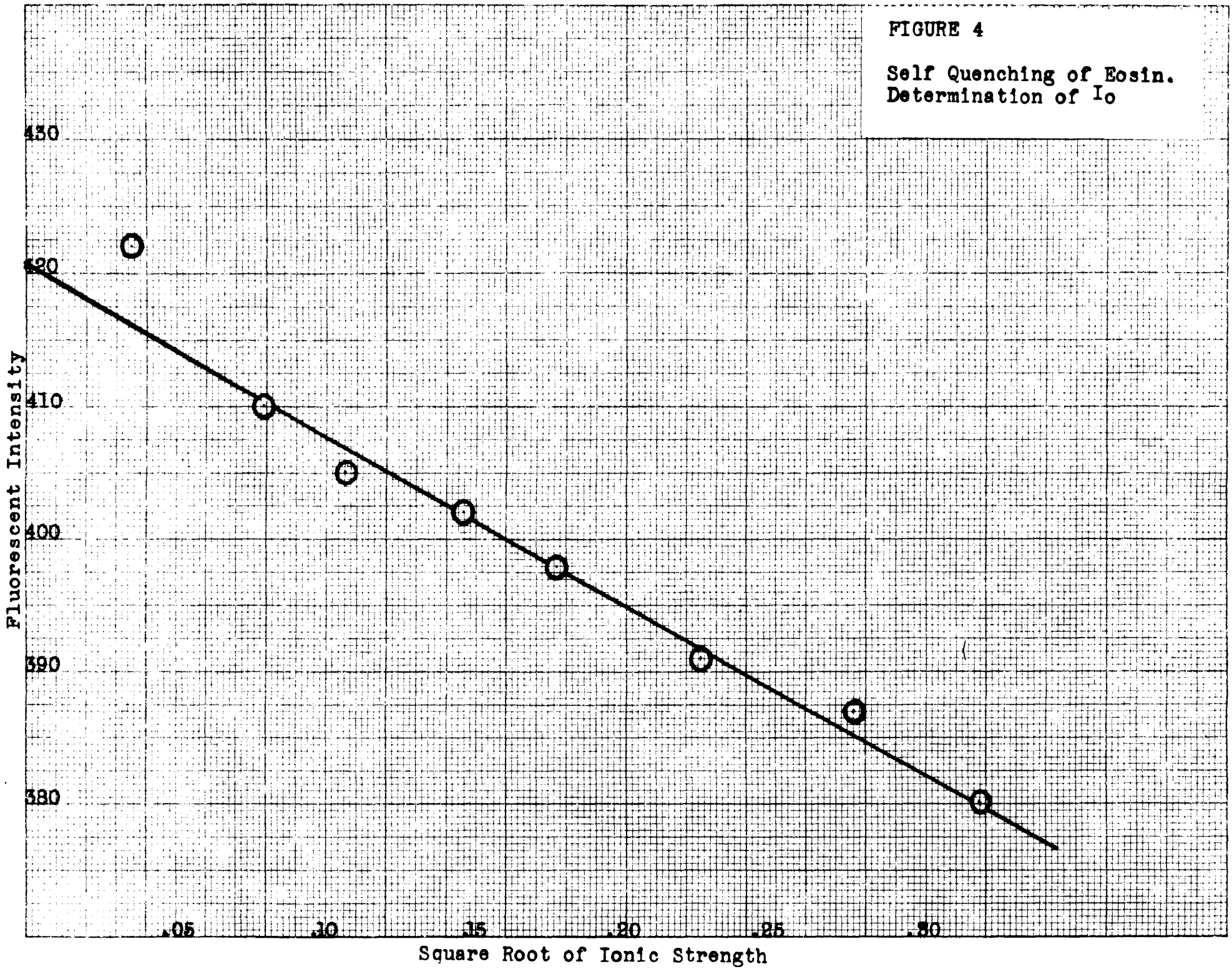


FIGURE 4
Self Quenching of Eosin.
Determination of I_o
430
420
410
400
390
380
Fluorescent Intensity
.05
.10
.15
.20
.25
.30
Square Root of Ionic Strength

Substituting observed values of I and $\sqrt{\mu}$ in Equation (96) and eliminating log $k_{Q(\mu=0)}^{s}$ from any two such Equations (96) gives the improved value of I_o. Experimentally, the I_o values were obtained by using those two values of I and $\sqrt{\mu}$ whose deviations from the curve as illustrated in Fig. 4 were a minimum. In this manner the values of I_o listed in Table III were obtained. Having obtained this improved value of I_o, self quenching constants were recalculated by the use of Equation (15). These final values of k_Q^s were also found to agree with Equation (95) and no change was required in the value of the parameter 2.5. (Table III, Figures 5 and 6.)

Although the data for each concentration of eosin (Table III) are consistent among themselves, they are not consistent regarding log $k_{Q(\mu=0)}^{s}$ when compared as a function of the concentration of the eosin. Umberger and La Mer[24] have shown that for the self quenching of uranin, log k_Q^s is linear in the concentration of the dye, for sufficiently low concentrations of the dye. The authors define the "true" quenching constant as the value of the quenching constant obtained by extrapolation to both zero ionic strength and zero concentration for the dye. The data available here for the self quenching of eosin are insufficient for the accurate extrapolation to zero concentration of eosin. A very rough approximation of the "true" self quenching constant was obtained from the data of Table III. $k_{Q(\mu=0)}^{s(c=0)} \sim 190$. In general, it can only be said that the self quenching of eosin is more efficient than that of uranin. ($k_{Q(\mu=0)}^{s(c=0)} = 8.2$)[24]

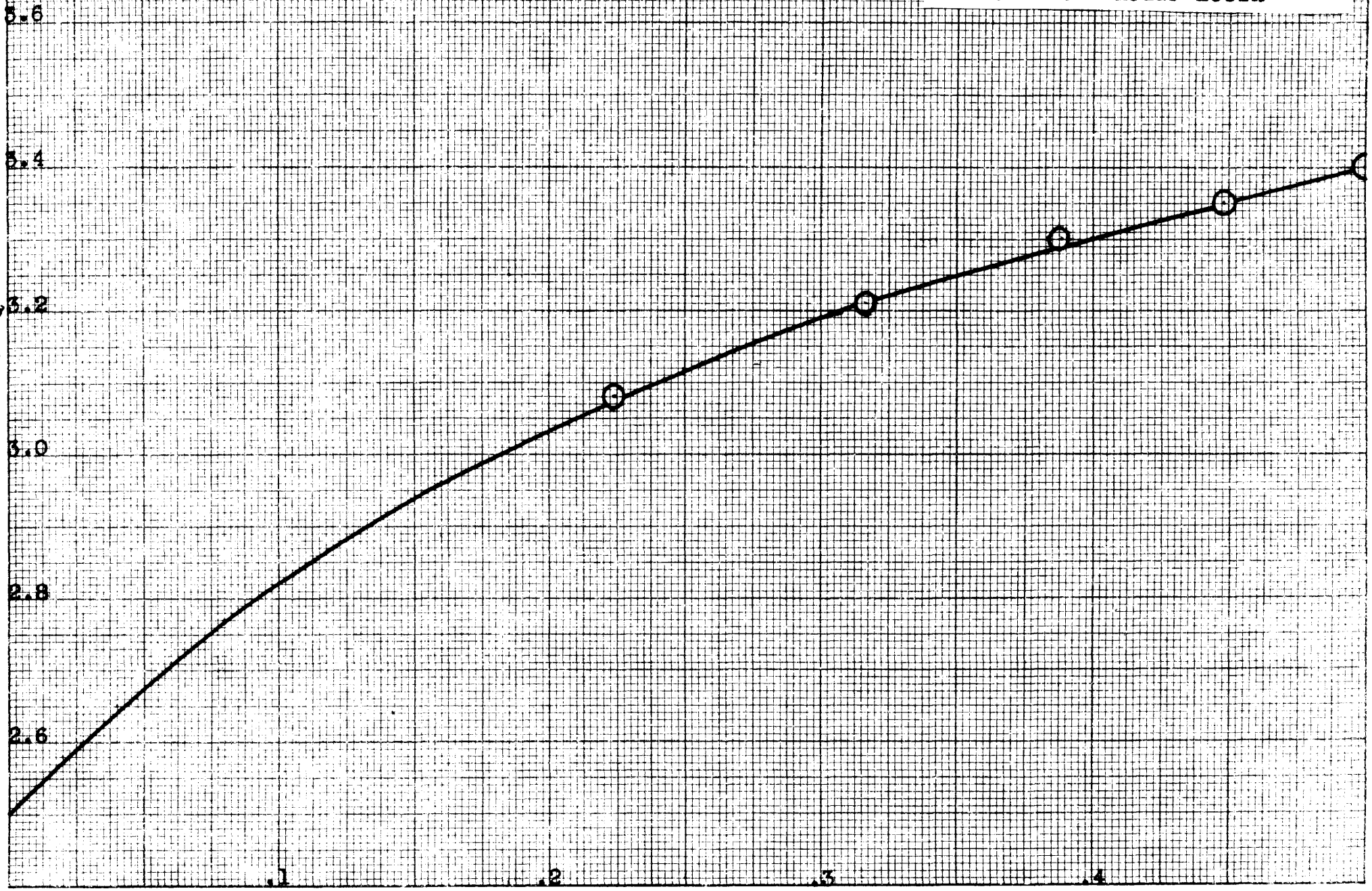
TABLE III

The Self Quenching of Rosin at 25.00° ± 0.05°C

(Rosin) moles/liter	$\sqrt{A}$	I	k_q^s	$\log k_q^s$	$\log k_{q(A=\infty)}^s$	Calculated by Equation (95)	
1×10^{-4}		(I_o=430)					
	0.035	422	190	2.28	-		
	.079	410	480	2.68	2.41		
	.106	405	610	2.78	2.44		
	.146	402	690	2.84	2.41		
	.177	398	800	2.90	2.40		
	.225	391	1000	3.00	2.41		
	.276	387	1110	3.04	2.38		
	.318	380	1310	3.12	2.40	2.41	.01
5×10^{-5}		(I_o=1098)					
	0.142	1077	440	2.64	2.22		
	.224	1035	1200	3.08	2.51		
	.317	1016	1620	3.21	2.51		
	.388	999	2000	3.30	2.51		
	.448	987	2240	3.35	2.49		
	.500	976	2500	3.40	2.49	2.50	.01
2×10^{-5}		(I_o=368)					
	0.224	362	800	2.90	2.32		
	.448	355	1850	3.27	2.42		
	.548	354	1950	3.29	2.36		

FIGURE 5

Salt Effect on Self Quenching
5×10^{-5} Molar Eosin



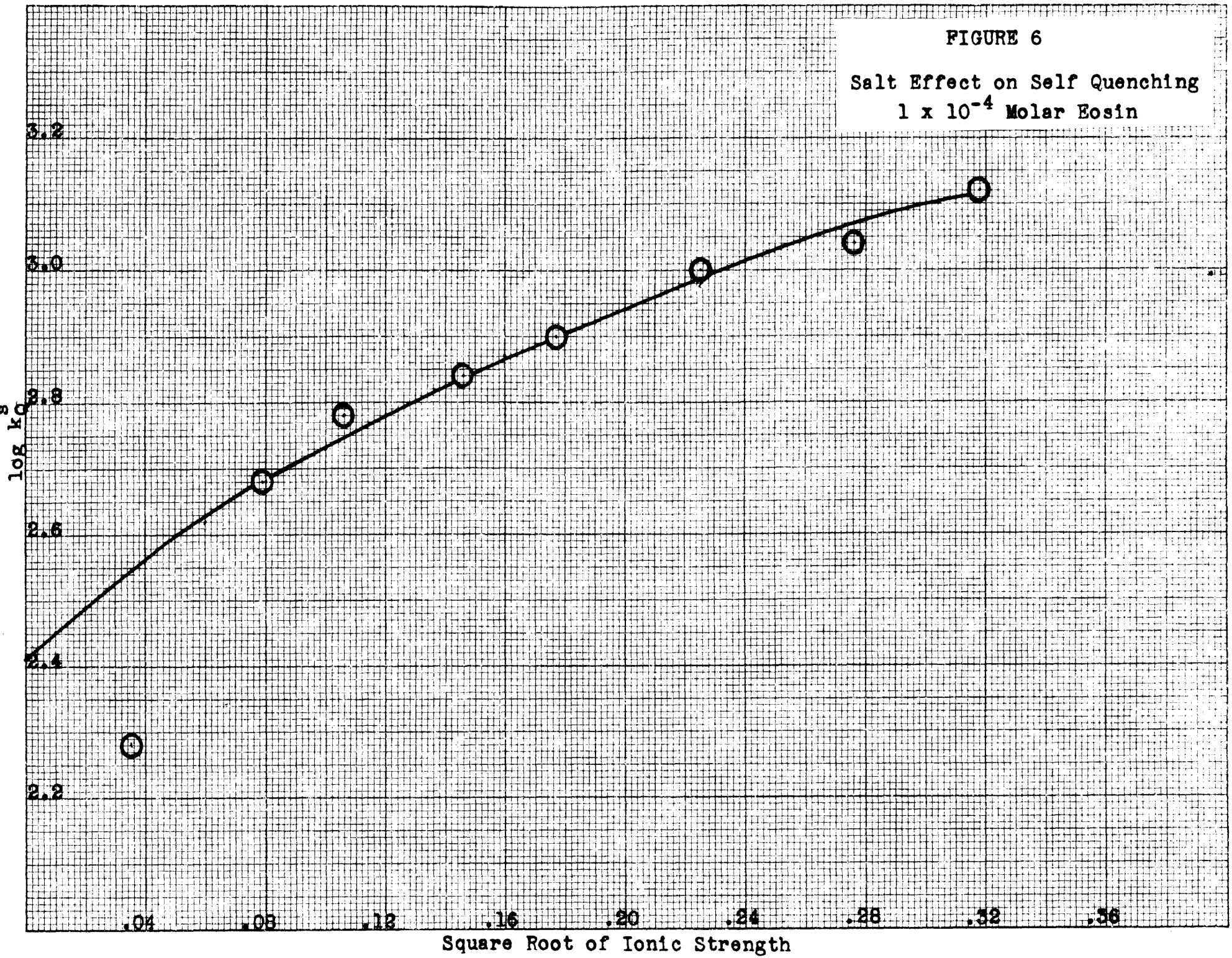


FIGURE 6
Salt Effect on Self Quenching
1 x 10^-4 Molar Eosin
3.2
3.0
2.8
2.6
2.4
2.2
log kQ
.04
.08
.12
.16
.20
.24
.28
.32
.36
Square Root of Ionic Strength

Iodide Ion Quenching of the Fluorescence of Eosin. The quenching of the fluorescence of eosin ion by iodide ion would be expected to follow the Debye-Huckel-Bronsted equation (Equation (27)) as found for other fluorescent ions quenched by foreign ions. This relation was found to hold for eosin ion quenched by iodide. Comparatively little work has been published on the iodide ion quenching constants of eosin. Pringsheim[38] (p. 323) gives a value of 3.7 for k_q. No conditions are given. Weber[13] has determined I/I_o as a function of potassium iodide concentration for 4.54×10^{-6} molar eosin at 15°C. Quenching constants calculated from Weber's data range from a minimum of 3.70 for 0.03 molar potassium iodide to a maximum of 11.0 for 1.203 molar potassium iodide. Weber's ionic strengths varied with the potassium iodide concentration, and the increase in k_q due to increasing quencher concentration is superimposed on the ionic strength effect.

In this investigation the iodide ion quenching of eosin was investigated as a function of eosin concentration, iodide ion concentration and ionic strength at 25°C in aqueous solution. Due to the low value of the quenching constant, and the fact that the quantity ΔV was proportional to the concentration of the iodide ion, solutions of ionic strength lower than 0.03 - 0.05 could not be investigated with the desired degree of accuracy. In establishing the effect of ionic strength, concentrations of potassium iodide greater than 0.10 molar were not used.

The data are given in Table IV and are plotted in Fig. 7 through 12. Since the iodide ion quenching reaction of eosin is one between a divalent and a univalent negative ion, it would be expected to follow Equation (27). In this case the $z_A z_B$ term will be 2. Trial and error calculations showed that in this case the denominator term has the value 2.5. Salt effects on the iodide quenching reaction can therefore be expressed by the equation

$$\log k_q = \log k_{q(\mu=0)} + 2.0\sqrt{\mu}/(1+2.5\sqrt{\mu}) \qquad (97)$$

The Debye-Huckel parameter "a" was calculated from the denominator term in Equation (97) and "a" = 7.6×10^{-8} cm. for all values of the eosin concentration. The same value of the parameter was thus obtained for both self quenching and iodide quenching of eosin. This parameter is thus independent of both the concentration of the eosin, and as far as investigated, of the nature of the quencher.

The log k_Q term, as it does for uranin, decreases as the eosin concentration decreases. (Fig. 12) Due to the low fluorescence intensity of the eosin, quenching constants for concentrations less than 5×10^{-6} molar could not be obtained. From Fig. 12, log $k_{Q(\mu=0)}^{(c=0)} = -0.022$ from which $k_{Q(\mu=0)}^{(c=0)} = 0.95$. This is the "true" quenching constant previously defined, and represents the quenching constant in water at 25°C. corrected for salt effects and the reabsorption of the fluorescent light by the solution.

TABLE IV

Summary of Ionic Strength Data at 25.00 ± .08°C

from Equation (97)

	[KI]	μ	$\sqrt{\mu}$	k_q	$\log k_q$	$\log k_{q(\mu=0)}$	$\overline{\log k_{q(\mu=0)}}$	$k_{q(\mu}$
.5 x 10^{-5}	.10	.100	0.317	2.32	0.366	0.022		
	.10	.200	.443	2.76	.441	.013	.020 ± .002	1.04
.0 x 10^{-5}	.05	.050	.224	2.27	.356	.069		
	.10	.100	.317	2.62	.418	.064		
	.10	.150	.388	2.85	.455	.061		
	.10	.200	.448	3.10	.491	.069		
	.10	.250	.500	3.30	.519	.073		
	.10	.500	.708	3.77	.576	.065	.067 ± .004	1.17
2 x 10^{-5}	.05	.050	.224	2.50	.398	.111		
	.10	.100	.317	2.79	.446	.092		
	.10	.200	.443	3.33	.529	.107		
	.10	.300	.548	3.67	.565	.103		
	.10	.500	.708	4.08	.611	.100	.103 ± .005	1.27
5 x 10^{-5}	.04	.040	.200	2.62	.418	.151		
	.05	.050	.224	2.78	.444	.157		
	.10	.100	.317	3.24	.511	.157		
	.10	.150	.388	3.46	.539	.145		
	.10	.200	.448	3.78	.578	.156		
	.10	.250	.500	3.98	.600	.154		
	.10	.300	.548	4.16	.619	.157		
	.10	.500	.708	4.74	.676	.165	.156 ± .004	1.43
10 x 10^{-5}	.01	.010	.100	2.08	.318	.158		
	.039	.039	.197	2.65	.423	.163		
	.05	.050	.224	2.62	.450	.163		
	.05	.100	.317	3.28	.516	.162		
	.05	.200	.448	3.73	.572	.148		
	.05	.250	.500	4.07	.610	.154		
	.05	.300	.548	4.21	.624	.161	.162 ± .002	1.45
25 x 10^{-5}	.03	.030	.173	2.66	.425	.177		
	.05	.050	.224	3.02	.479	.189		
	.075	.075	.273	3.13	.496	.170		
	.10	.201	.448	3.95	.597	.174		
	.10	.251	.501	4.19	.622	.177	.177 ± .004	
0	0	0	0	0.95	- 0.022	Extrapolated Value		

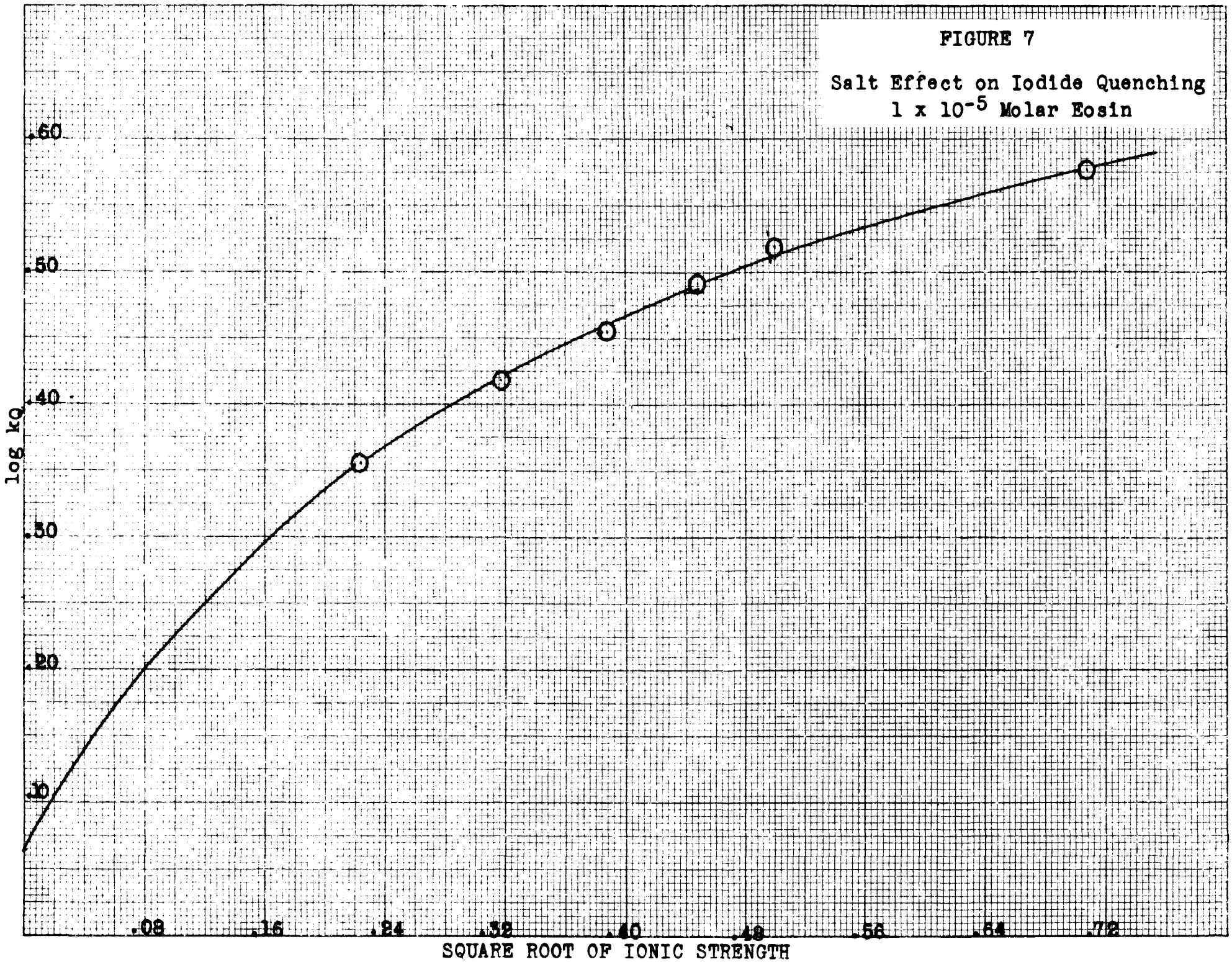
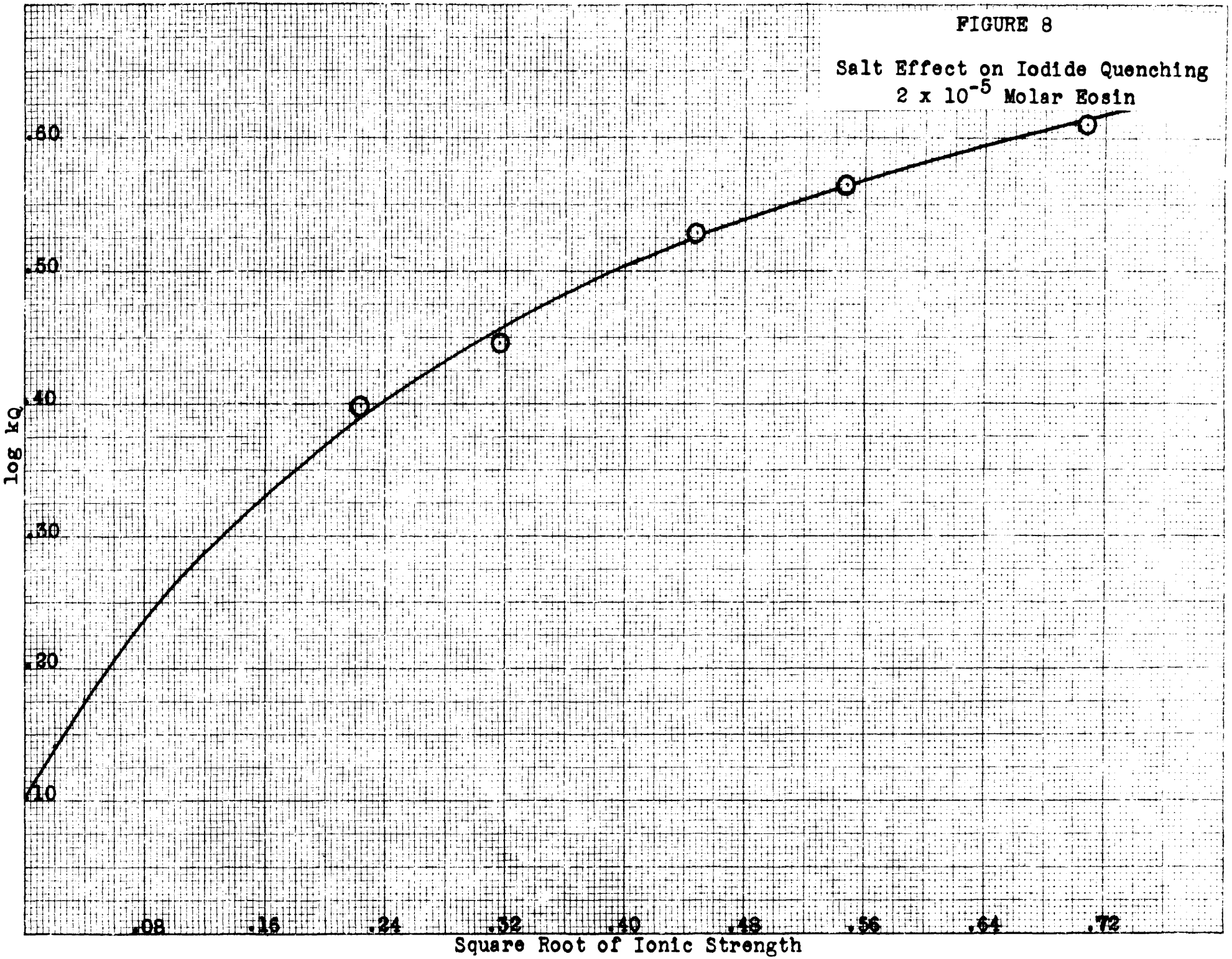


FIGURE 7
Salt Effect on Iodide Quenching
1 x 10-5 Molar Eosin
.60
.50
.40
.30
.20
.10
log kQ
.08
.16
.24
.32
.40
.48
.56
.64
.72
SQUARE ROOT OF IONIC STRENGTH

FIGURE 8

Salt Effect on Iodide Quenching
2×10^{-5} Molar Eosin



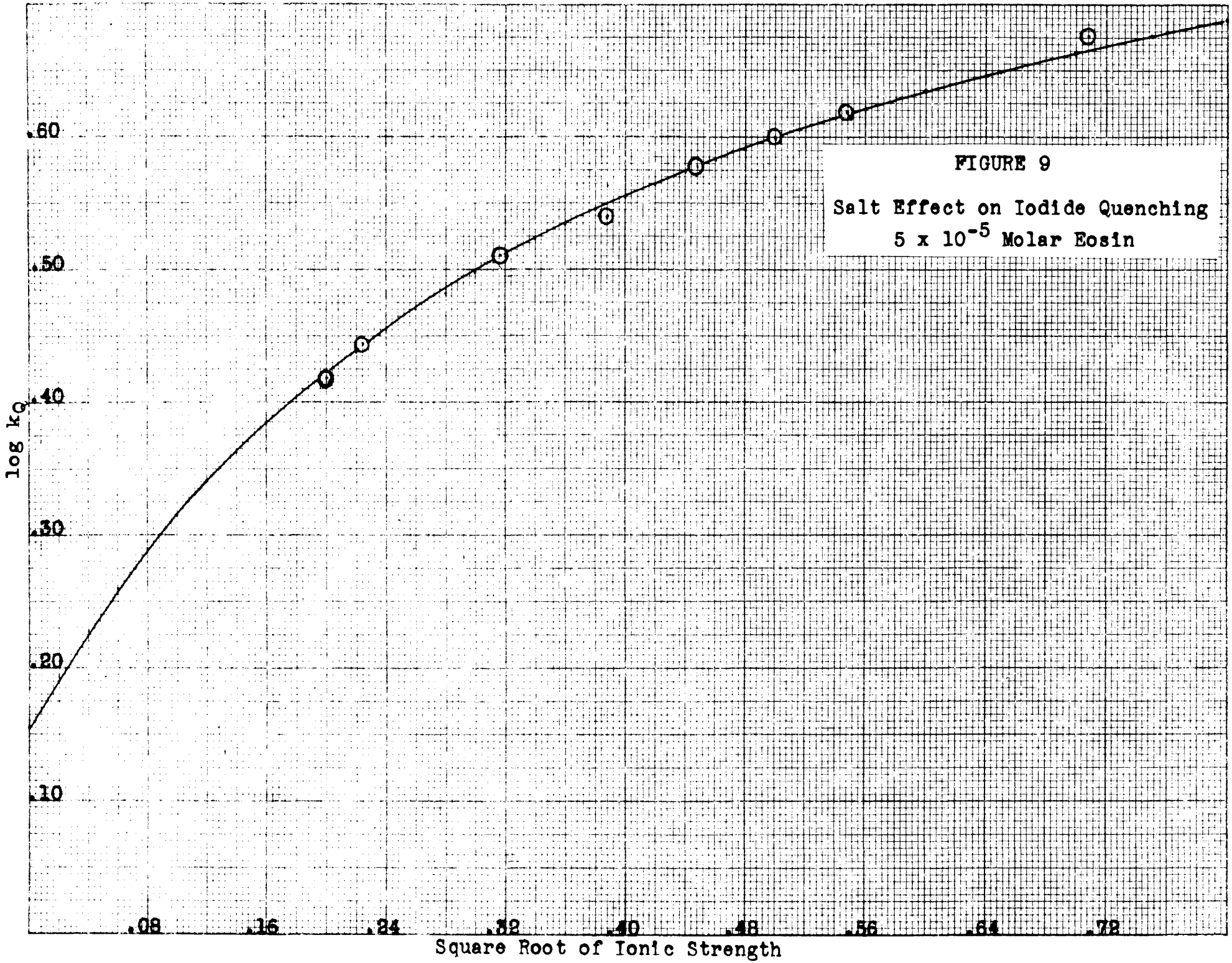


FIGURE 9

Salt Effect on Iodide Quenching

5×10^{-5} Molar Eosin

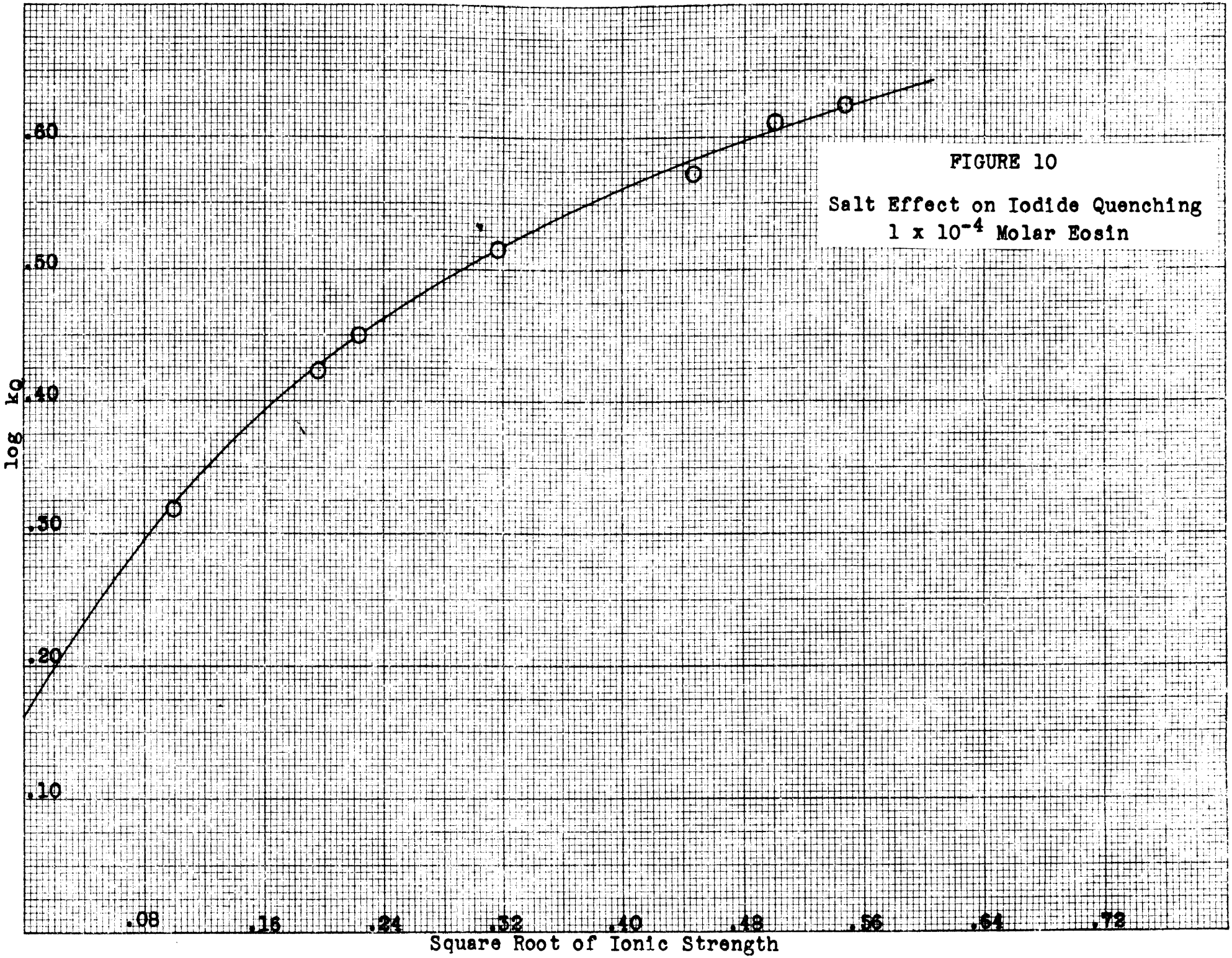


FIGURE 10

Salt Effect on Iodide Quenching
1×10^{-4} Molar Eosin

FIGURE 11

Salt Effect on Iodide Quenching
2.5×10^{-4} Molar Eosin

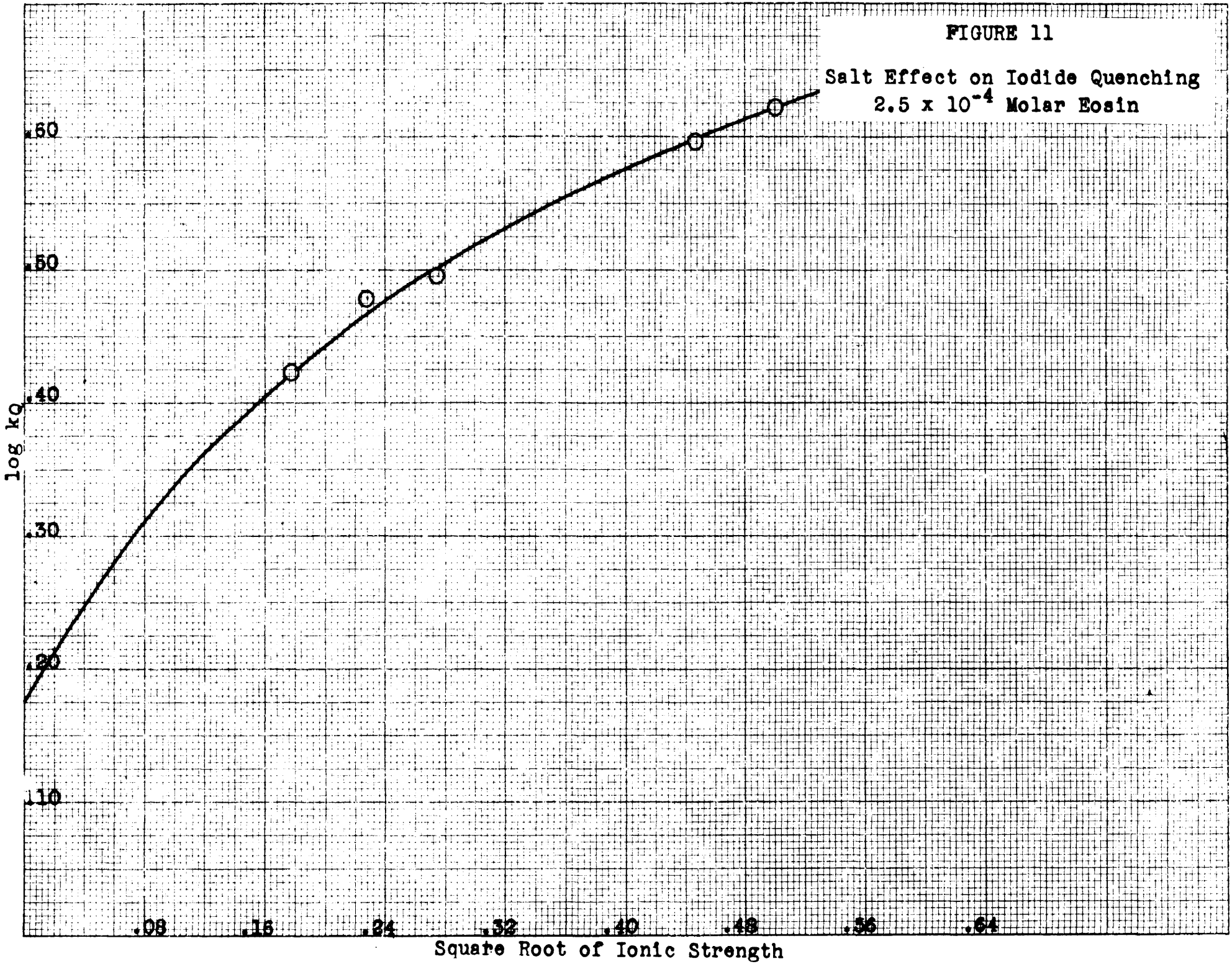
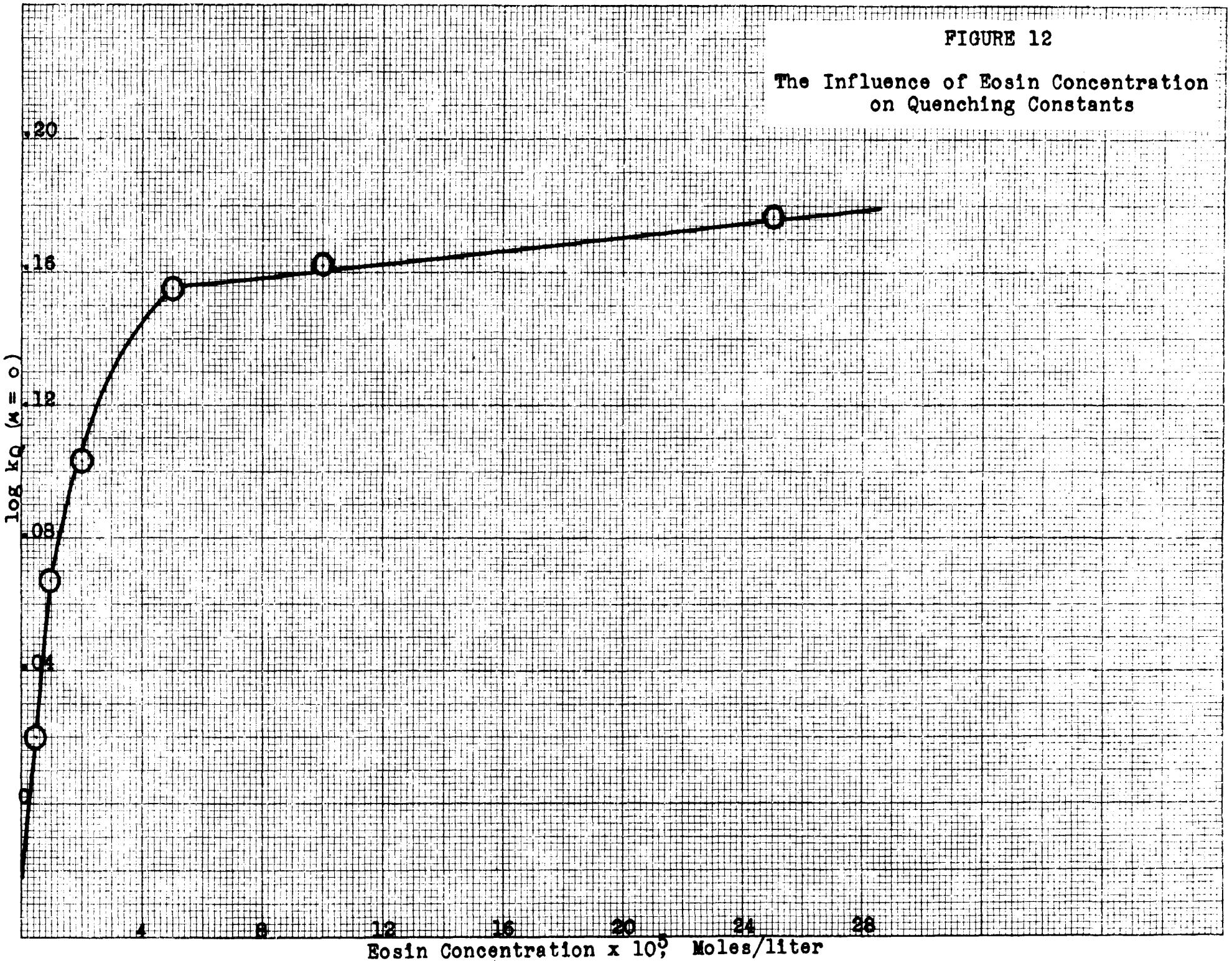


FIGURE 12

The Influence of Eosin Concentration on Quenching Constants



An independent check of $k_Q^{(c=0)}{}_{(\mu=0)}$ should be possible using Perrin's equation[15]

$$\frac{1}{p} = \left(\frac{1}{p_0}\right)\left[1 + \left(1 - \frac{p_0}{3}\right)\left(\frac{RT\tau}{\eta V}\right)\right] \qquad (98)$$

where p is the degree of polarization, p_o is the limiting polarization, V is the volume occupied by one mole of the fluorescent material and τ is the average life. In the form used by Weber[13]

$$\frac{\tau_0}{\tau} = \frac{1/p_i - 1/p_0}{1/p - 1/p_0} \qquad (99)$$

p_i is the polarization of the unquenched solution and p is the polarization of the quenched solution. From the Stern-Volmer Equation (18) the following equation is obtained

$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + \tau_0 k[I^-] \qquad (100)$$

Using equation (99) and (100) it is apparent that quenching constants can thus be obtained from polarization measurements. The data of Weber, however, lead to inconsistent results, even in his own measurements. Therefore, no attempt is made here to apply this independent method of calculation.

Comparison of the Theoretical and Measured Quenching Constants for the Iodide Quenching of Eosin. The application of Equation (94) to the iodide ion quenching of eosin in water at 25°C. yields $k_Q(\mu=0)$(theoretical) = 8:00 as compared to the observed value of 0.95. As pointed out previously,[24] discrepancies in these values can be due to any number of causes. Among these are chemical energy of activation,

inaccuracies in the estimation of the values used for ionic radii, non-equality of diffusion and quenching radii and orientation effects. Orientation effects would be expected to play a larger role in the quenching of eosin than in the quenching of uranin due to the distribution of the bromine atoms around the eosin molecule. The relative magnitudes of the observed and theoretical quenching constants show that a quenching action occurs in approximately one out of every eight encounters.

The Influence of the Potassium Iodide Concentration on the Quenching Constant. The failure of the Stern-Volmer equation at high concentrations of quencher has been ascribed to static quenching[12, 14] and also whenever possible to ionic screening effects.[24] Static quenching of eosin by potassium iodide is very small, however, and the evidence for it is not conclusive.[13] In the case of the eosin-iodide reaction, static quenching can not be more than 1% of the total quenching.

Previously, where effects of quencher concentrations in ionic systems have been investigated, no control has been maintained over the ionic strength, and this effect has been superimposed on the concentration effect. To study the effect of quencher concentration, ionic strengths must therefore be kept constant.

In investigating the effect of potassium iodide concentration on the quenching constant, varying concentrations of potassium iodide were employed in isoionic strength solutions of 0.50 and 0.25 at 25°C. (Table V)

TABLE V

The Effect of Iodide Ion Concentration on the Quenching Constant.

(Eosin) = 1×10^{-4} molar T = 25.00 ± 0.05°C.

	[KI] moles/liter	k_Q
1 μ = 0.25	0.020	3.84
	.050	4.00
	.100	4.07
	.150	4.05
	.200	4.11
	.250	4.12
2 μ = 0.50	0.050	4.38
	.200	4.64
	.300	4.83
	.400	5.02

Values of k_q are linear in (I^-) (Fig. 13) at both ionic strengths employed. The data may be expressed analytically as

$$k_q = 0.80\,[I^-] + 3.96; \quad \mu = 0.25 \qquad (101)$$

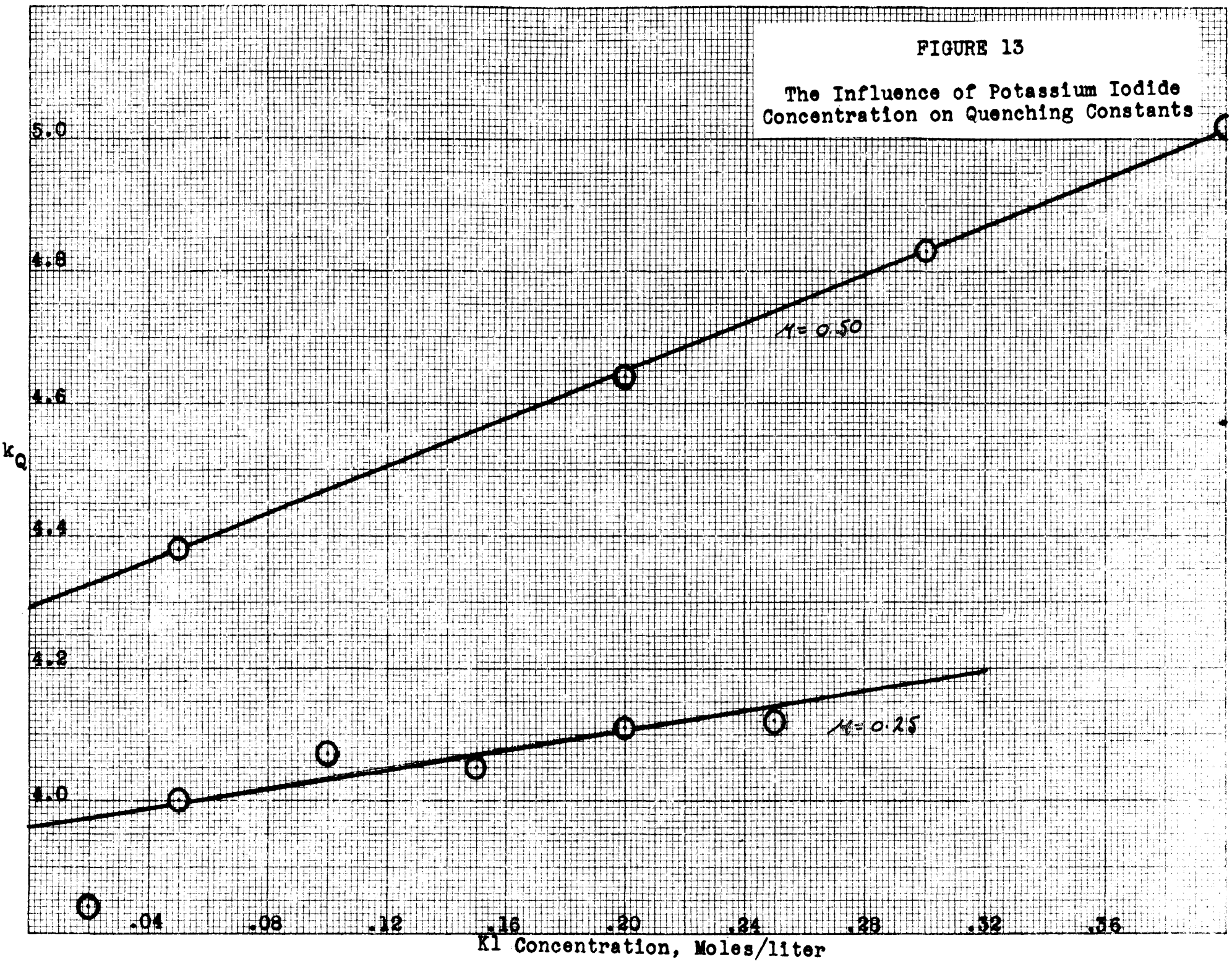
$$k_q = 1.83\,[I^-] + 4.29; \quad \mu = 0.50 \qquad (102)$$

These equations are in agreement with that predicted by Rollefson and Rose[23] requiring a linear relationship between k_q and (I^-). The influence of ionic strength on the rate at which iodide concentration influences the quenching constant is marked. A 100 per cent increase in ionic strength causes a 129 per cent increase in the slope of the curve. The increase in rate with increasing ionic strength can be due to the reduction of repulsion between dye and quencher ion caused by the screening effect of the additional potassium ion atmosphere about the negative ions.

The Influence of Viscosity on Quenching as a Function of Dielectric Constant. Quenching constants were obtained in solutions of dielectric constant 55, 65 and 75 for 5×10^{-5} molar eosin, 0.10 molar potassium iodide at ionic strength of 0.1 at 25°C. The ideal treatment of the data would be to compare $k_q^{(\epsilon=0)}$ over a range of ionic strength for the various dielectric constants and viscosities. Practically in the case of the eosin-iodide quenching reaction, this is impossible. The increasingly low values

FIGURE 13

The Influence of Potassium Iodide Concentration on Quenching Constants



of the quenching constants observed as the viscosity was increased made such extrapolations unreliable, and all data involving viscosity, temperature and dielectric constant effects are given for the concentrations of the reactants specified above. A severe limitation is placed upon this method, in that, having set the dielectric constant at a specified value, no control can be maintained over the viscosity. There is thus unfortunately no overlap in viscosities between dielectric 75 and dielectrics 65 and 55. The data are tabulated in Table VI. An investigation of the data shows that they may be divided into two categories. A "normal" category involving those solutions containing sucrose, glycerin or ethylene glycol and an additional category containing those systems whose dielectric constants were adjusted with ethanol, methanol, acetone or dioxane. This specific solvent effect becomes most evident in the cases of ethanol and methanol at dielectric constant 55, and is so marked that the general shape of the curves obtained plotting k_q against $1/\eta$ is distorted. (Fig. 14)

At dielectric constants 65 and 75 ethanol and methanol appear to belong to the regular series.

Specific solvent effects for methanol have been previously recognized.[36] The viscosities of aqueous solutions of methanol, ethanol and acetone show maxima when considered as functions of concentration, and the essential nature of the systems depends on the occurrence of water-rich or solvent-rich mixtures with identical macroscopic viscosities. At dielectrics 75 and 65

TABLE VI

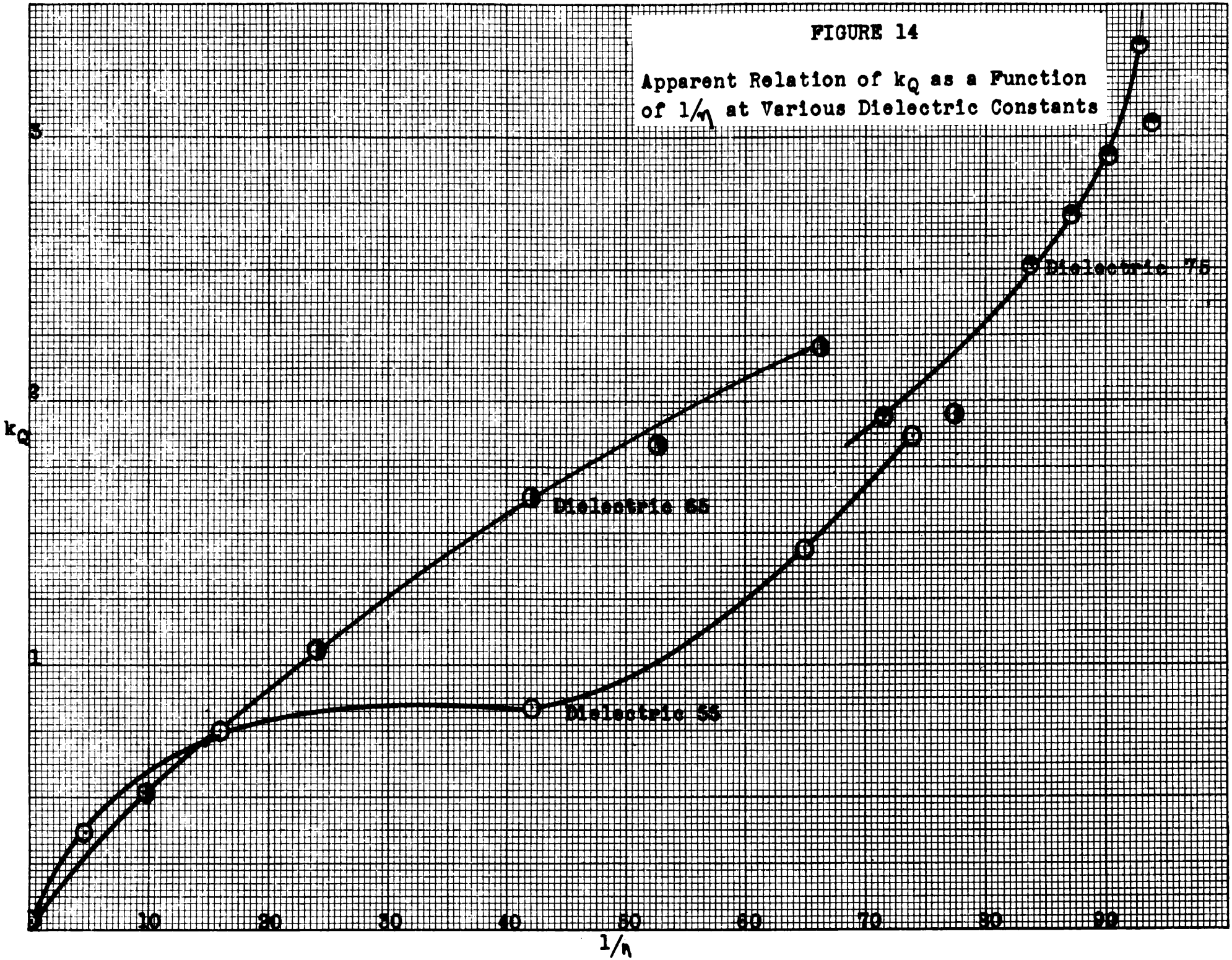
The Influence of Viscosity and Dielectric Constant on Quenching Constants at 25°C.

(Eosin) = 5 x 10^{-5} molar; (KI) = 0.10 molar; μ = 0.10

Solvent	Dielectric constant	η poise	$1/\eta$	k_q	k_q ther.	ρ	$k_q(\mu=0)$ calc.
Sucrose	55	10	0.10	0.03	0.06	0.50	0.0084
Glycerin	55	0.2121	4.71	.37	2.88	.129	.10
Glycol	55	.0625	16.0	.75	9.77	.087	.21
Acetone	55	0.0135	74.0	1.87	45.2	.041	.52
Methanol	55	.0154	65.0	1.43	39.7	.036	.40
Ethanol	55	.0237	42.1	0.79	25.8	.031	.25
Sucrose	65	.1008	9.9	0.52	5.24	0.099	.18
Glycerin	65	.0414	24.2	1.06	12.7	.084	.38
Glycol	65	.0237	42.2	1.63	22.2	.073	.58
Acetone	65	.0129	77.5	1.95	40.8	.048	.69
Methanol	65	.0151	66.3	2.21	34.9	.063	.78
Ethanol	65	.0189	52.8	1.83	27.8	.066	.65
Sucrose	75	0.0140	71.6	1.93	14.2	.136	.31
Glycerin	75	.0119	83.9	2.52	16.7	.151	1.06
Glycol	75	.0115	87.2	2.72	17.3	.157	1.15
Acetone	75	.0107	93.3	3.08	18.6	.165	1.29
Methanol	75	.0108	92.7	3.35	19.4	.182	1.41
Ethanol	75	.0111	90.4	2.93	18.0	.163	1.25
Dioxane	55	-	-	(53.7)			
"	65	-	-	(54)			
"	75	.00951	105.1	(99.6)			

FIGURE 14

Apparent Relation of k_Q as a Function of $1/\eta$ at Various Dielectric Constants



where the k_q of ethanol and methanol solutions are in relative agreement with the other solvents, the viscosities are on the water-rich portion of the viscosity composition curve. At dielectric 55, where the deviation is most marked, the viscosities are those occurring at about the maxima or on the alcohol-rich portion of the curve.

The viscosities of aqueous solutions of sucrose, glycerin and ethylene glycol show no maxima and increase regularly with increasing solvent concentration. Acetone[37] has been stated to lower the fluorescence of uranin.

Dioxane solutions gave totally unexpected behavior. Whereas the fluorescent intensities of unquenched aqueous dioxane solutions were constant under continuous illumination, the intensities of quenched dioxane systems were of a lower order of magnitude than quenched solutions using other auxiliary solvents, and increased steadily with time. (Table VIII, Fig. 23.) Consequently successive determinations of quenching constants on the same solution gave progressively lower values. Determination of quenching constants by extrapolation to zero time gave values 10 to 30 times as high as those determined in other solvents.

Discussion of Dielectric Effects. The theoretical quenching constants calculated from Equation (84) for 25°C and at $\mu = 0.10$, when plotted as functions of the solvent fluidity, (Fig. 16) show three distinct straight lines with a common intersection at the origin. The slopes of these lines increase with decreasing dielectric constant in

FIGURE 15

The Effects of Dielectric Constants and Viscosity on Quenching Constants "Normal" Series

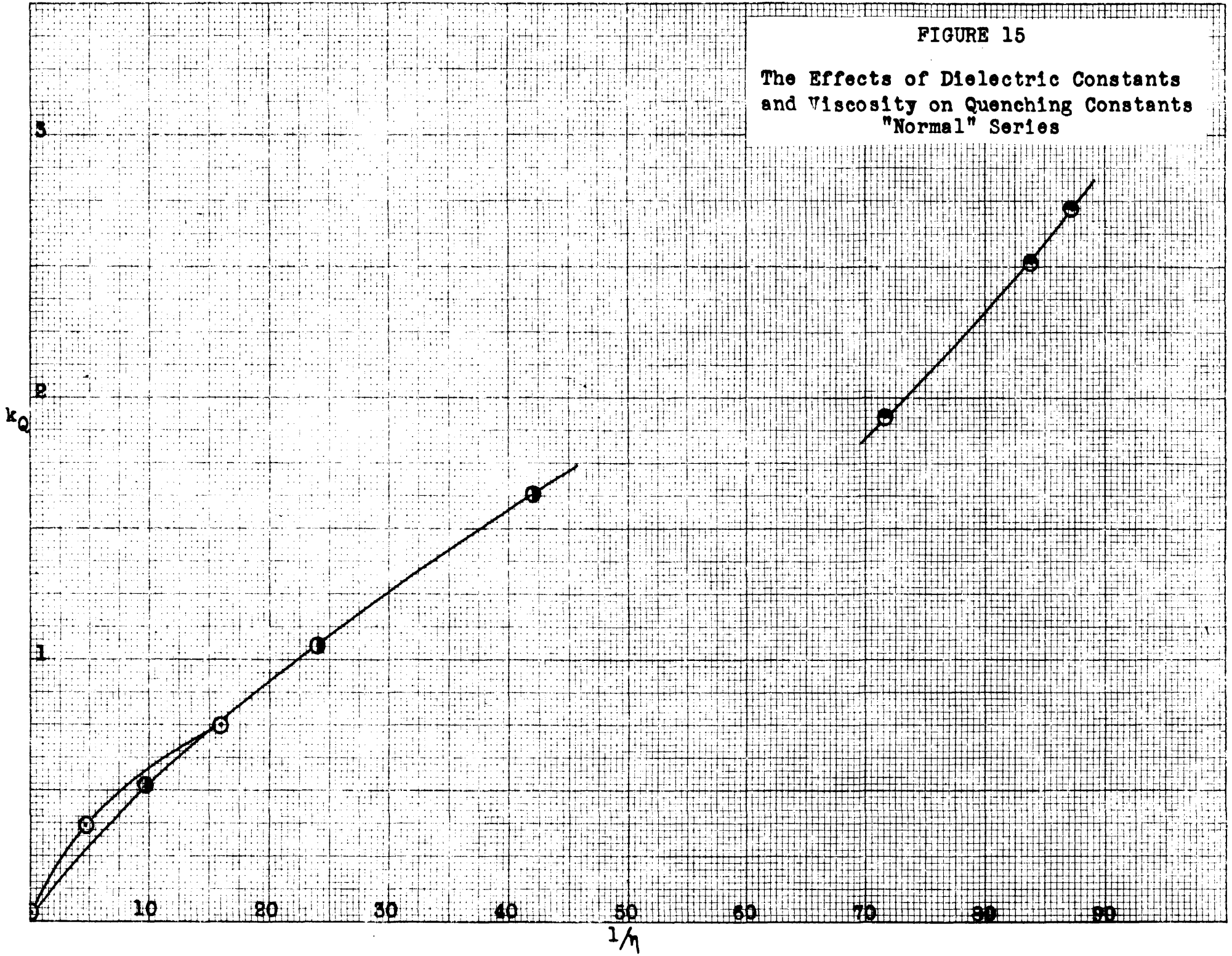


FIGURE 16

Theoretical Quenching Constants as Functions of $1/\eta$ at Various Dielectric Constants at 25°C Calculated By Equation 94

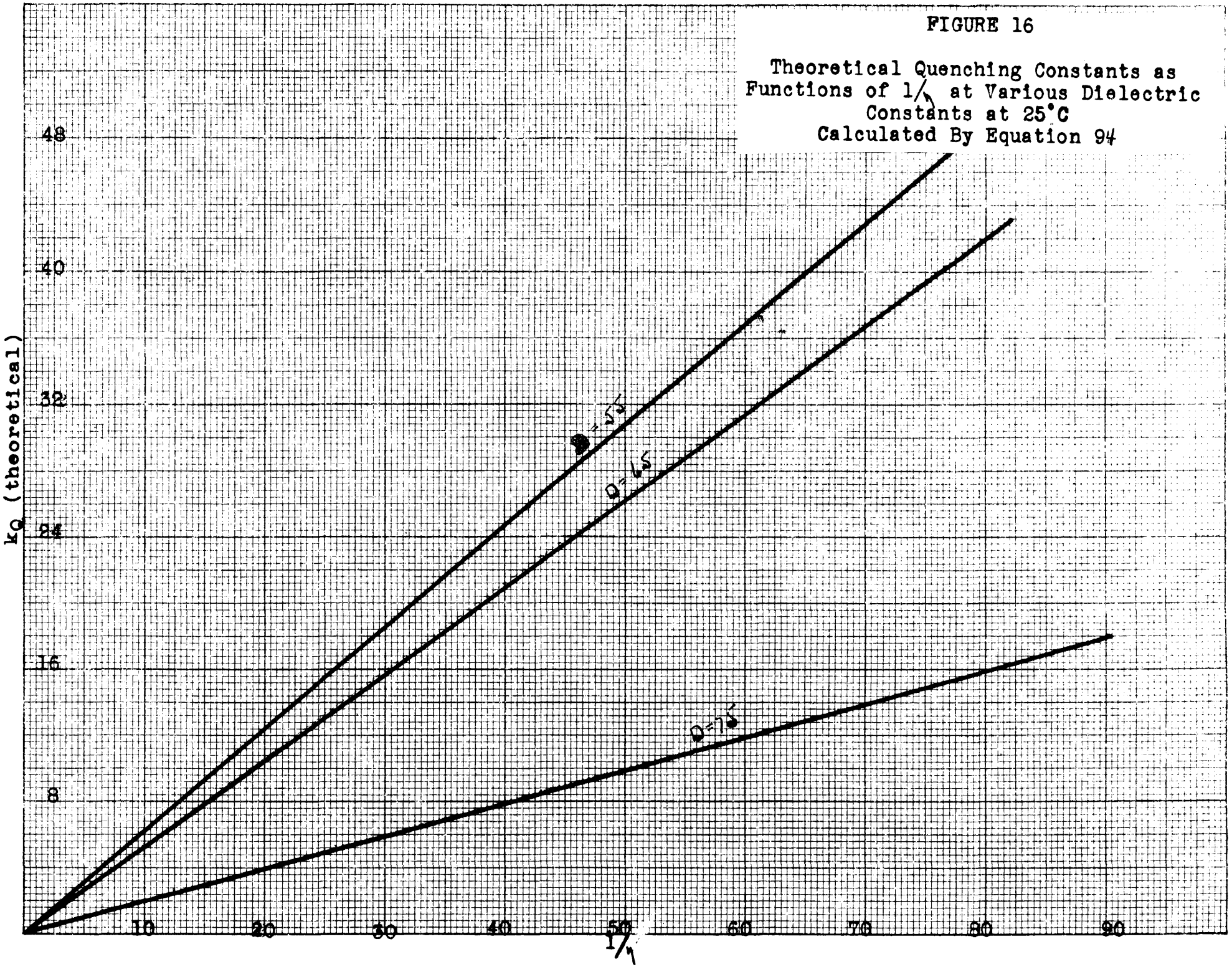
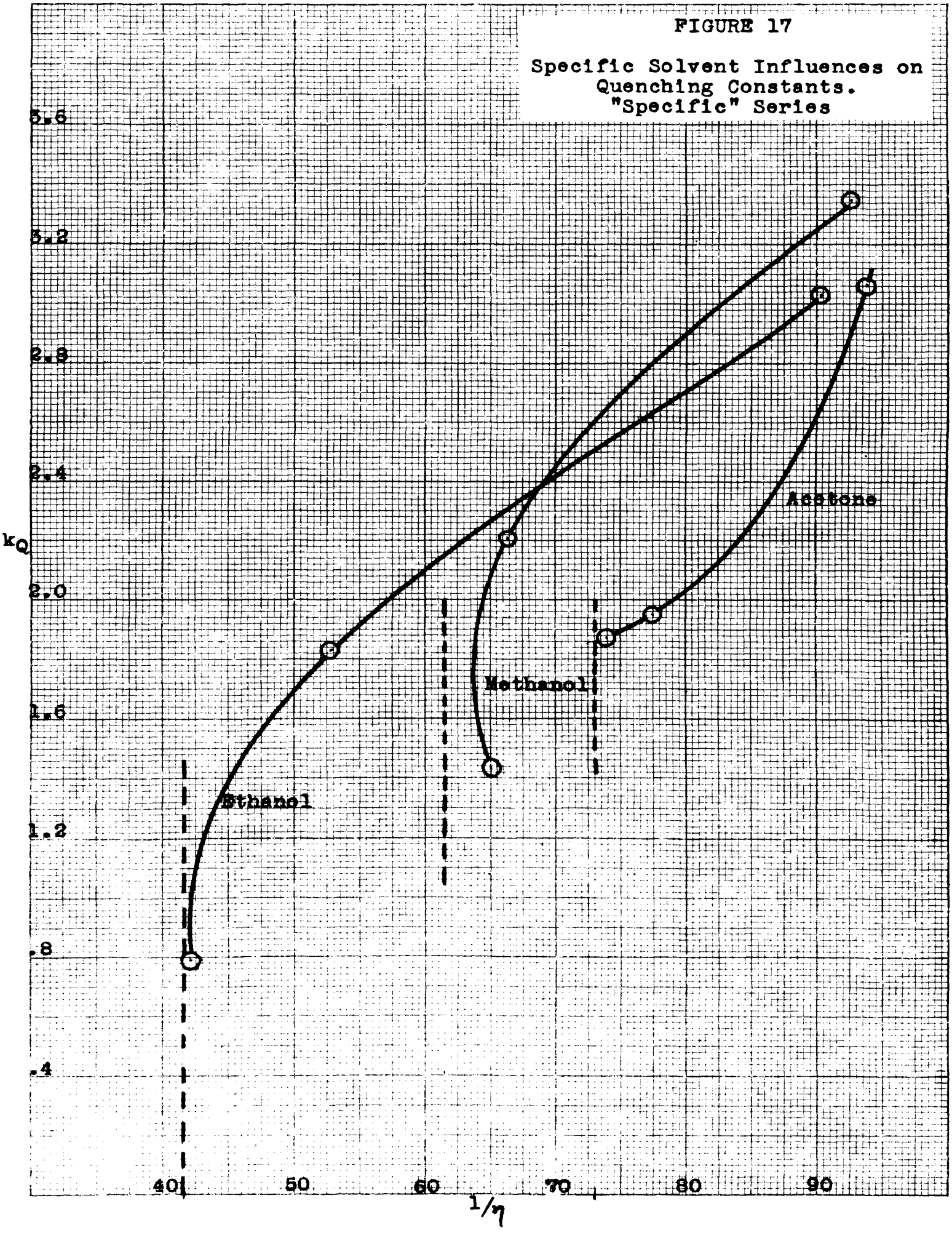


FIGURE 17

Specific Solvent Influences on Quenching Constants. "Specific" Series



isoviscous solutions. This appears to be in opposition to the predictions of the Bronsted-Christiansen-Scatchard theory. However, it must be remembered that the data were obtained at an ionic strength of 0.10, and that the theoretical calculation is for this ionic strength. The Bronsted-Christiansen-Scatchard theory is for a reaction at zero ionic strength. These facts will be referred to again shortly.

A mathematical formulation for the existence of this family of straight lines with the parameter D is difficult, but with the aid of certain assumptions usually valid only in a more restricted sense, it may be accomplished.

Collins and Kimball[53] have shown that the expression for the solution to Fick's diffusion equation

$$\frac{dc}{dt} = \bar{D} \nabla^2 c \qquad (103)$$

where $\bar{D}$ is defined by Equation (43) can be given by

$$k_D = 4\pi R^2 \bar{D} \left(\frac{\partial c}{\partial w}\right)_R \qquad (104)$$

Hodges and La Mer[56] define k_D by the equation

$$k_D = 4\pi R^2 N \bar{D} / 1000 a \qquad (105)$$

The factor "a" is equivalent to the distance between equilibrium positions of the molecules involved in viscous flow. Exact values of "a" are unknown, but they should be of the same order of magnitude as R. Equation (104) can then take the form

$$k_D = 4\pi R N \bar{D} / 1000 \qquad (106)$$

To allow for the influence of the ionic atmosphere, Umberger and La Mer[24] introduced the factor

$$f = R\int_{R}^{\infty} \frac{e^{v/kT}}{r^2}\,dr \tag{68}$$

which they evaluated to

$$f = e^{-\kappa L}\left(e^{L/R}-1\right)\Big/ L/R \tag{69}$$

Equation (106) then becomes, when these substitutions are made

$$k_D = \frac{4\pi \bar{D} N L e^{\kappa L}}{1000\,(e^{L/R}-1)} \cdot \tag{107}$$

Since both L and K are functions of the dielectric constant, it follows that quenching constants must also be functions of the dielectric constant. Due to the L term in the function $(e^{L/R}-1)$ quenching constants would be functions of dielectric constant even at zero ionic strength. The dubious assumption is now made that if the exponential terms are expanded in power series, terms of second powers and higher may be ignored. Since both κL and L/R have values of about 2, over dielectric range 55 - 75 and a temperature range 25 - 45°C, the error in this assumption is apparent. However, assuming this is possible, equation (107) becomes

$$k_D = \frac{4\pi \bar{D} N L(1+\kappa L)}{\frac{1000\,L}{R}} = \frac{4\pi \bar{D} N R(1+\kappa L)}{1000} \tag{108}$$

$$\kappa = \left(\frac{8\pi\epsilon^2 N^2 \mu}{1000 D R_g T}\right)^{1/2} \qquad (90) \text{ and } \qquad L = 2\epsilon^2/DkT \qquad (91)$$

The symbols have their conventional meaning, except R is the "reaction distance" of Umberger and La Mer,[24] while R_g refers to the molar gas constant. Evaluating constant terms, equation (108) becomes

$$k_D = 4\pi \bar{D} R N \left(1 + \frac{5.39 \times 10^6 \sqrt{\mu}}{(DT)^{3/2}}\right) \qquad (109)$$

This equation predicts that at constant temperature and dielectric constant, a plot of k_D against $1/\eta$ will be a straight line passing through the origin, and that as the dielectric increases the slope will decrease. However, it also predicts that for temperature changes at constant dielectric, k_D as a function of T will have the general form

$$k_D = f\left(T/\eta + c/\eta T^{1/2}\right) \qquad (110)$$

and will be non-linear in T/η. It also postulates that at $\mu=0$ there will be no dielectric constant effect.

The experimental data obtained for the "normal" solvents at 25°C. and dielectric constants 55, 65 and 75 (Table VI) are plotted in Fig. 15 as functions of the solvent fluidity.

The relative positions of the theoretical curves in Fig. 16 and of the experimental curves in Fig. 15 do not prove that the data fail to obey Scatchard's equation. These theoretical values and the observed values are for the

specific case of quenching at an ionic strength of 0.10. It has been shown[64] that Scatchard's equation is valid only at zero ionic strength, and that at finite ionic strengths the relationship between log k and 1/D may be the reverse of that between log k_o and 1/D.

Calculations of log $k_{Q(\mu=0)}$ for the various dielectric constants at 25.0°C. were carried out using the corrections terms 2.48 and 2.49 given by Amis.[55] These calculated values are given in Table VI. When these $k_{Q(\mu=0)}$ values are plotted as functions of $1/\eta$, the order with respect to dielectric constant is reversed over that for the order obtained at an ionic strength of 0.10. Thus the Bronsted-Christiansen-Scatchard theory holds in this case also. Since the calculated values of k_Q in Table VI are based on only one experimental point, their use in further discussion is hardly justified. They do, however, indicate the reasons why the experimental data were not obtained at very low ionic strengths. Lowering the ionic strengths would have necessitated lowering the concentration of potassium iodide also, with a consequent lowering of the observed ΔV values. This effect superimposed on the salt effect, would have led to such inaccuracies in the measurement of these low quenching constants that the results could not have been considered at all reliable.

As mentioned previously, the requirement of setting the dielectric constant placed a severe limitation on the range of viscosity permitted. In Fig. 15 there is no overlap between the curve for dielectric constant 75 and those of dielectric

constants 65 and 55. In addition, the short range of the viscosity covered does not permit an investigation of the shapes of the extremes of the curves. It is unfortunate that in the systems investigated there is no ratio of solvent-water which gives high viscosities with high dielectric constants. This renders it impossible to compare quantitatively the quenching constants at various dielectric constants in isoviscous media.

It is, however, apparent that the relative positions of the curves in Fig. 15 are in agreement with the relative positional requirements of the theoretical treatment as shown in Fig. 16. There is thus a dielectric effect on the quenching constant in the iodide-eosin reaction, but it apparently has less impact than the viscosity, especially at high viscosities.

The value of the ratio k_q (calc)/k_q(obs)[24] gives the number of encounters required for one quenching reaction. It is impossible to state that these values are functions of the dielectric constant. The data are subject to too much scatter. On the average, there is one quenching for every nine encounters in the "normal" series, and one for every six encounters in the series showing specific solvent effects, at low viscosities where their behavior is "normal". As the concentrations of these solvents is increased and the specificity comes more into play, the values increase. For ethanol the trend with increasing concentration gives a quenching for every 6, 15 and 32 encounters, while for methanol the values are 6, 16 and 28 and for acetone 6, 21 and 24. This order is also the order of dielectric constants 75, 65 and 55 respectively.

Temperature Coefficients of Quenching. In isodielectric media of 55, 60 and 65 (glycerin-water) the quenching constants showed a positive temperature coefficient (Fig. 18, 19 and 20)(Table VII). The dielectric effect is masked out by the simultaneous changes in temperature and viscosity. Under the conditions of the investigation, the change in the factor $(DT)^{3/2}$ is only a maximum of about 50 per cent, whereas the viscosity changes by a factor of 20 and the ratio T/η by a factor of 3000. Consequently k_Q as a function of T/η is less marked by changes in the dielectric constant. The theoretical curves from Equation (94) are practically colinear for low values of T/η (Fig. 21).

Consequently, regarding the precision available in quenching measurements, k_Q should be nearly independent of dielectric constant with changing temperature. The experimental findings are in agreement with this fact. All experimental points fall along the same curve, independently of the dielectric constants over the range of dielectric constant and temperatures investigated. (Fig. 20)

The calculation of the energy of activation by measurements of the probability of reaction per encounter has been shown to be feasible by Williamson and La Mer.[25] (Equation 82) The function $\log (1/p - 1)$ should be linear in $1/T$, and the slope is proportional to $(E_a - E_v)$. The sign of this slope determines the relative magnitudes of E_a and E_v. The data of Table VII are treated in this manner (Fig. 22) and since the slope is negative, the existing energy of activation is smaller than the existing energy of diffusion.

TABLE VII

The Influence of Temperature - Viscosity Changes on the Quenching Constant.

Temperature °C.	Dielectric Constant	η poise	T/η	k_q	k_q theor	p	$\log(1/p-1)$
25.00 ± .05	55	0.2121	1406	0.37	2.87	0.129	0.830
25.0 ± .05	65	.0414	7205	1.06	12.15	.087	1.019
35.0 ± 0.1	55	.0814	3785	0.68	7.14	.095	0.971
35.0 ± 0.1	65	.0190	16220	1.44	22.2	.065	1.159
45.0 ± 0.2	55	.0386	8240	1.01	14.45	.070	1.124
45.0 ± 0.2	60	.0191	16625	1.88	21.1	.075	1.082
45.0 ± 0.2	65	.0106	30000	2.69	39.0	.069	1.130
25.00 ± .05	78.5	.00895	33300	3.24	36.0	.090	1.004 (Water)

FIGURE 18

Temperature Coefficients of Quenching

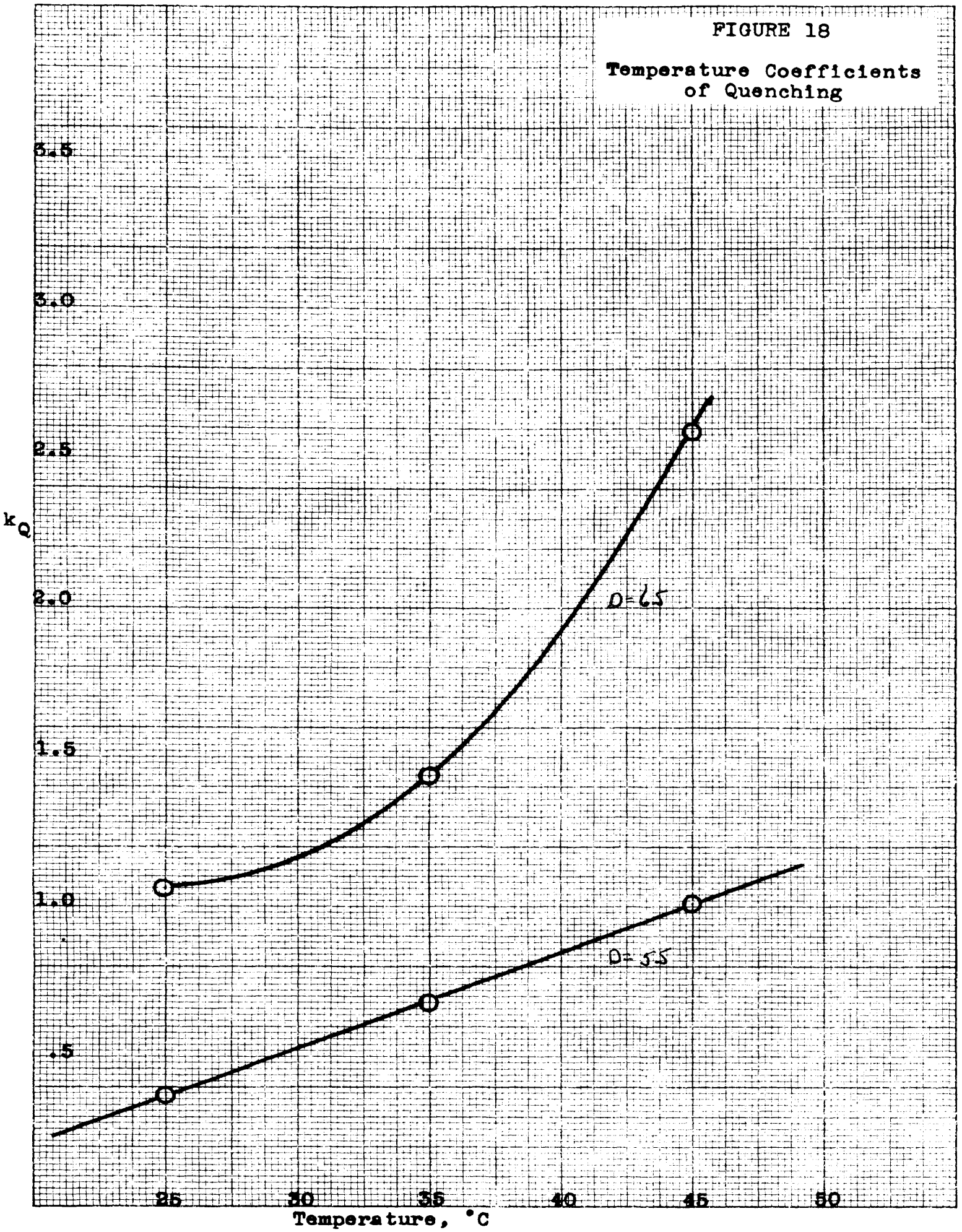
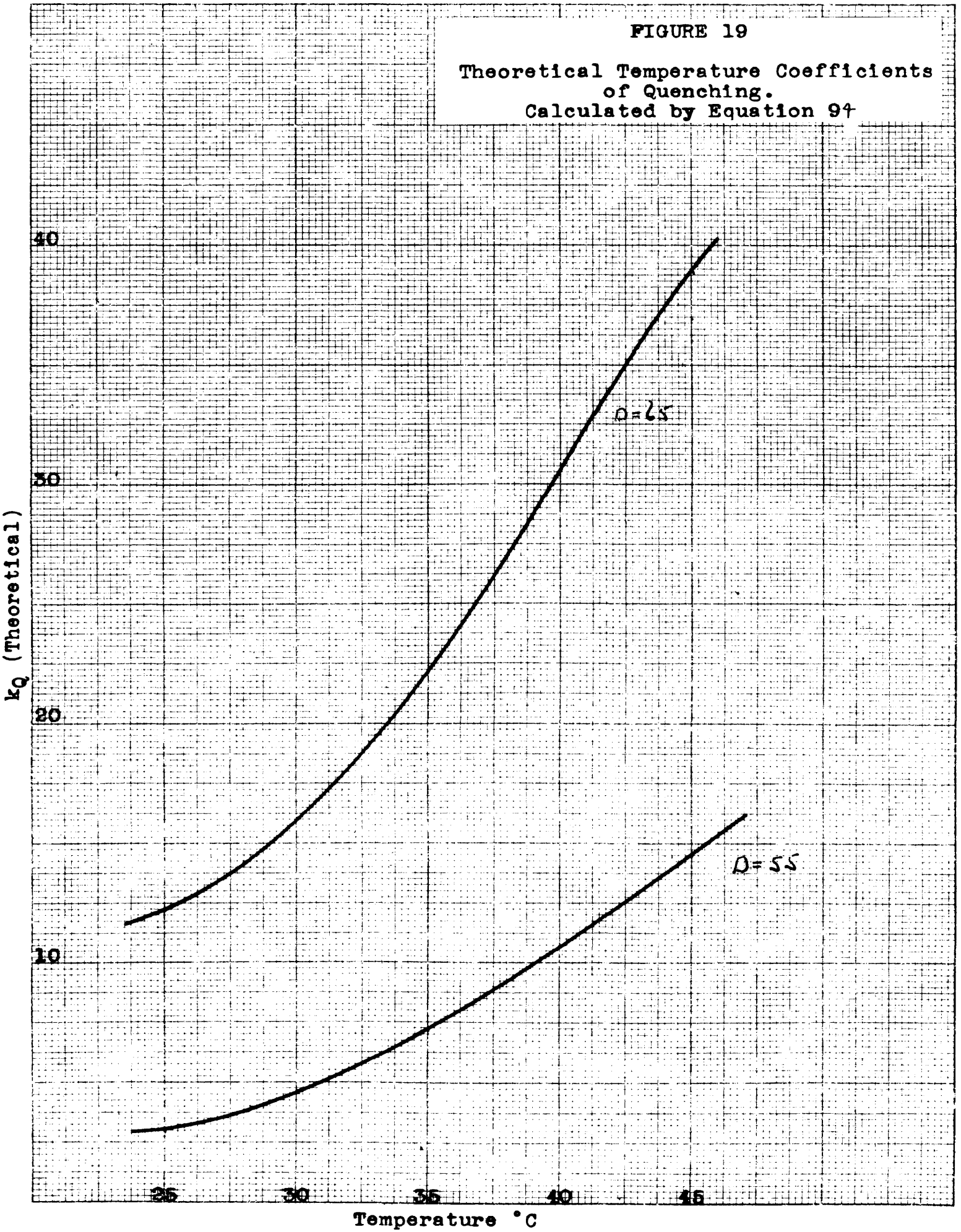


FIGURE 19

Theoretical Temperature Coefficients of Quenching. Calculated by Equation 94



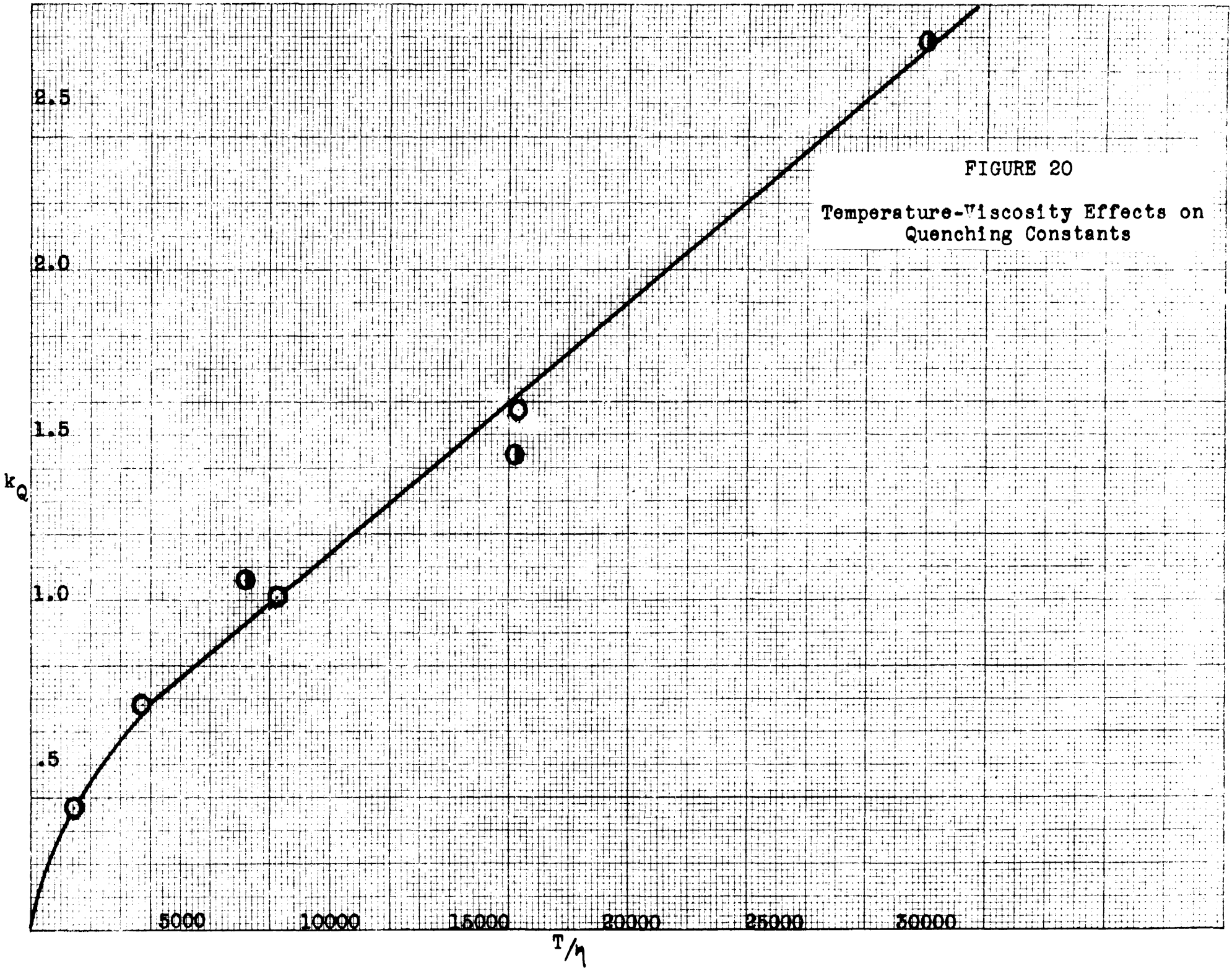


FIGURE 20

Temperature-Viscosity Effects on Quenching Constants

FIGURE 21

Theoretical Variation of k_Q with T/η
Calculated by Equation 94

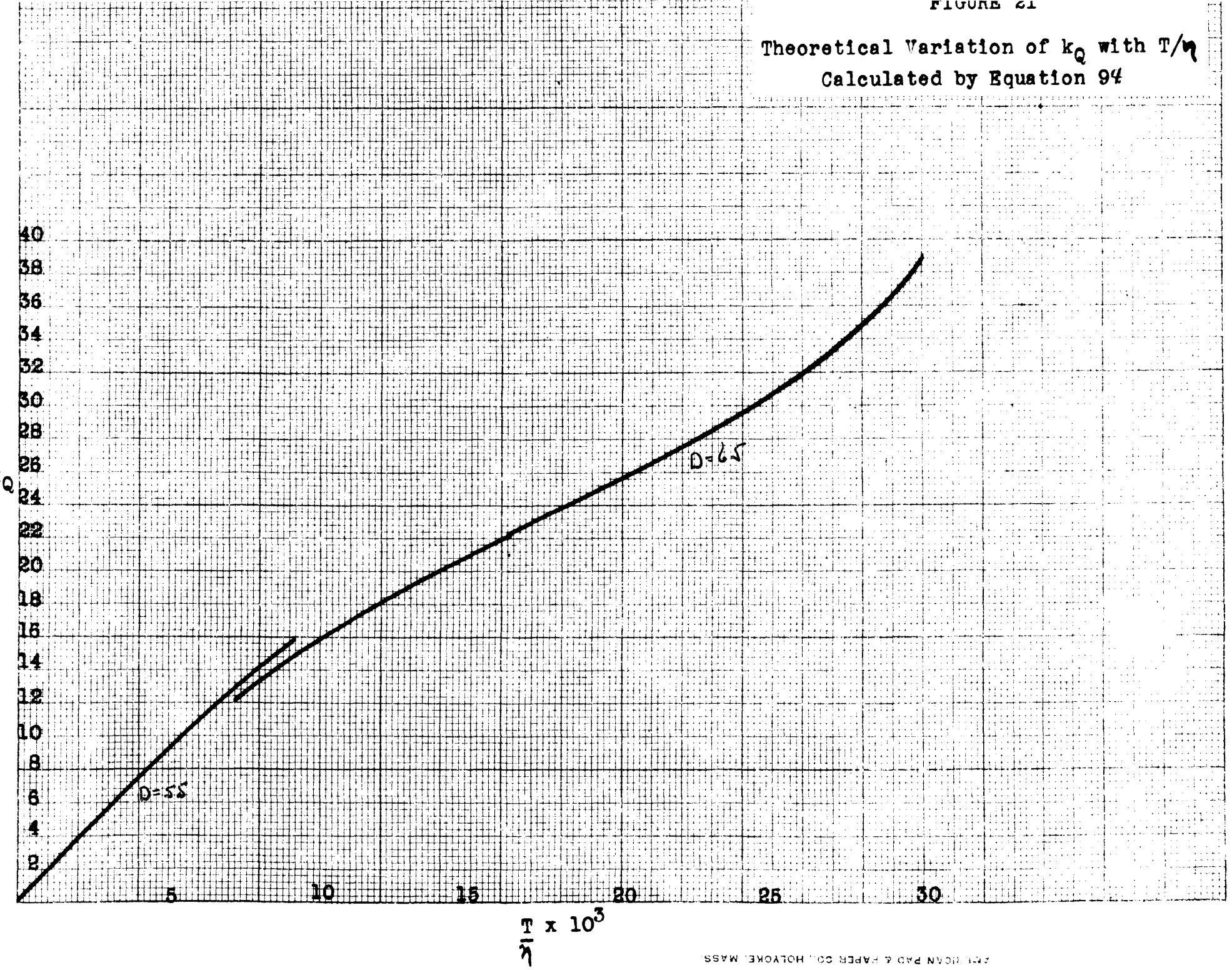


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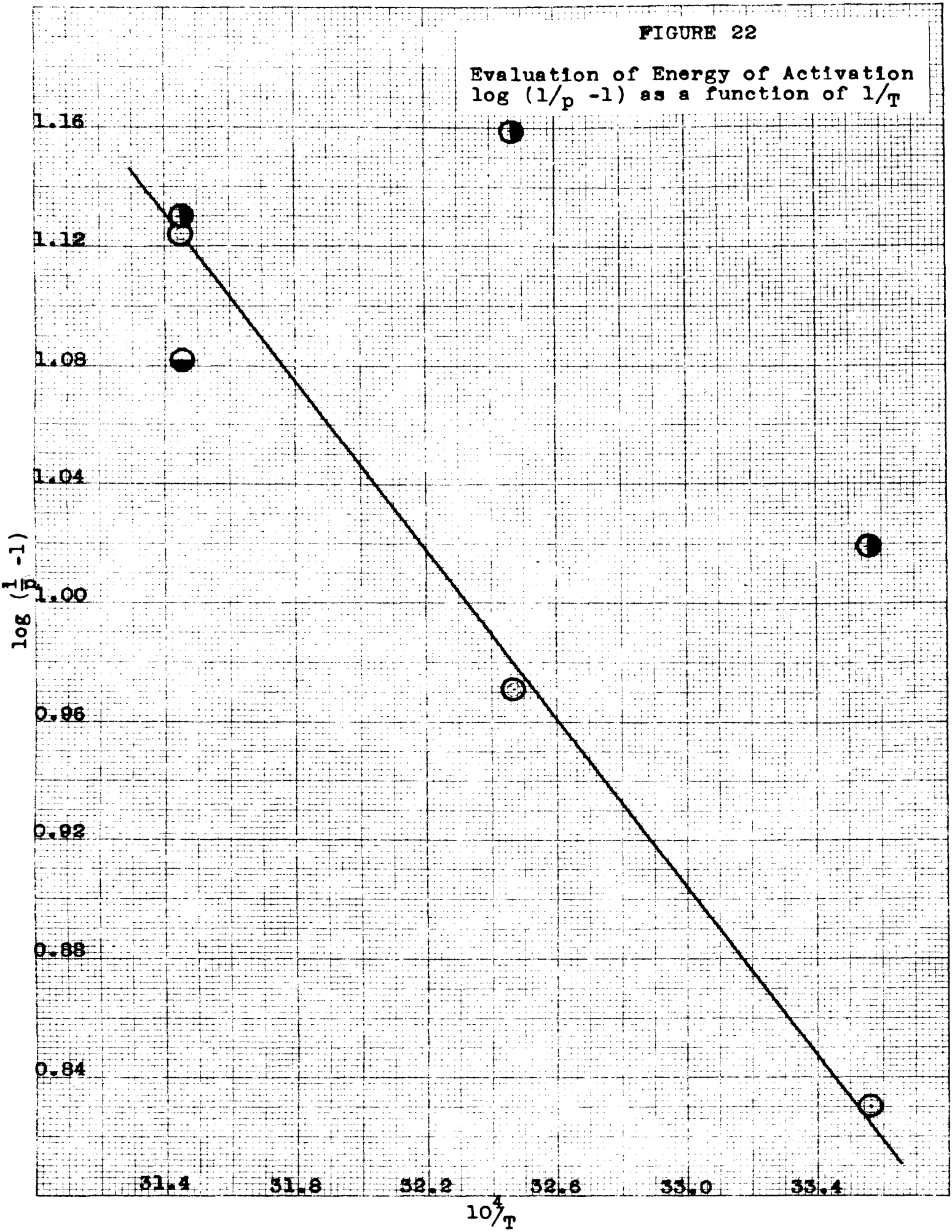
FIGURE 22

Evaluation of Energy of Activation
log $(1/p - 1)$ as a function of $1/T$



The data of Fig. 20 suggest that the constants for dielectric 65 may be subject to experimental error at 25° and 35°C. This effect is magnified in the plot of $\log(\eta_0/\eta - 1)$ as a function of 1/T (Fig. 22), and does not necessarily indicate a dielectric effect. A further confirmation of this is the data for all dielectrics at 45°C, which cluster around the value $\log(\eta_0/\eta - 1) = 1.12$.

The value of $(E_a - E_v) = -6.5 \times 10^3$ calories, and the intercept $\log C/p = 5.62$, from which $P \approx 2.4 \times 10^{-6}$. Choosing a value of $E_v = 4000$ calories, $E_a = 2500$ calories, and the quenching of eosin by iodide is activation controlled as well as diffusion controlled. The low values of P indicate that orientation factors are of not inconsiderable magnitude.

The existence of the specific effects due to adjusting the dielectric constant with ethanol, methanol, acetone or dioxane indicates that quenching constants depend on factors other than those previously discussed. It cannot be categorically stated that such an additional effect is absent in the sucrose, glycerine and ethylene glycol systems. The departure from linearity observed at both low and high viscosities may be due to such an effect.

The existence of maxima in the viscosities of aqueous solutions of methanol, ethanol and acetone requires, since quenching constants are functions of viscosity, that the path traced by k_q as a function of $1/\eta$ return on itself as the viscosity of the solution passes through its maximum value.

TABLE VIII

Changes in Fluorescent Intensity of Aqueous Dioxane Solution (D = 65) Under Continuous Irradiation

(Eosin) = 5 x 10^{-5} molar. (KI) = 0.10 molar; μ = 0.10

Time, Minutes	Fluorescent Intensity (Galvanometer deflections)
0	4.30
2	4.65
4	4.90
6	5.05
8	5.13
10	5.36
12	5.50
16	5.70
18	5.75
24	5.99
30	6.10
38.5	6.30
44	6.50
48	6.50
54	6.70
59	6.75

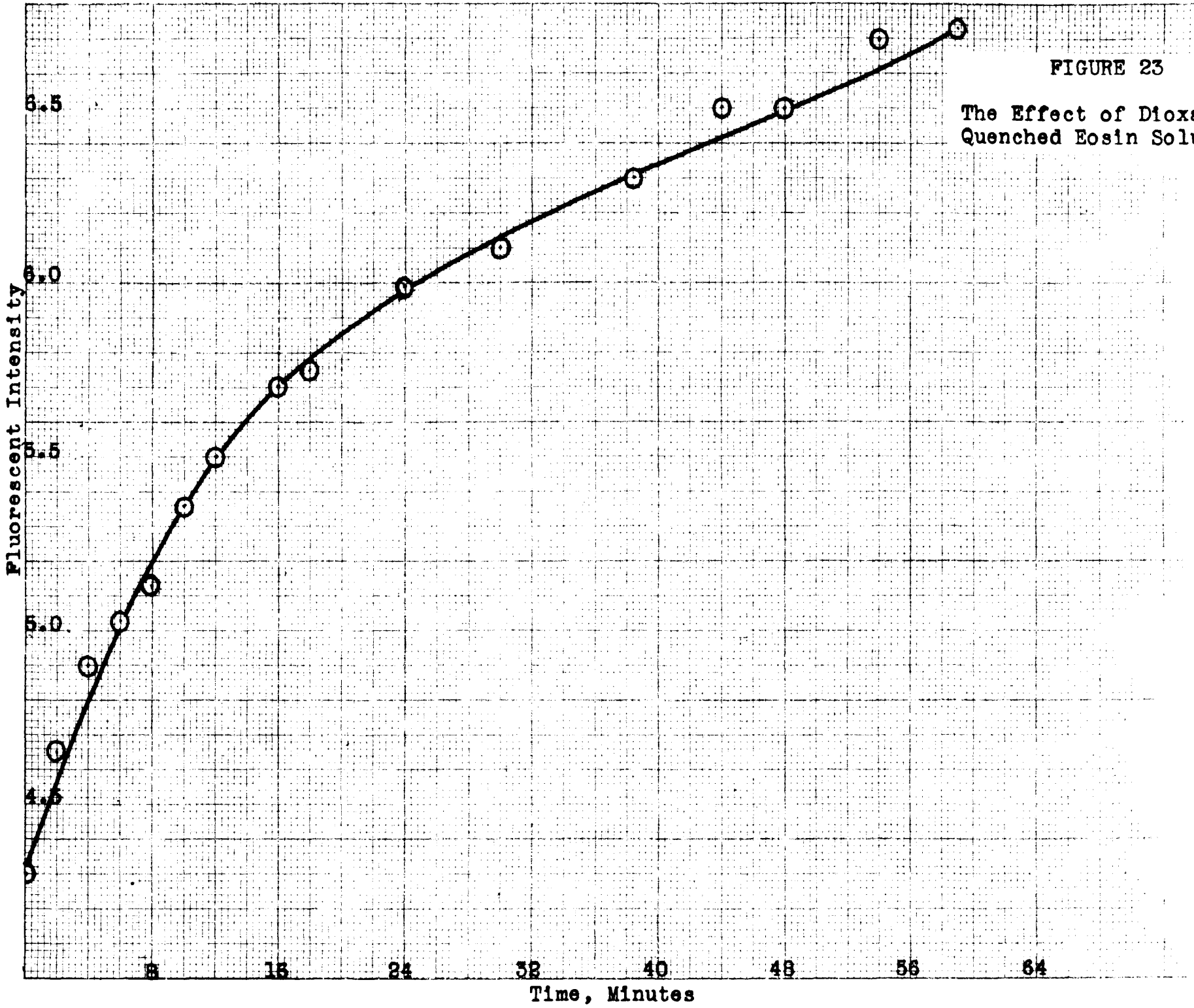


FIGURE 23

The Effect of Dioxane on Quenched Eosin Solution

Since the water-rich solution must necessarily differ from the alcohol-rich solution, it would be naive to expect the trace of the curve to reverse 180 degrees at the point where $1/\eta$ is at its minimum value. The curves should, however, begin to slope more sharply around this point prior to reversing direction. This is confirmed by the behavior of both ethanol and methanol. This change in path of these curves indicates that insofar as this quenching reaction is concerned, the viscosity effect has more impact on the value of the quenching constant than does the dielectric constant. (Fig. 17)

The behavior of the dioxane-water system finds no ready explanation. The system under investigation was of an exceedingly complex nature, in that in addition to the eosin, water, dioxane and potassium iodide, it was saturated with atmospheric oxygen. The steady increase of fluorescent intensity with time (Fig. 23) under constant irradiation suggests either the formation of a new fluorescent compound, or a loss of the quencher. A possible explanation is a photo-induced oxidation involving dioxane peroxide and eosin. An oxidation of the iodide to iodate, which is not a quencher for eosin[11] is a plausible explanation, but in the absence of direct evidence is pure speculation. The cause of the high magnitude of the quenching constants obtained compared with the conventional values of the other solvents is even more obscure. The fluorescent intensities

of unquenched dioxane solutions were not significantly different from unquenched solutions obtained using other solvents. The quenched solutions, however, had quite low fluorescent intensities. The effect can thus be ascribed to neither the dioxane alone nor to the potassium iodide alone, but must be due to some interaction between these two. The effect can hardly be ascribed to the viscosity effect. At dielectric constant 75 the fluidity of the dioxane solution was 105.1. The increase over the next highest member of this dielectric constant (methanol: $k_Q = 3.35$; $1/\eta = 92.7$) is only 13.5 per cent. The quenching constant of dioxane increases by a factor of 30 over the same fluidity range.

SUMMARY

The literature of the quenching of fluorescence in solution as a kinetic process has been reviewed. Quenching has previously been shown to be diffusion controlled and quenching constants are given in very general terms by

$$k_Q = p \tau_0 k_D$$

where p is the probability of reaction per encounter and is a function of orientation, activation energy and other factors. τ_0 is the average life of the unquenched activated dye, and k_D is the encounter frequency and is a function of temperature, viscosity, ionic strength and dielectric constant of the medium.

These concepts have been applied to the quenching of the eosin ion by iodide ion in aqueous and aqueous-non aqueous solvent pairs. An improved sensitive fluorimeter was constructed with a very high degree of stability.

Based on spectrophotometric data, it was shown that the quenching of eosin by iodide ion could not be due to the formation of a non-fluorescent complex between the dye and the quencher.

The self-quenching of eosin shows a positive primary salt effect which is accountable for in terms of the Debye-Huckel-Bronsted equation

$$\log k_Q^s = \log k_Q^{s(\mu=0)} + 4.0\sqrt{\mu}/(1+2.5\sqrt{\mu})$$

indicating that self-quenching is explainable by the mechanism

$$Eo^{=*} + Eo^{=} \longrightarrow 2Eo^{=} + heat$$

Iodide ion quenching also shows a positive primary salt effect in agreement with the Debye-Huckel-Bronsted equation

$$\log k_q = \log k_{q(\mu=0)} + 2.0\sqrt{\mu}/(1+2.5\sqrt{\mu})$$

from which the mechanism for quenching

$$Eo^{=*} + I^{-} \longrightarrow Eo^{=} + I^{-} + heat$$

is postulated.

Quenching constants are functions of the concentration of the dye, due to the reabsorption of the emitted fluorescent light in its passage through the solution. This effect is removable by extrapolation to zero concentration of the dye.

Quenching constants increase linearly with increasing concentration of quencher at constant ionic strength. The rate of change of k_Q with quencher concentration is higher at higher ionic strengths.

Comparison of observed and theoretical quenching constants show that quenching of eosin occurs in one out of every eight encounters with an iodide molecule.

In isodielectric media, the constants increase with increasing fluidity of the system. The shape of the curve obtained when k_Q is plotted as functions of fluidity is determined by the organic solvent used to adjust the dielectric constants and viscosity. It was found that the solvents used to make these adjustments could be divided into two sets:

the "normal" series which consists of the compounds sucrose, glycerine and ethylene glycol, the viscosity of whose aqueous solutions increase continually with concentration; and the specific series which consist of ethanol, methanol and acetone, whose viscosity composition curves show maxima.

The predictions of theory regarding the influence of the dielectric constant have been shown to be obeyed in the "normal" series. In the specific series the deviations are due to the existence of the maximum, and have been shown to occur most rapidly at this point. In general the dielectric constant has less effect on quenching than does the viscosity.

Aqueous solutions of dioxane are characterized by quenching constants estimated as 10 to 30 times as high as those found with other solvents, together with a constant change in fluorescent intensity of quenched solutions under continuous radiation. A possible explanation in terms of photo-induced oxidations has been advanced.

The eosin-iodide quenching reaction has a positive temperature coefficient. Due to the relative changes in dielectric constant, temperature and viscosity, the values of k_Q fall along a single curve when k_Q is plotted as a function of T/η . Viscosity-temperature changes have a greater influence on quenching constants than do dielectric constants.

The quenching of eosin by iodide was found to be activation controlled as well as diffusion controlled, and the energy of activation is 2.5 kilocalories.

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ABSTRACT

Norman E. Sharpless, Ph.D. 1951 (B.S. The George Washington University)
Title of thesis: The Quenching of the Fluorescence of the Eosin Ion by the Iodide Ion.
Thesis directed by Professor William J. Svirbely
Major: Physical Chemistry, Department of Chemistry
Minors: Organic Chemistry, Analytical Chemistry
Pages in thesis, 102. Words in abstract, 479

The quenching of the fluorescence of eosin ion by iodide ion was investigated as a function of eosin concentration, ionic strength, iodide ion concentration, dielectric constant, viscosity and temperature.

For the measurement of quenching constants, a fluorimeter was constructed of an unusually high degree of stability and sensitivity.

Theoretical quenching constants can be expressed by an equation of type

$$k_q = p(1-d)\,\gamma_0\,k_D$$

where k_D is the encounter frequency, expressed in terms of ionic interaction factors, and viscosity. $p(1-d)$ is the probability of any encounter resulting in quenching and γ_0 is the average life of the excited molecule.

By means of absorption spectra it was shown that the quenching could not be attributed to the formation of a complex between the dye and the quencher.

The quenching constant of the eosin ion by the iodide ion shows a positive salt effect which can be expressed at 25°C by the equation

$$\log k_q = \log k_{q(\mu=0)} + 2.0\sqrt{\mu}/(1+2.5\sqrt{\mu})$$

while the self quenching constant at 25°C can be expressed by the equation

$$\log k_q^s = \log k_{q(\mu=0)}^s + 4.0\sqrt{\mu}/(1+2.5\sqrt{\mu})$$

Quenching constants for eosin are functions of the concentration of the eosin. This effect is due to the reabsorption of the fluorescent light by the solution and may be removed by extrapolation to zero concentration of the dye. The resulting figure is the "true" quenching constant. The "true" quenching constant for the iodide quenching of eosin in water at 25°C is

$$k_{q(\mu=0)}^{(c=0)} = 0.95$$

Comparison with the theoretical equation indicates that a quenching action occurs in one out of every eight encounters.

In aqueous- non-aqueous isodielectric solutions, the quenching constants increase with increasing fluidity of the solution, but the actual behavior of the system depends on the choice of the non-aqueous solvent. A "normal" series, consisting of solutions whose viscosities increase regularly from that of water to that of the solvent, behaves as predicted by the theoretical equation. A "specific" series, consisting of solutions whose viscosity-composition curves show maxima, possesses, in general, a sharp decrease in quenching constants around the point where the viscosity is a maximum. The theoretical equation predicts a family of curves with different slopes set by the value of the dielectric constant. This effect was found to hold for the "normal" solutions. In general, the viscosity of the

solution has a greater impact on the quenching constants than does the dielectric constant.

The iodide ion quenching of eosin has a positive temperature coefficient. The theoretical equation in this case indicates that changes in the ratio T/η are more important than changes in the dielectric constant, and that within experimental error, especially at high viscosities, the dielectric constant effect will not be measurable. This was confirmed experimentally, since all quenching constants seemed to fall along a single curve when k_q was plotted against T/η.

The activation energy for the eosin-iodide quenching reaction was found to be about 1.8 kilocalories.

Solutions containing thionine were found to behave abnormally. Quenched solutions had very low fluorescent intensity which increased with time. A hypothesis is presented on a photo-induced oxidation of iodide in post-olation.

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