JAN. 16, 1969

TO DR. BUSSE,

WHO HAS BEEN AND ALWAYS WILL BE A SOURCE OF INSPIRATION TO ME. MY PROFOND GRATETUDE FOR ALL YOUR KIND NESSES.

Parviz Farid

THE ION PAIR FORMATION BY SOME AZO SULFONATES

by

Parviz Javid

Under the Supervision of Professor Takeru Higuchi

This work is concerned with the determination of various parameters which influence the formation and extraction of ion pairs. A typical azo mono sulfonic acid dye, tropaeolin OO and its ortho hydroxy analogs were utilized in these studies. The factors investigated were: (1) the effect of dye concentration on the partition coefficient; (2) the effect of buffer concentration on the extraction of the dye amine; (3) the effect of pH on the partitioning of the dye; (4) the effect of concentration and nature of inorganic and organic cations present on the partitioning; (5) the effect of organic masking or solvating agent in the non-aqueous phase.

It was observed that the partition coefficient of the dye was independent of concentration when it was partitioned between an aqueous solution and an organic phase consisting of 16% W/V n-amyl alcohol and chloroform. The linearity of the partition coefficient in the concentration range used (up to 5×10^{-5} M/L) indicated that the dye did not undergo appreciable association and that, therefore, partition coefficient could be calculated directly using the analytical concentrations. Similarly, the buffer

concentration had no effect on the observed partition coefficient, providing that the cation concentration was kept constant.

Further work on the effect of pH on the partitioning of the dye showed a dramatic effect due to variation of hydrogen ion concentration. The observed partition coefficient was found to be independent of pH in a region above pH 5. Below this there was a marked increase in partition coefficient reaching a maximum of approximately pH 1.2. At a pH of less than one there appeared to be a slight decrease in partitioning which might be attributed to the protonation of the dye. The profile would indicate that the dissociated form of the dye does not partition significantly whereas the undissociated form contributes mainly to the observed partitioning.

Studies dealing with the effect of inorganic cations indicated that under conditions of constant pH the addition of sodium markedly increased the partition coefficient of the dye when the dye was present in its dissociated form and had negligible effect at low pH where the predominant specie present was the undissociated acid. This would indicate that at high pH the dye is extracted as a sodium ion pair. This was found to be true for both tropaeolin OO and its hydroxy analog. Previous studies cited suggested that divalent cations did not form extractable ion pairs. Consequently, a study identical to that of the sodium investigation was carried out using Ca^{++} as the inorganic cation. It was found that partitioning was independent of added calcium and was essentially equal to the value which was extrapolated to zero sodium concentration.

Studies of the effect of organic cations were conducted using a series of amines, specifically d-amphetamine, methyl amphetamine, dimethyl amphetamine, and tetrabutyl ammonium chloride. Studies using these compounds were carried out using an organic phase consisting of 1.8% W/V amyl alcohol in chloroform. In the case of the 1^0, 2^0, 3^0 amines the order of extraction was $3^0 > 2^0 > 1^0$. The order of extraction does not correlate with the order of the pKa's of the amines which is $2^0 > 3^0 > 1^0$, indicating steric effects are more influencial than basicity of the nitrogen. The enhanced partition of the quaternary might be attributed to the higher molecular weight and increased number of lipophilic groups around the nitrogen.

The effect of the solvating and masking agent in the organic phase was investigated using various concentrations of chloroform in cyclohexane. Cyclohexane was chosen since it alone was not capable of extracting ion pairs. Consequently, the effect of the chloroform could be determined as an independent variable. It was found that the primary amine required greater solvation or shielding than the other compounds, possibly due to the lack of inherent alkyl groups on the amino function.

From these studies, it can be suggested that this technique may be utilized for separating rather similar structures and that the separation can be modified by consideration of the variables studied. The technique offers a rather sensitive method for analysis of various amino compounds.

APPROVED____________________

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THE ION PAIR FORMATION BY SOME AZO SULFONATES

by

PARVIZ JAVID

A thesis submitted in partial fulfillment of the
requirements for the degree of
DOCTOR OF PHILOSOPHY
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1969

TO MY PARENTS

AND MY BROTHER,

DR. MANUCHER JAVID,

AND HIS FAMILY

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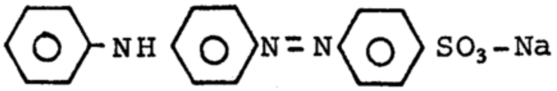
INTRODUCTION

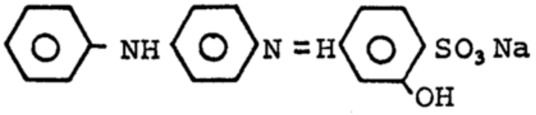
The acid-dye method (1) is a widely used assay for determination of basic nitrogen compounds. It is based on the observation that acid-dyes, high molecular weight sulfonic acids such as bromocresol purple, bromothymol blue, chlorophenol red, bromocresol green bromophenol blue, methyl orange, eosin, and tropaeolins form readily detectable ion pairs with many amines which extractable into an organic phase. The concentration of the ion pair in the organic phase can be determined colorimetrically. Therefore, by extracting an aqueous mixture of an amine and an excess concentration of a dye with an organic phase, the concentration of the nitrogenous compound initially present can be determined.

Since many pharmaceuticals such as alkaloids, antihistamines, anticholinergics, tranquilizers, energizers, etc., contain basic nitrogens, this method could be used effectively for the quantitative determination of these compounds in pharmaceutical preparations.

Although the colorimetric determination of amines by ion-pair formation has been used for a long time, the physical chemistry and a systematic approach to elucidate factors influencing the ion-pair formation have only lately been studied. For most cases the approach seems to have been empirical in nature.

The purpose of this investigation was to study two typical acid-dye compounds, tropaeolin OO* and its ortho-hydroxy analog,


Tropaeolin OO


Hydroxy tropaeolin OO

to determine:

a. the influence of the hydroxy group adjacent to the sulfonic acid group on partitioning of the dye between water and chloroform

b. the pH profile of the partioning of the dye

c. the dependence of the partitioning on organic and inorganic cations

d. the effect of the concentration of the dye on partitioning

e. influence of the total buffer concentration.

Since in the acid-dye method the blank consists of extraction of the dye with no amine added, control of these variables leads to the determination of the best conditions for the assay. The ideal case is when blank absorption in the organic phase is negligible, and amine-dye ion pair extraction is complete with respect to amine.

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* Sodium p-[(p-amino-phenyl)-azo] benzene-sulfonate. This compound is also known as: diphenyl amine orange, orange G-S, orange N, CI acid orange 5, C.I. No. 13080 orange IV, fast yellow, and acid yellow.

The second phase of this study dealt with ion-pair formation of the two dyes with inorganic cations and primary, secondary, tertiary, and quaternary amines to find the relationship between structure of amine and its extraction into the organic layer as an ion pair. The effect of adding a masking agent to the organic phase to enhance solvation of the ion pair in organic phase and the binding number were also determined.

PAST WORK

The first indication of ion-pair formation was made by Bjerrum (2) who pointed out the probability of a pair of oppositely charged ions being able to come sufficiently close together in such a way that the force of attraction between them would be strong enough to overcome the effect of thermal agitation.

The effect of ion-pair formation may be calculated on the basis that all oppositely charged ions within a certain distance are capable of association as ion pairs. According to Bjerrum (2) there is a critical distance q

$$q = \frac{Z^+Z^-e^2}{2DkT}$$

Z^+, Z^- = valences of anion and cation respectively

e = protonic charge

D = dielectric constant

k = Boltzman constant

Fuoss and Kraus (3) illustrated the effect of dielectric constant of an ion pair by using tetraisoamylammonium nitrate asn an example.

Bjerrum derived another equation which was simplified by Fuoss (4,5) to:

$$1/K = \frac{4\pi N\ a^3 b}{3000}$$

K = dissociation constant of the ion pair

N = Avogadro's number

a = distance of closest approach

$$b = \frac{Z^+Z^-e^2}{aDkT}$$

Reiss (6) derived a relationship for determination of effective range of the distance for ion-pair formation. He derived an equation for ion-pair formation with these advantages:

1. The law of mass action is not invoked.
2. The theory is thoroughly consistent in the assumption that interaction is confined to the nearest neighbor of the opposite sign.
3. The distributed densities of positive ions in the neighborhood of negative ions is conserved. This is in good agreement with Fuoss' simple approach.

Batson and Krauss (7) showed that organic salts of large molecules do exist largely as ion pairs in solutions of low dielectric constant. Mukerjee (8,9), studying the analysis of anionic surfactants by cationic dyes, suggested that among factors affecting the ion-pair formation (complex between dye and surfactant), dielectric constant was the determining factor in the amount of the compound extracted in the organic phase. Other influencing factors were:

1. concentration of the dye used
2. concentration of the surfactant
3. the nature of organic solvent
4. volume ratio of organic to aqueous phase.

He proposed this equilibrium scheme:

$$\begin{tikzcd}
\mathrm{COH\,(aq)} \arrow[r, shift left] \arrow[d, shift left] & \mathrm{CCl\,(aq)} \arrow[l, shift left] \arrow[r, shift left] \arrow[d, shift left] & \mathrm{CD}_{\mathrm{aq}} \arrow[l, shift left] \arrow[r] \arrow[d, shift left] & \mathrm{NaD}_{\mathrm{(aq)}} \arrow[r] \arrow[d, shift left] & \mathrm{HD\,(aq)} \arrow[d, shift left] \\
\mathrm{COH\,(org)} \arrow[r, shift left] \arrow[u, shift left, "K_1"'] & \mathrm{CCl\,(org)} \arrow[l, shift left] \arrow[r, shift left] \arrow[u, shift left, "K_2"'] & \mathrm{CD}_{\mathrm{(org)}} \arrow[l, shift left] \arrow[r, shift left] \arrow[u, shift left, "K_3"'] & \mathrm{NaD}_{\mathrm{(org)}} \arrow[l, shift left] \arrow[r, shift left] \arrow[u, shift left, "K_4"'] & \mathrm{HD}_{\mathrm{(org)}} \arrow[l, shift left] \arrow[u, shift left, "K_5"']
\end{tikzcd}$$

where

NaD = anionic detergent

CCl = cationic dye

D = detergent ion

C = colored dye

K_1 = extraction constant of the basic form of the dye

K_2 = partition of the dye between the organic and aqueous phase

K_3 = equilibrium partition of the dye-surfactant

K_4 = equilibrium partition of detergent (surfactant)

K_5 = equilibrium partition of unionized dye

The best condition for ion-pair extraction is when K_2 and K_4 are minimum. K_1 is important only in the basic solutions. K_5 is important in the case when HD is a weak acid and solution is acidic.

McClellan and Freiser (10) have shown that when ion pairs involving inorganic cations are extracted, the limiting step is the formation of coordination compounds in water. Review of other works is in good agreement with Freiser's findings (11). Kraus (12) discussed the basic concept of ion association, and Morrison and Freiser (13-16) studied its application in extraction.

Application of the Acid-Dye Method

Prudhomme (17) found that alkaloids such as quinine form chloroform soluble complexes with acid-dyes such as eosin.

Auerbach (18) used bromthymol blue and bromophenol blue for colorimetric determination of some germicidal quaternary ammonium salts in dilute solutions. He found that cations of composition $(R_1R_2R_3R_4N)^+$ where R_1, R_2, and R_3 are methyl or higher and R_4 is  CH_2 or long-chain aryl alkyl can form ion pairs with the dye, which can be extracted in alkaline solutions. The advantage of this method was that 50 to 60 non-quaternary amines tested all gave negative results under alkaline conditions. He preferred bromophenol blue because it gave a low blank reading with ethylene dichloride which was used as the solvent.

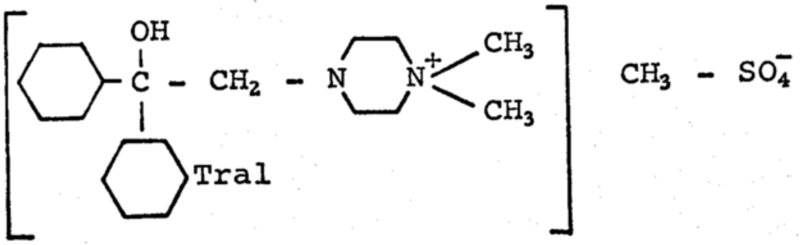
Brodie, Udenfriend, and Dill (19) estimated cinchonine in plasma at the level of 50γ per liter by using methyl orange solution as pH 5 and benzene as the non-aqueous phase.

Durick, King, Ware, and Cronheim (20) worked with atropine, hyoscyamine, and scopolamine at ten milligrams per liter with bromocresol purple as the dye. They obtained accuracy of 15% at pH 5.5. Ballard, Issacs, and Scott (21) reviewed the extraction of quaternary ammonium salts and some amines by acid-dye indicators such as bromothymol blue and determined the effect of dye concentration, solvent, phase volume ratio, time, and other parameters.

Thomis and Kotonis (22-24) used tropaeolin OO and other acid dyes for determination of compounds such as strychnine, codeine, quinine, emetine, procaine, atropine, and spartein. The effect of pH on ion-pair extraction was determined.

Mukerjee and Mysels (17, 18) studied the ion-pair formation of methylene blue, pinacyanol, and bromophenol blue as the dyes and sodium lauryl sulfate, sodium laurate, cetyl trimethylammonium bromide, and dodecylammonium chloride as surfactants. Sensitivity of the method was in the order of 10^{-6} - 10^{-7} moles per liter.

Helgren, Theivagt, and Campbell (25) devised a method for analytical determination of a cholinergic drug Tral (hexocyclium) based on ion-pair formation with bromthymol blue .



using chloroform as the organic phase using a modification of Mukerjee's (9) scheme:

$$\begin{tikzcd}
DNa\,(H_2O) \arrow[r] \arrow[d, shift left] & RD\,(H_2O) \arrow[r, shift left] \arrow[d, shift left] & RX\,(H_2O) \arrow[l, shift left] \arrow[d, shift left] \\
DNa\,(CHCl_3) \arrow[u, shift left, "K_2"'] \arrow[r, shift left] & RD\,(CHCl_3) \arrow[u, shift left, "K_1"'] \arrow[l, shift left] \arrow[r, shift left] & RX\,(CHCl_3) \arrow[u, shift left, "K_3"'] \arrow[l, shift left]
\end{tikzcd}$$

DNa = bromothymol blue anion

RX = Tral

RD = dye-amine complex

K_2 and K_3 represent partitioning of the dye and Tral, respectively

K_1 = partitioning of Tral dye complex

They suggested that the choice of solvents, pK of the indicator, and pH of the aqueous phase were important factors in favoring the partition in organic phase. They studied the effect of pH, shaking time, and interfering materials. The composition of Tral-dye complex according to the authors is 1:1. When using the acid-dye method, they found that common tablet excipients under the assay condition showed no effect except carbowax 6000, which at pH 8.5 combined with bromothymol blue to form a chloroform soluble complex.

Divatia and Biles (26) isolated di-n-propylamine, diisopropylamine, di-n-butylamine, diisobutylamine, bi-n-amylamine, diisoamylamine, and dihexylamine salts of tropaeolin OO. They found that the distribution constant of these salts between water and chloroform, or carbon tetrachloride, or both, was governed by molecular weight and branching of the aliphatic amine, the relative concentration of amine and the dye, and the dielectric constant of the solvent. They found a direct relationship between the logarithm of the distribution constant and molecular weight of amine. They also showed the monomeric nature of ion pairs in the organic layer.

Higuchi (27) suggested that stoichiometric complexes between the ion pair and the solvating species in the organic phase are more responsible for favoring distribution coefficient than dielectric constant (8,9,25). Hull and Biles (28) studied the ion-pair formation of diisooctylamine and diisoamylamine with azo dyes, tropaeolin O, tropaeolin OO, methyl orange, and sodium azo benzene sulfonate, using amyl, hexyl, or lauryl alcohol as solvating agents in benzene. They confirmed Higuchi's (27) supposition about the importance of proton-donor molecule in aiding the extraction of amines. They determined the binding number and overall stability constants.

Biles, Plakogiannis, and Wong (29) determined apparent partition coefficient of alkyl sulfate salts of methantheline, propentheline, benzomethamine, isopropamide, oxyphenonium, tridihexethyl, homatropine, 2-PAM(paralidoxime). They made a comparison of molecular structure to the apparent partition coefficient. Tropaeoline OO was used for determination of the amine present. The comparison of molecular structure to the apparent partition coefficient is given. They found no temperature effect in the temperature range of 4^0 - 45^0C.

Schill (30) investigated factors involved in photometric determination of amines and quaternary ammonium compounds with bromothymol blue. He studied such effects as pH and interfering ions in the aqueous phase, specifically Na^+, K^+, $NH_4{}^+$. He also studied association of bromothymol blue in aqueous solutions (31), partition coefficient of bromothymol blue between water and methylene chloride (32), extraction conditions and extraction constants (33), dissociation constants of amines (34), and association in aqueous solutions between bromthymol blue and quaternary ammonium ions (35).

Higuchi, Michaelis, Tan, and Hurwitz (36) studied the ion-pair formation of dextromethorphan (a tertiary amine) and the role of dipolar protodotic agents (masking agents). They also studied the effects of various anions on the partition coefficients. They also derived equations to relate partition coefficients and binding numbers to variables such as anion concentration, pH, and solvent composition.

Tan (37) investigated the physical-chemical aspects of distribution of ion pairs between immicible solvents, including pH, ionic strength, polymerization, and effect of the masking agents on ion-pair formation of dextromethorphan in chloroform, normal-amyl alcohol, and in cyclohexane. The concentration of each phase was determined by direct measurement of the absorbance in each phase, but when p-tertiary butylphenol was added as solvating agent, the organic phase was determined by non-aqueous titration. When p-tertiary butylphenol and dimethylcaprylamide were both present in the system, the apparent partition coefficient was determined by acid-dye method (28-29). He also determined extraction of free base in organic phase which interferes with ion-pair determination.

Michaelis reported the kinetics and thermodynamics of ion-pair formation of dextromethorphan. A review of the mechanism of ion-pair formation is also given (38,39).

Roubal (40) used the hydroxy naphthyl sulfonates as acid-dyes and studied the effect of isomeric structural changes on the extractability of a limited number of amines. He also determined the effect of pH, and of inorganic and organic cations on the partition profile. He also found that temperature change had a marked effect on partitioning.

EXPERIMENTAL

Equipment

Cary Model 11 MS and Cary 14 spectrophotometers were used for all absorbance readings.

Measurements of pH were made on a Beckman research pH meter equipped with calomel electrode and appropriate glass electrode. pH was controlled within 0.01 unit.

Shaking was carried out on a Burrell wrist-action shaker.

Separatory funnels used were all globe type.

Reagents

Redistilled water was prepared by distillation from acid permanganate in all-glass distillation apparatus to remove impurities.

Chloroform (analytical reagent, Mallinkrodt) was shaken two times with sulfuric acid and then washed several times with double-distilled water to remove the preservative.

n-Amyl alcohol (Eastman Organic Chemicals), boiling point 136°.

Cyclohexane (Aldrich) 99+% - B.P. 81-81.5 n_D^{20} 1.4225.

d-Amphetamine sulfate and d-desoxyephedrine sulfate (Aldrich Chemical Company) were prepared by addition of sulfuric acid to an alcoholic solution of vacuum distilled amines. d-Amphetamine sulfate was recrystallized two times from hydro-alcoholic solution. In the case of d-desoxyephedrine sulfate, an ethanol-diethyl ether mixture was used for recrystallization.

N,N-dimethyl amphetamine hydrochloride (Smith, Kline and French Research Laboratories).

Tropaeolin OO (Eastman Organic Chemicals) was purified by recrystallization from water (4 times).

Ortho-hydroxytropaeolin OO was prepared from 4-amino 2-hydroxy sulfonic acid (41) according to the procedure described in "Processes of Dye Chemistry" (42) and recrystallized 4 times from water. It showed a positive phenol test and showed a single spot in thin layer chromatography.

Calcium salt of tropaeolin OO was prepared by recrystallization of tropaeolin OO with equal mixtures of calcium chloride solution and ethyl alcohol. Percent calcium calculated for Tr_2Ca = 5.38%, found 5.39%.*

Procedure

The apparent partition coefficients were determined by a single extraction method. Aqueous solutions were prepared by dissolving the proper amounts of the required components in distilled water which was saturated with the organic phase. In turn, the organic phase was saturated with distilled water.

Twenty-five milliliters of each phase were placed in a separatory funnel and shaken for an hour at 25°C. Enough time was allowed for complete separation of the phases. The absorbance of each phase was measured against a blank

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* Medical Science Laboratories, Medical School, Univ. of Wisconsin, Madison, Wisconsin.

prepared in analogous manner.

The absorbance maximum for dissociated tropaeolin OO in the water layer was found to be 440 mμ and that of hydroxytropaeolin OO to be 444 mμ. Both compounds obeyed Beer's Law in the concentration range used. There was no shift in the maximum when inorganic anions or organic amines were present. The absorbance maximum below pH 2.3 was found to be 530 mμ for both compounds.

The apparent pK_a values of tropaeolin OO and hydroxytropaeolin OO in the system were determined spectrophotometrically and found to be 1.8. The recorded pH values listed are the readings after shaking.

The buffers used in the studies were acetate and phosphate buffers. The concentration of the buffer was .01 molar in all cases except for the calcium and amine studies; in these cases the buffer concentration was 0.004 moles per liter.

Since sodium ions had increased the partition coefficient of both tropaeolin OO and hydroxytropaeolin OO, partition coefficient values were extrapolated to the zero sodium ion concentration in determining the effect of pH on partitioning.

RESULTS AND DISCUSSION

Effect of Concentration of the Dye and Buffer Concentration on Partition Coefficient of Tropaeolin OO and Hydroxytropaeolin OO.

Effect of the Dye Concentration - The partition coefficient of the dye at pH 6.27 and 4.0 (acetate buffer) was found to be independent of the dye when buffer and Na^+ concentrations were kept constant. This can be seen in Figure 1 by plotting the partition coefficient as a function of the total initial dye concentration. The constant value of the partition coefficient would indicate that the dyes do not associate in the range of the concentration employed.

Effect of Total Buffer Concentration - The change in concentration of the buffer when Na^+ concentration was kept constant had no detectable effect on the partitioning of both dyes studied at pH = 6.27 (acetate buffer), proving that partition coefficient of the dye is independent of total buffer concentration.

pH Profile of the Extraction of Tropaeolin OO and Hydroxy-Tropaeolin OO.

Figures 2 and 3 show the partitioning of tropaeolin OO and hydroxytropaeolin OO as a function of pH. The curves indicate the following. Above pH 3.5 the dyes are in the dissociated state and are not extractable into chloroform and amyl alcohol mixture. Below pH 3.5 the undissociated form of the dye is extracted, as evidenced by an increase

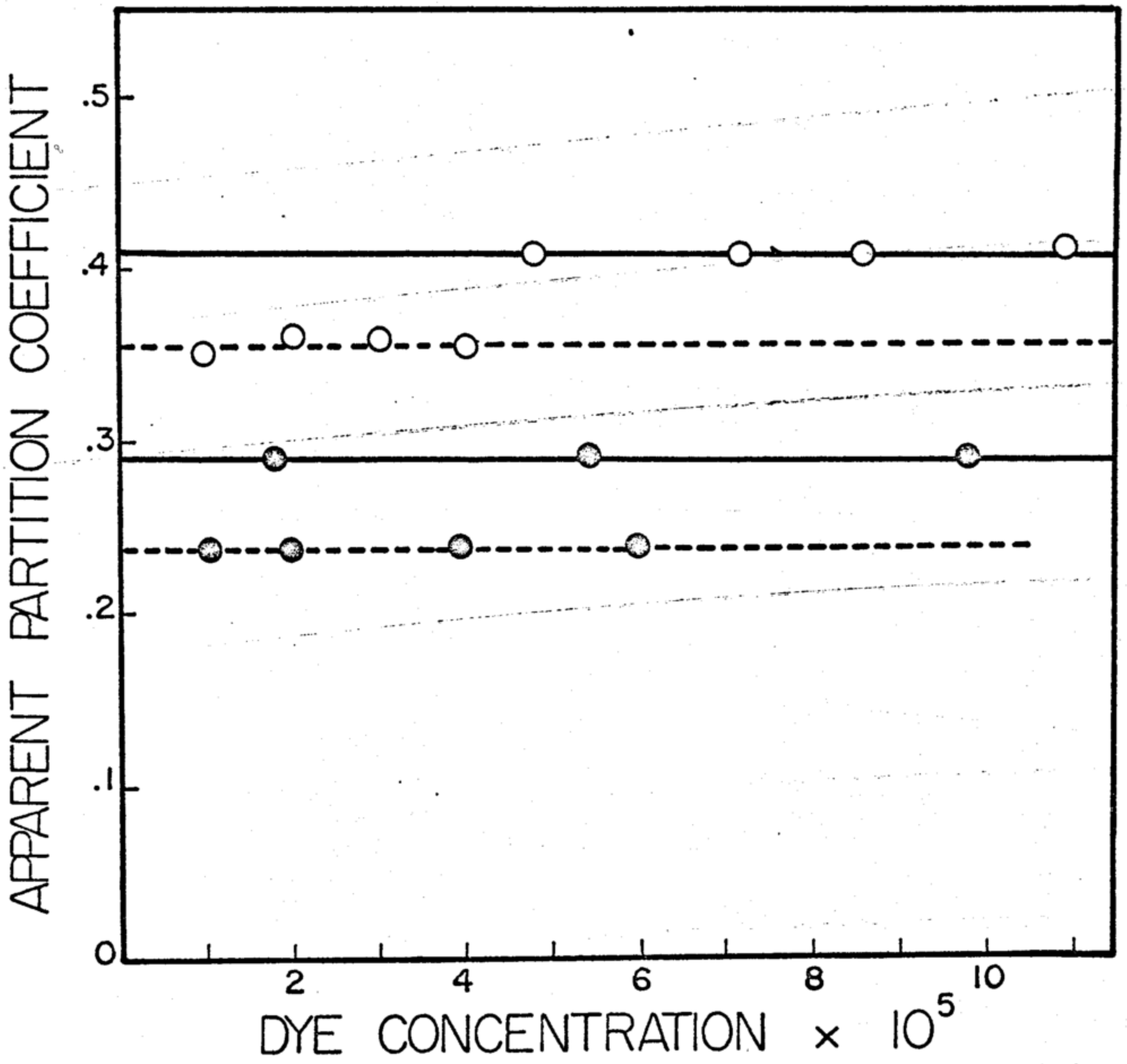

Figure 1. Effect of sulfonate concentration on partition coefficient. Na^+ concentration 0.1 M/L. Organic phase: 16% W/V n-amyl alcohol in chloroform.

○——○ pH 4.05 Hydroxytropaeolin OO
○- - - -○ pH 6.27 Hydroxytropaeolin OO
●——● pH 4.05 Tropaeolin OO
●- - - -● pH 6.27 Tropaeolin OO

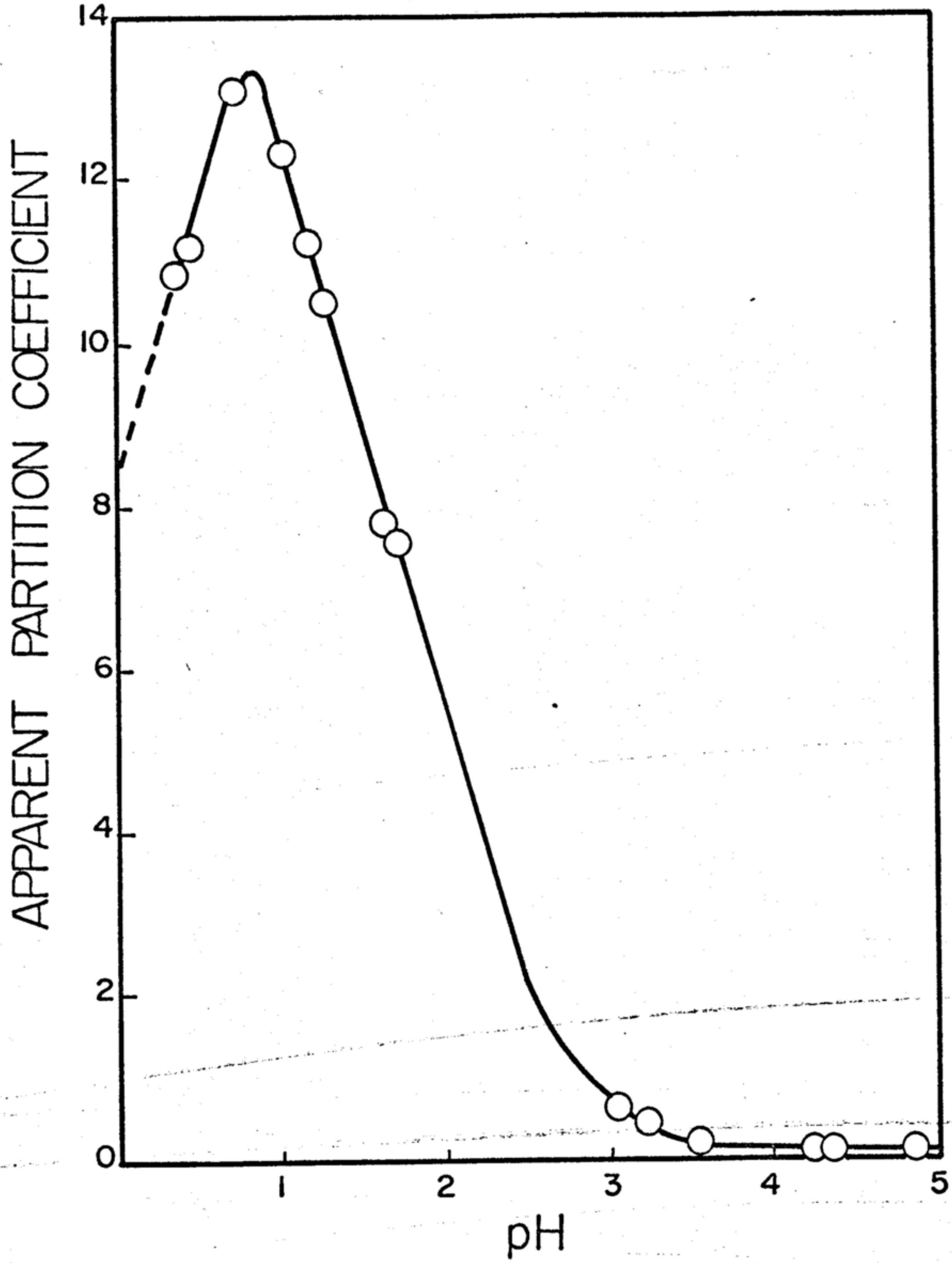

Figure 2. pH profile of the distribution of tropaeolin 00 between water and 16% W/V n-amyl alcohol in chloroform.

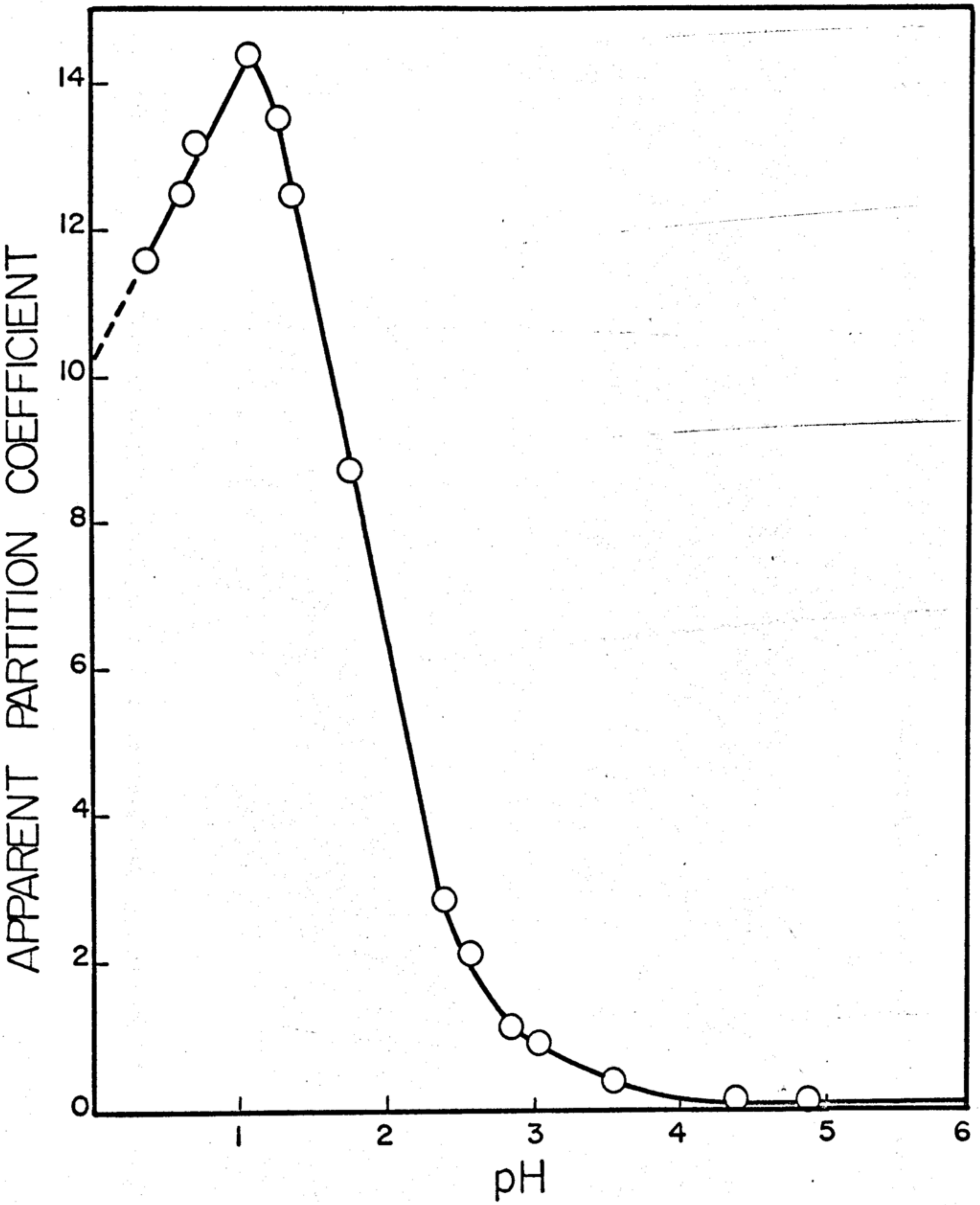

Figure 3. pH profile of the distribution of hydroxy tropaeolin OO between water and 16% W/V n-amyl alcohol in chloroform.

in the partitioning of both compounds. The decrease of the partition coefficient at low pH would suggest that protonation of the diphenylamine portion of the molecule occurs. The pK_a of diphenylamine is .85 (43), and the charged species would not be expected to be extractable.

A further indication of extraction of free acid at pH lower than 3.5 may be given by making a plot of log p.c. vs pH. It is possible to obtain a mathematical expression relating partition coefficient and pH, starting from the equation

$$pH = pK_a + \text{Log}\,\frac{(\text{salt})}{(\text{acid})} \qquad (1)$$

$$\text{Log}\,\frac{(\text{acid})}{(\text{salt})} = -pH + pK_a \qquad (2)$$

Assuming that the acid form is the only form extracted in the organic phase, and salt remains in the H_2O phase, we can rewrite equation 2 as

$$\text{Log}\,\frac{(\text{dye})_{\text{org}}}{(\text{dye})_{H_2O}} = -pH + \text{constant} \qquad (3)$$

or

$$\text{Log apparent p.c.} = -pH + \text{constant}$$

Therefore, plot of Log p.c. vs pH should give a straight line with a slope of (-1).

The plot of log p.c. vs pH (Figure 4) below the region which is independent of pH shows a straight line relationship, but the slope has a value of less than (-1). This can be explained by considering that first of all the equation holds for weak acids, and sulfonic acids are usually strong

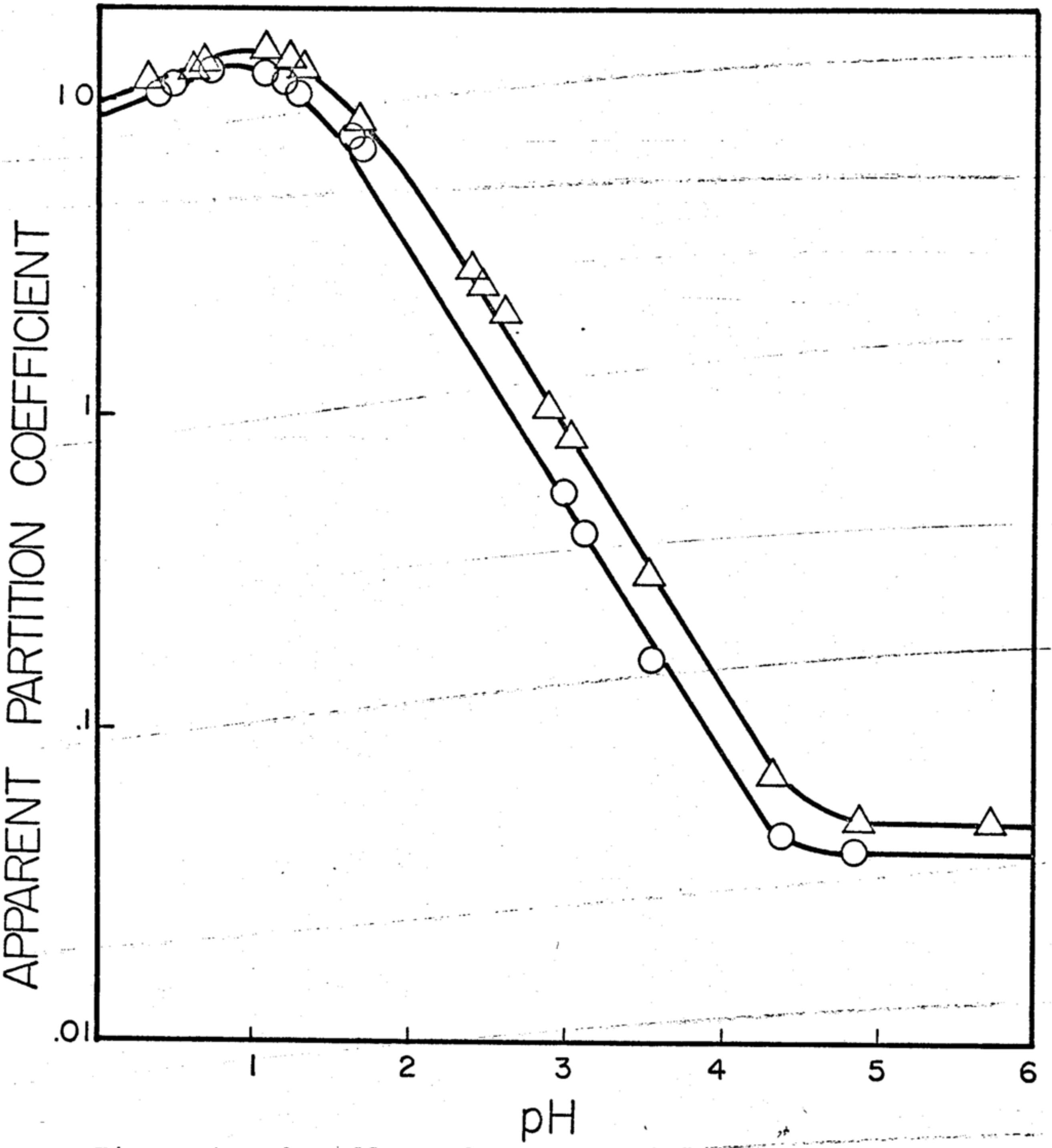

Figure 4. The effect of pH on partition coefficient of the sulfonates.

○ Tropaeolin OO

△ Hydroxy tropaeolin OO

Organic phase 16% W/V n-amyl alcohol in chloroform acids; secondly, the protonation of the dye molecules at low pH would reduce the apparent partition coefficient. The higher partition coefficient of hydroxytropaeolin OO may be explained by the spacial configuration of the molecule, resulting in intermolecular hydrogen bonding between the phenolic hydroxy and sulfonate portion of the molecule which will enhance partitioning.

Effect of Inorganic and Organic Cations on the Formation of Ion Pairs with Tropaeolin OO and Hydroxytropaeolin OO

Although much of the previous acid-dye work cited in the literature concerns the extraction of organic cations, such as amines, as ion pairs with organic or inorganic anions, one must also consider the extraction of inorganic cations. The equations formulated by Higuchi, et al., (36) can be generalized as follows.

$$C^{+}_{aq} + X^{-}_{aq} \overset{K_e}{\rightleftharpoons} (C^{+}X^{-})_{org} \qquad (4)$$

where

C^{+}_{aq} represents any monoprotic cation in the aqueous phase

X^{-}_{aq} represents any monovalent anion in the aqueous phase

$(C^{+}X^{-})_{org}$ represents the ion pair in the organic layer

K_e is the extraction constant for the system

It would appear from earlier work (36,37,38) that in order to have significant extraction the organic phase must contain some agent which will complex or solvate the ion pair to stabilize it in the organic phase. This can be represented as

$$(C^+X^-)_{org} + nM \overset{K_c}{\rightleftharpoons} (C^+X^- \cdot M_n)_{org} \qquad (5)$$

where

M represents the complexing agent in the organic phase

n being the number of moles interacting with the ion pair

$(C^+X^- \cdot M_n)_{org}$ being the solvated ion pair

K_c being the equilibrium constant for interaction

Equations 4 and 5 can be combined to give

$$K_o = K_e K_c = \frac{[C^+X^- \cdot M_n]_{org}}{[C^+]_{aq} \cdot [X^-]_{aq} \cdot [M]^n} \qquad (6)$$

where K_o can be considered as the overall equilibrium constant.

The equation can be further simplified if one keeps the total cation concentration a constant and varies the anion concentration. Under these conditions it is possible to determine the change in the apparent partition coefficient of the cation since under experimental conditions:

$$PC_{cat} = \frac{[C^+X^- \cdot M_n]_{org}}{[C^+]_{aq}} \qquad (7)$$

Therefore, equation 3 can be rewritten as:

$$PC_{cat} = K_o [X^-]_{aq} \cdot [M]^n \qquad (8)$$

or conversely keeping the anion constant and varying the cation, one can determine the effect of the cation on the partition coefficient of the anion

$$PC_{an} = \frac{[C^+X^- \cdot M_n]_{org}}{[X^-]_{aq}} \qquad (9)$$

and

$$PC_{anion} = K_o [C^+]_{aq} \cdot [M]^n \qquad (10)$$

Equations 8 and 10 predict a linear dependence of the partition coefficients of each species as a function of variation in concentration of the other ion.

Effect of Inorganic Cations - Since most studies dealing with ion-pair partitioning are carried out in buffered solutions, it was of interest to determine the effect of the inorganic cations present in the buffer on the dye partitioning. This effect is important since the nature of the inorganic cations would have an effect on the blank extraction in the absence of the organic cations.

Studies were carried out on both azo dyes in a pH range of 6.27 to 1.72, keeping the dye concentration constant at 4.2×10^{-5} moles per liter. The sodium ion concentration was varied from 5×10^{-3} to 2.5×10^{-1} moles per liter by addition of sodium chloride. The organic phase was 16%W/V n-amyl alcohol in chloroform. The results are shown in Figures 5 and 6. Table 1 summarizes the effect of pH on the observed extraction constant, K_e. As is clearly evident, there is a marked dependency of the partition coefficient of both compounds on the sodium concentration at the higher pH values. This can be attributed to the ion-pair formation of the ionized dye anions with sodium cations. At low pH values where the dyes to a great extent exist as undissociated acids, there is little or no sodium dependency as might be anticipated. At intermediate pH values the slopes reflect the changes in ratio between the dissociated and undissociated species present.

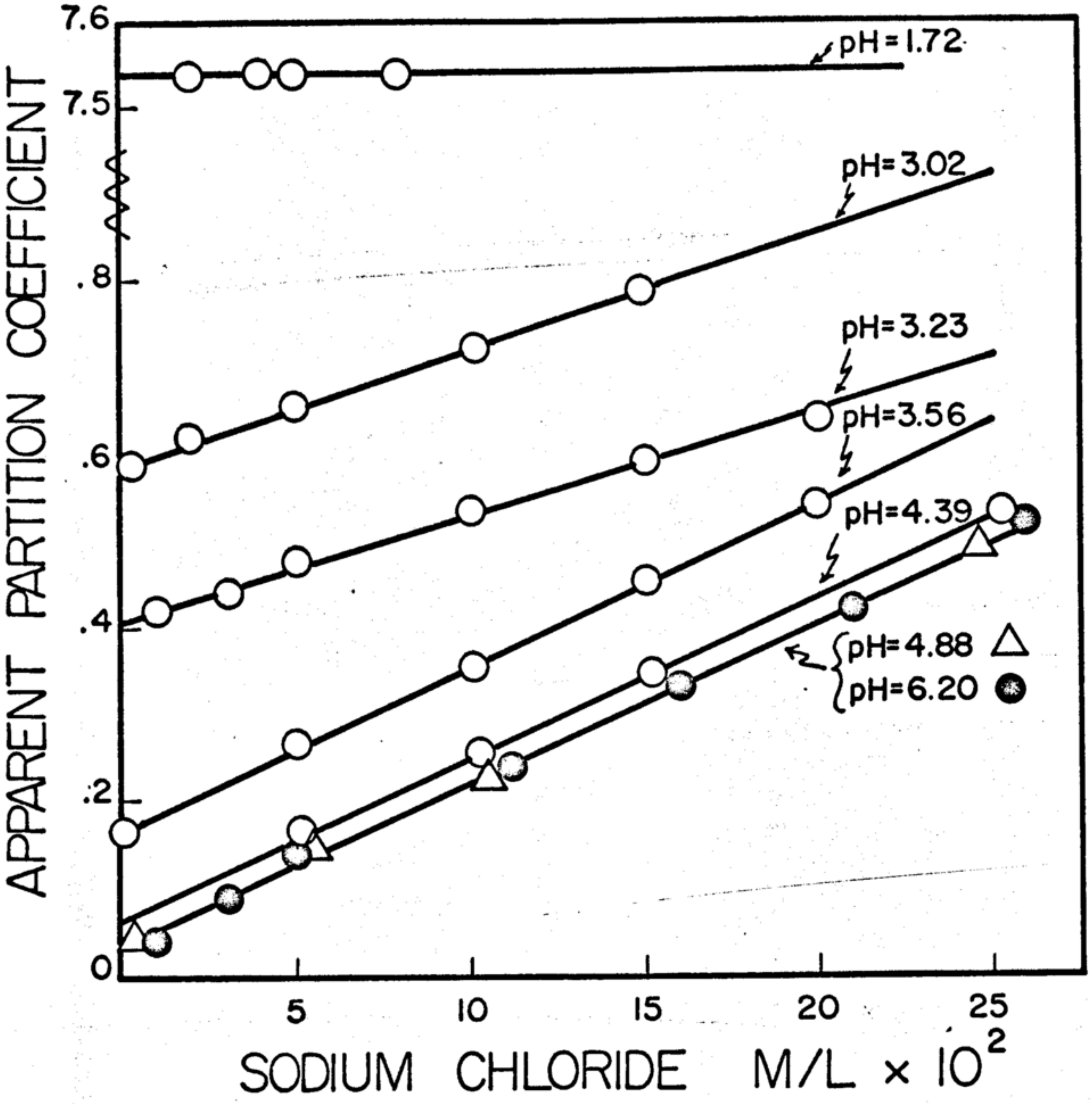

Figure 5. Effect of sodium ion concentration on the partition coefficient of tropaeolin OO. Organic Phase: 16% W/V n-amyl alcohol in chloroform. Tropaeolin OO concentration 4.2 x 10^{-5} M/L phosphate and acetate buffers.

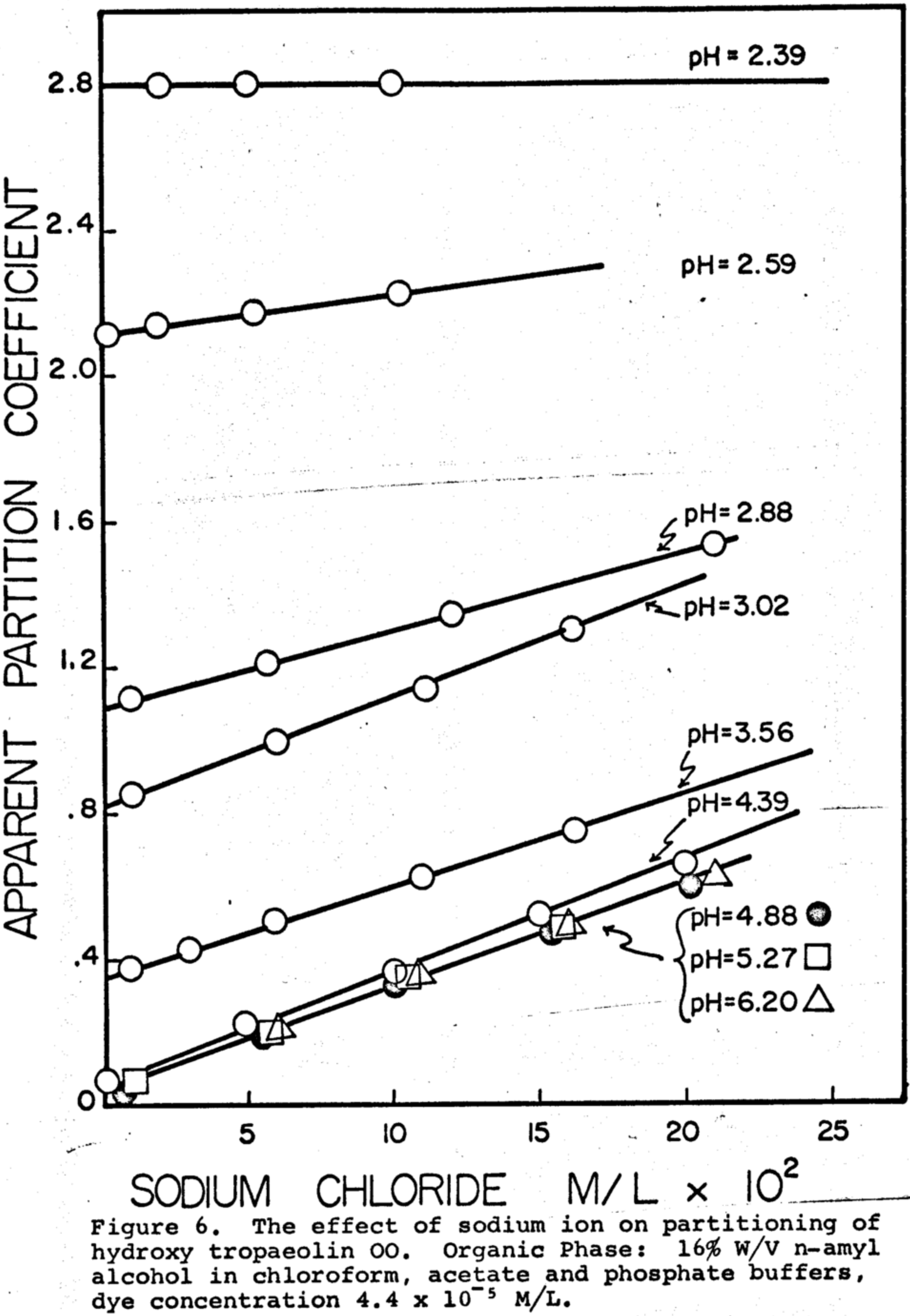

Figure 6. The effect of sodium ion on partitioning of hydroxy tropaeolin OO. Organic Phase: 16% W/V n-amyl alcohol in chloroform, acetate and phosphate buffers, dye concentration 4.4 x 10^{-5} M/L.

Table 1. Extraction Constant (K_e) of Tropaeolin OO and Hydroxytropaeolin as a Function of pH Using Acetate and Phosphate Buffers. Organic Phase: 16% W/V Amyl Alcohol in Chloroform

pH	Tropaeolin OO	Hydroxytropaeolin
6.27	1.85	2.8
4.88	1.85	2.8
4.29	1.90	2.90
3.56	1.9	2.4
3.27	1.26	-
3.02	1.35	2.6
2.88	-	2.15
2.59	-	1.0
2.39	-	0
1.72	0	0

The extrapolation of the lines to zero sodium concentrations were used to generate the pH profiles shown in Figures 2 and 3. It should be noted that at the higher pH values where essentially all of the dyes are in the ionized form, the extrapolation to zero sodium should pass through the origin. However, it will be noted that a finite intercept is observed. The cause of the intercept is unclear since changes in pH in a limited range do not affect the values as would be expected if the deviation were due to extraction of the unionized acid. This intercept cannot be explained as the effect of the impurity because it remains constant on multiple extraction.

In previous studies it was shown (37,40,44,45) that divalent ions did not form extractable ion pairs. Therefore, it was felt that use of these salts in buffer systems would minimize variations in the blank extractions as seen in the case of the presence of sodium cations. The use of calcium buffers was investigated using a calcium acetate buffer system at pH 4.05 and 5.05 with 1.2×10^{-5} moles per liter of tropaeolin OO and varying the calcium ion concentration from 1×10^{-2} to 5×10^{-2} M/L. The organic phase was 16% W/V n-amyl alcohol in chloroform, the same as used in the sodium ion studies. The zero slope of the lines shown in Figure 7 confirms that the divalent calcium ion does not form extractable ion pairs with the dye. The intercepts at zero calcium ion concentration are the same as those for the sodium data previously presented.

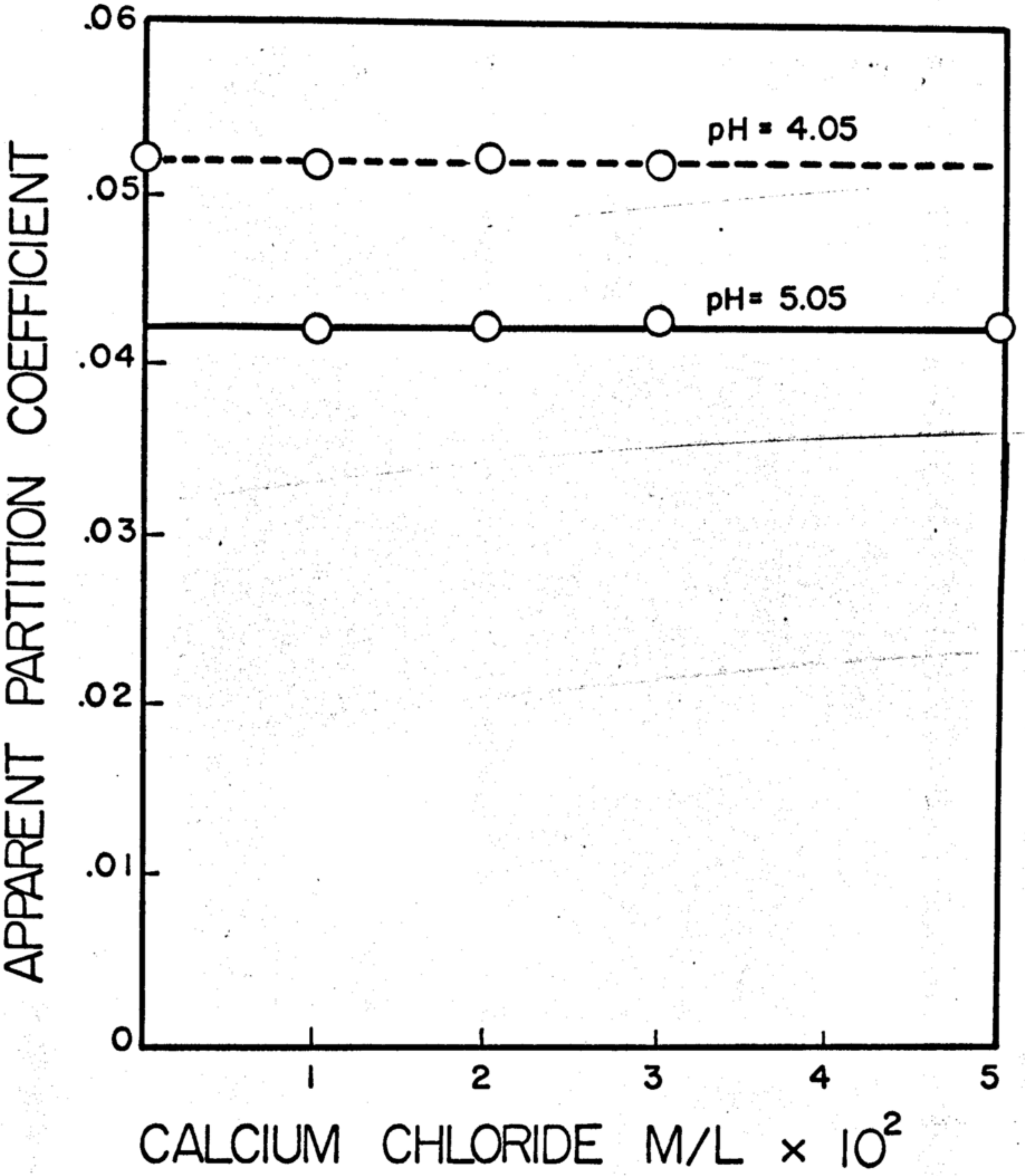

Figure 7. Effect of calcium ion on the partitioning of tropaeolin OO at pH 5.05 and 4.05 calcium acetate buffer. Organic phase: 16% W/V n-amyl alcohol in chloroform.

Similar data were obtained at pH 5.05 when the calcium salt of tropaeolin OO was used and increasing the calcium concentration by addition of $CaCl_2$ as shown in Figure 8. When the total calcium ion concentration exceeded 5×10^{-2} M/L calcium tropaeolin OO precipitated. This would limit the use of this approach in analytical methods.

Formation of Ion-Pairs by Organic Cations with Tropaeolin OO and Hydroxytropaeolin OO

From previous work (26,28,36,37,38,39,40,44) it is apparent that extraction of organic cations such as protonated amines will be highly influenced by the nature of the cation as well as the anion and composition of the organic phase. To determine the effect of the ortho hydroxy substitution on tropaeolin OO as well as the variation in the amines, four basic compounds were tested:

(1) d-Amphetamine
(2) N-methyl amphetamine
(3) N,N-dimethyl amphetamine
(4) Tetrabutyl ammonium chloride

Preliminary data indicated that the primary amine (d-amphetamine) and the secondary amine (N-methyl amphetamine) exhibited rather low partition coefficients in chloroform. This suggested that chloroform alone did not sufficiently solvate the ion pair and that a stronger masking agent was required. Studies were carried out using a mixture of 1.8% W/V n-amyl alcohol in chloroform and determining the partition coefficients of the amines at varying dye concentrations. The results are summarized in Figures 9 - 12.

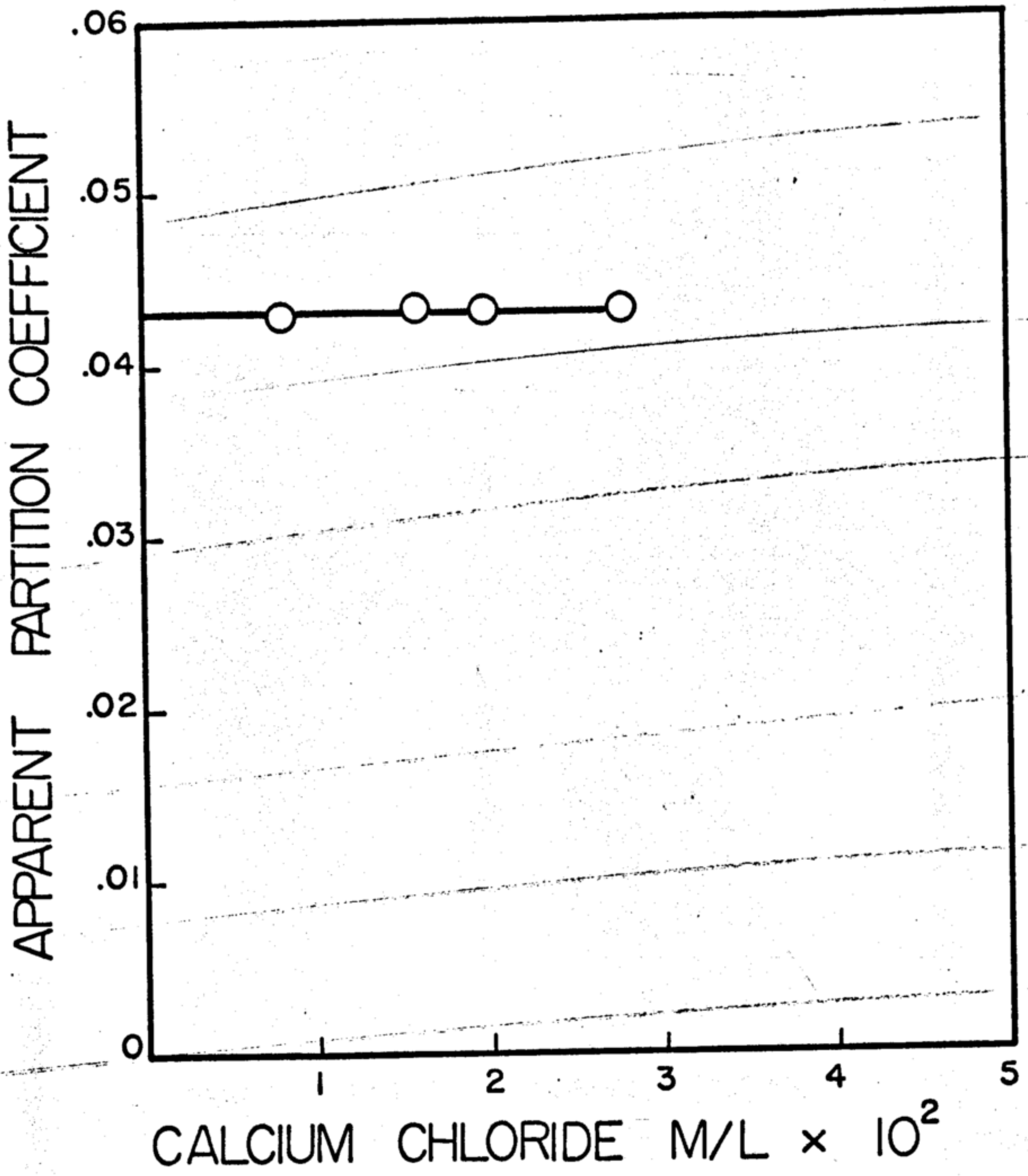

Figure 8. The effect of calcium on partition coefficient of calcium salt of tropaeolin OO $Ca(Tr)_2$, calcium acetate buffer at pH 5.05. Tropaeolin concentration 1.2×10^{-5} M/L. Organic phase: 16% W/V n-amyl alcohol in chloroform.

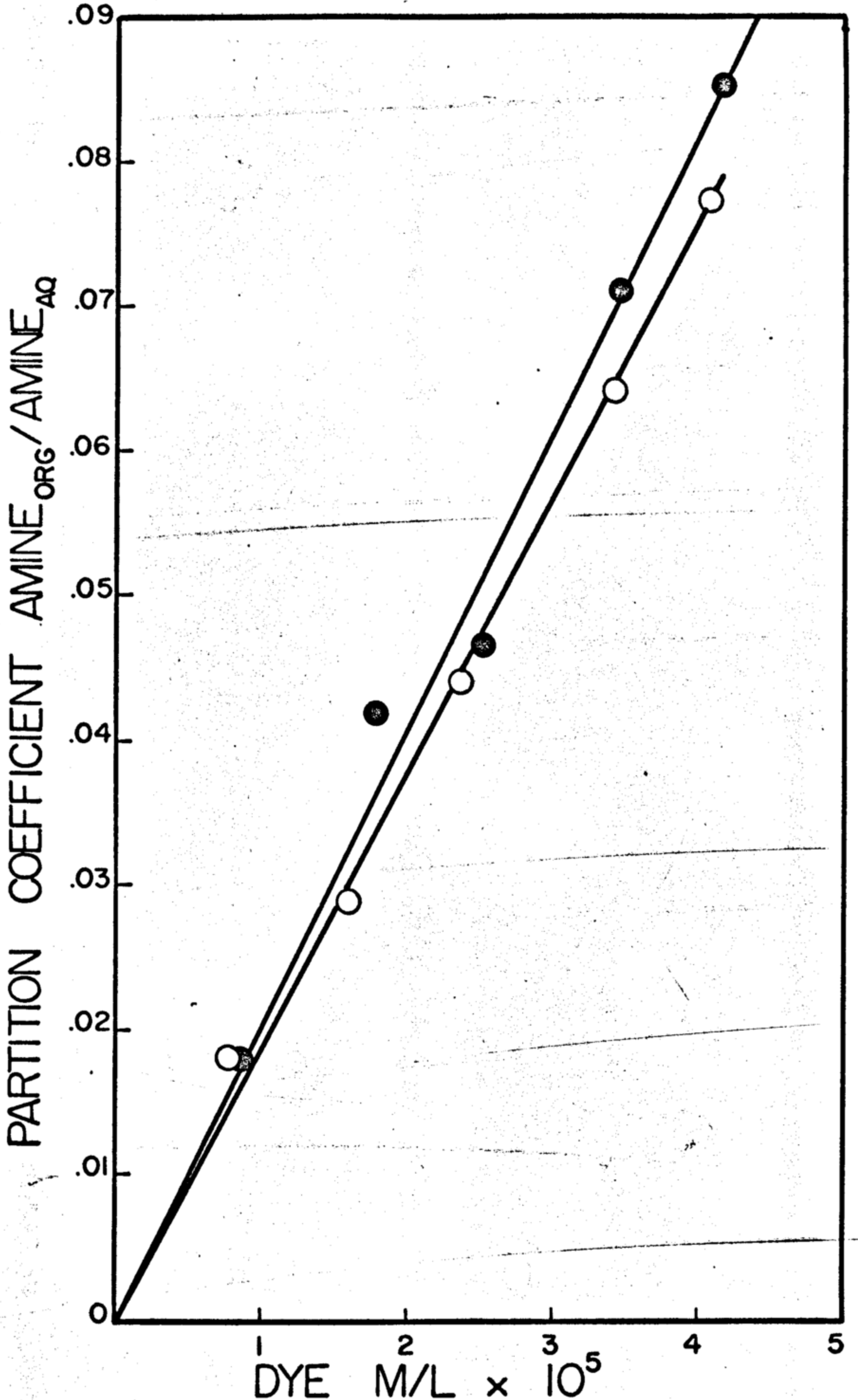
.09
.08
.07
.06
.05
.04
.03
.02
.01
0
1
2
3
4
5
PARTITION COEFFICIENT AMINE$_{ORG}$/AMINE$_{AQ}$
DYE M/L × 10^5

Figure 9

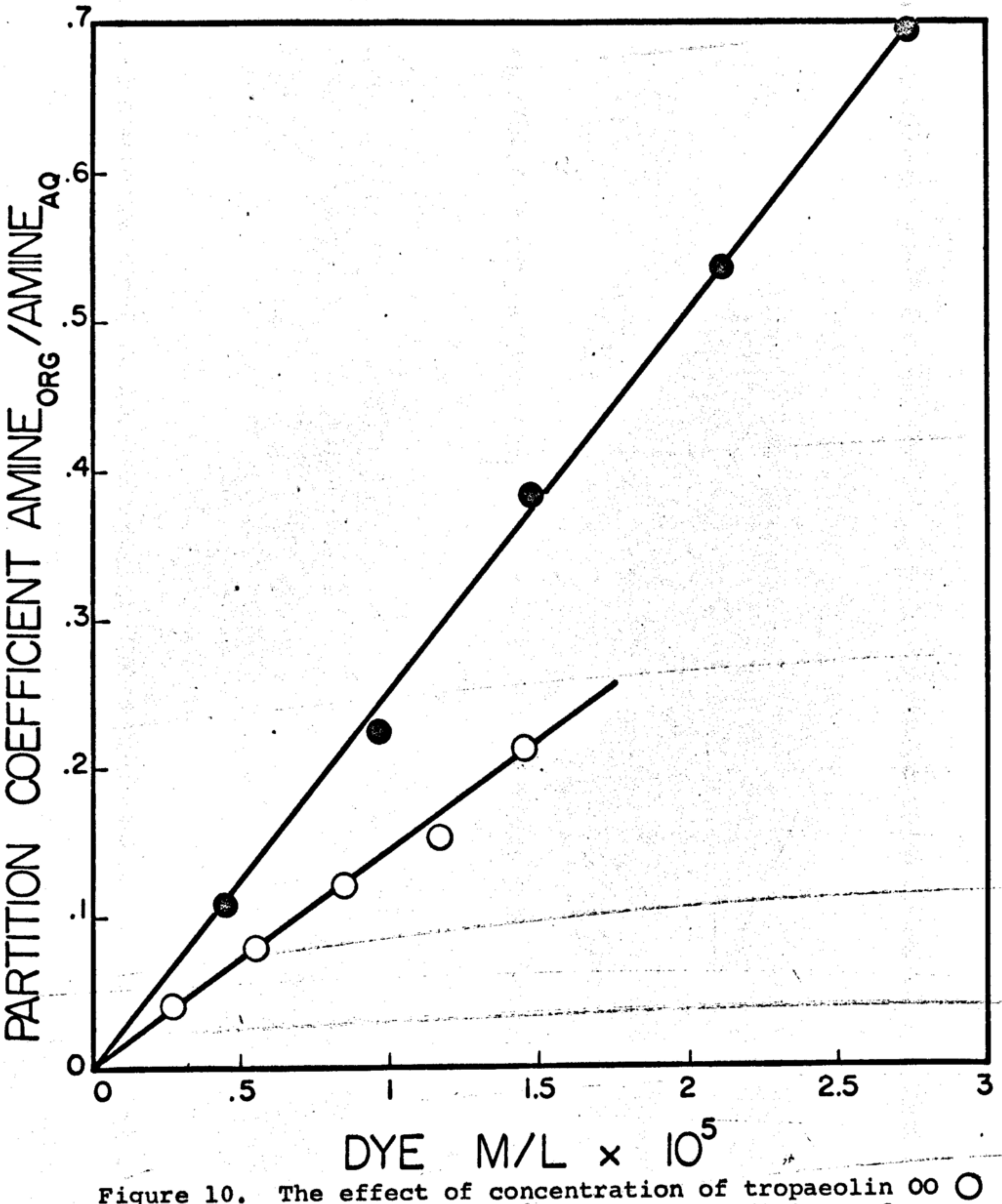

Figure 10. The effect of concentration of tropaeolin OO ○ and hydroxy tropaeolin ● on partition coefficient of n-methyl amphetamine. Organic phase: 1.8% W/V n-amyl alcohol in chloroform. pH = 4.2 (acetate), Na^+ = .004 M/L, amine = 5.2×10^{-5}.

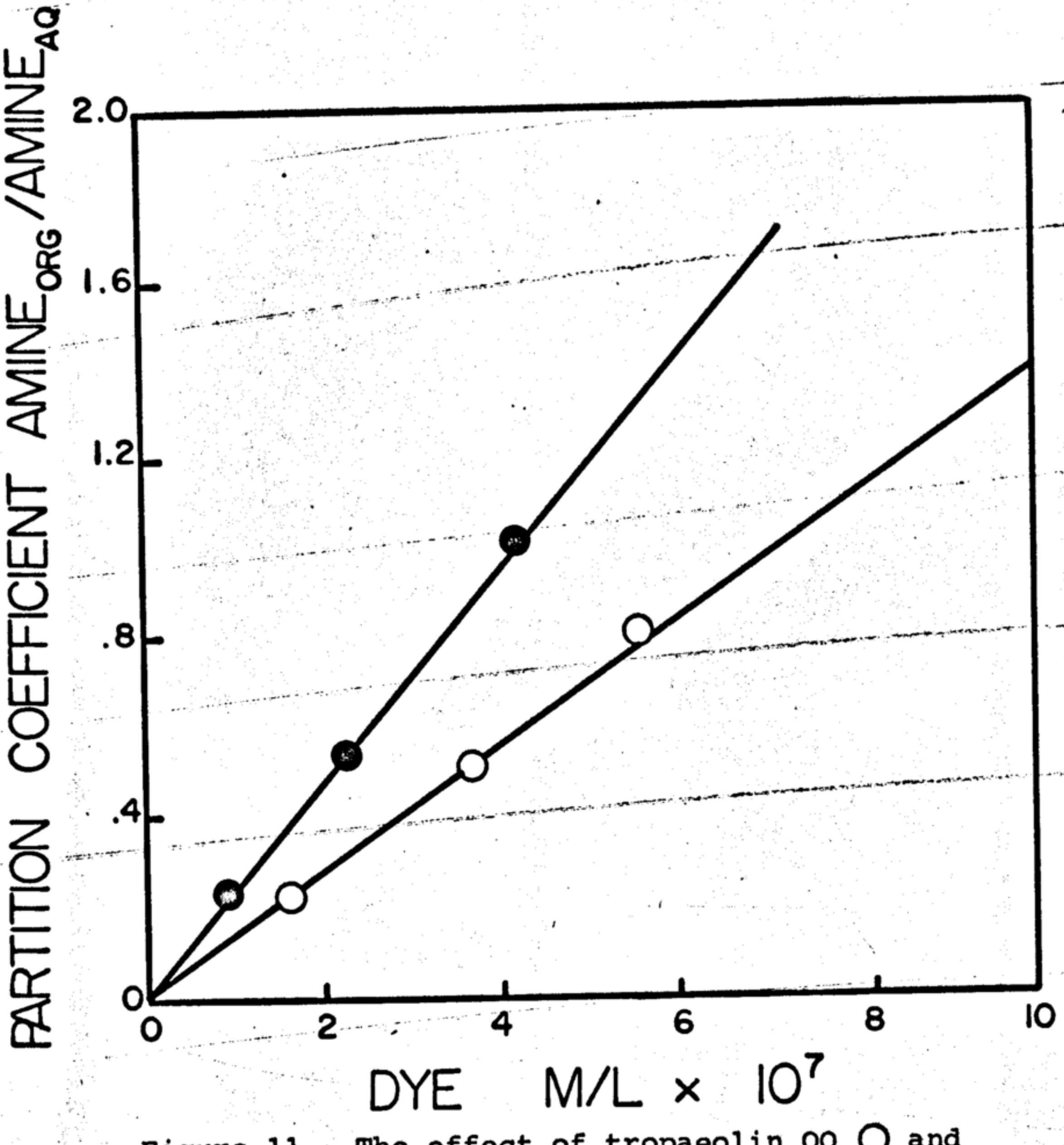

Figure 11. The effect of tropaeolin OO ○ and hydroxy tropaeolin OO ● on partition coefficient of dimethyl amphetamine. Organic phase: 1.8% W/V n-amyl alcohol in chloroform, pH 4.2 (acetate), Na$^+$ conc. = .004 M/L, amine conc. 5×10^{-5}.

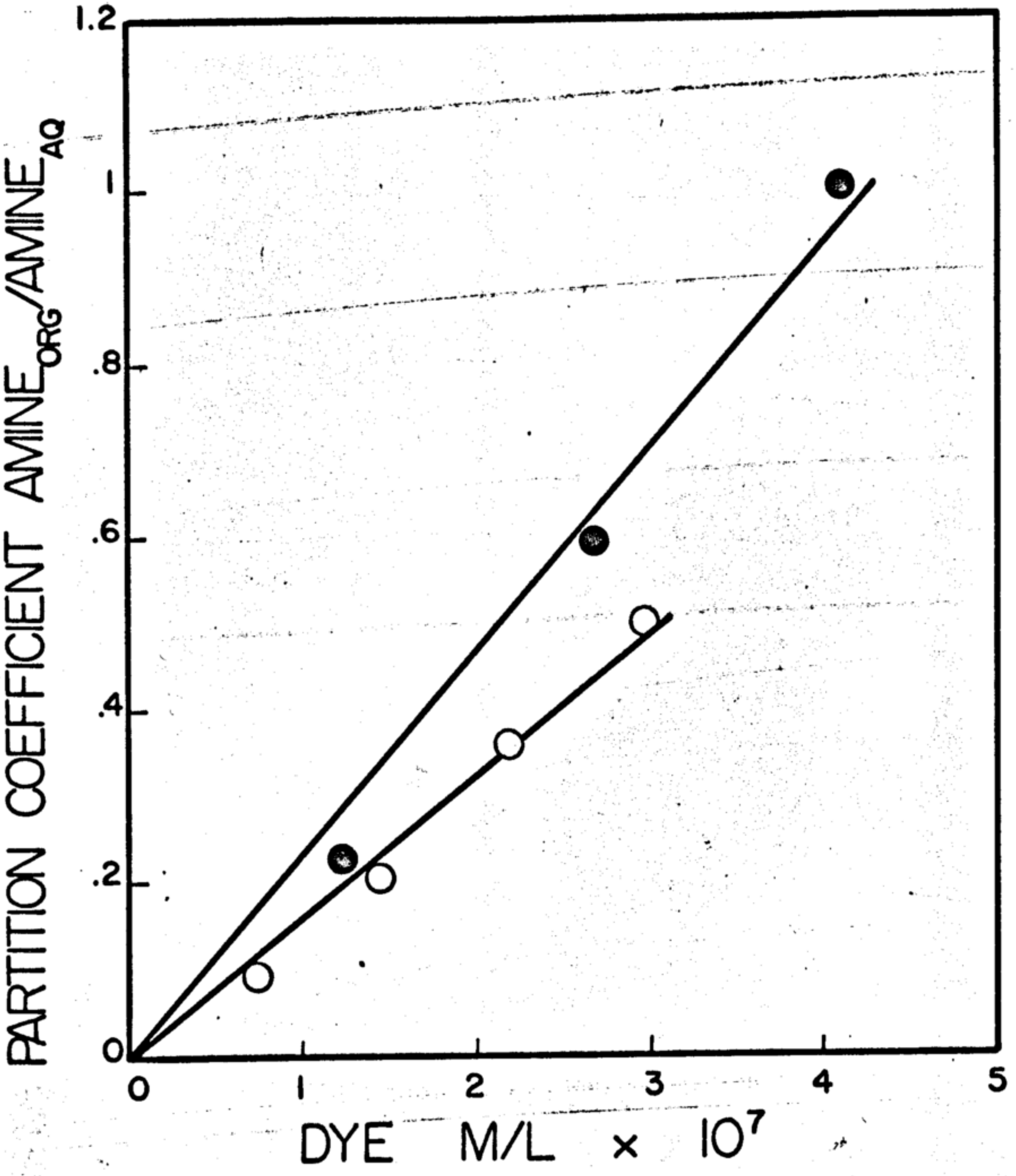

Figure 12. The effect of concentration of tropaeolin OO ○ and hydroxy tropaeolin OO ● on the partition coefficient of tetrabutyl ammonium chloride. Organic phase: 1.8% W/V n-amyl alcohol, in chloroform, pH = 4.2, Na^+ = .004 M/L, amine = 5.0 x 10^{-5} M/L.

Examination of the data indicates that the addition of methyl groups on an amino nitrogen dramatically increases the partition coefficient. The effect appears to be associated with a steric effect rather than changes in the basicity of the nitrogen since the order of increasing partition coefficients was $3^0 > 2^0 > 1^0$ whereas the changes in pKa values are $2^0 > 3^0 > 1^0$. It can be speculated that the additional methyl group enhances the shielding of the charge and consequently favors partitioning into the organic phase. It is probably more likely, however, that the observed effects can be largely attributed to increased hydrophobic interaction between the alkyl groups and the aqueous environment.

The importance of the ortho hydroxy group was not as great as anticipated except in the case of the secondary amine. This again may be due to a favored steric orientation which would enhance shielding by the hydroxy group. It is readily apparent that the n-amyl alcohol acts to shield the charge and enhances partitioning.

To study the effect of steric changes of the amines and dyes on the binding number or molecularity of the masking agent with the ion pairs, a series of experiments were run at varying chloroform concentrations using higher amine concentrations. It was found that cyclohexane did not extract ion pairs; therefore, it was possible to study the effect of chloroform by varying its concentration in cyclohexane.

If one takes the logarithm of equation 8, we find that

$$\log PC_{cat} = \log K_0 + \log (X^-)_{org} + n \log (M) \qquad (11)$$

when the concentration anion is kept constant a plot of log PC_{cat} versus log (M) should yield a straight line with a slope of $\underline{n}$ which is the binding number of chloroform with the ion pairs. Data for the various compounds plotted in accord with equation 11 is shown in Figures 13 - 16. In addition to evaluation of the values for $\underline{n}$, one can compare the values for K_0, the overall extraction and binding constant by extrapolation to 1 molar chloroform concentration. These results are compiled in Table 2.

* * * * * *

Table 2. The Effect of Variation of Cation Structure on the Binding Number and K_0

Compound	Binding Number		K_0	
	Tropaeoline OO	Hydroxy-tropaeoline OO	Trop.	Hydroxy-trop.
d-Amphetamine	4	4	.00218	.003
Methyl amphetamine	2.9	3	.157	.23
Dimethyl amphetamine	3.2	3.7	7.75	3.73
Tetrabutyl ammonium chloride	3.5	3.5	24.3	53.3

* * * * * *

From these studies it is evident that different types of amines can be readily separated by the use of ion-pair extraction and that the partition coefficients can be highly influenced by the nature of the organic phase.

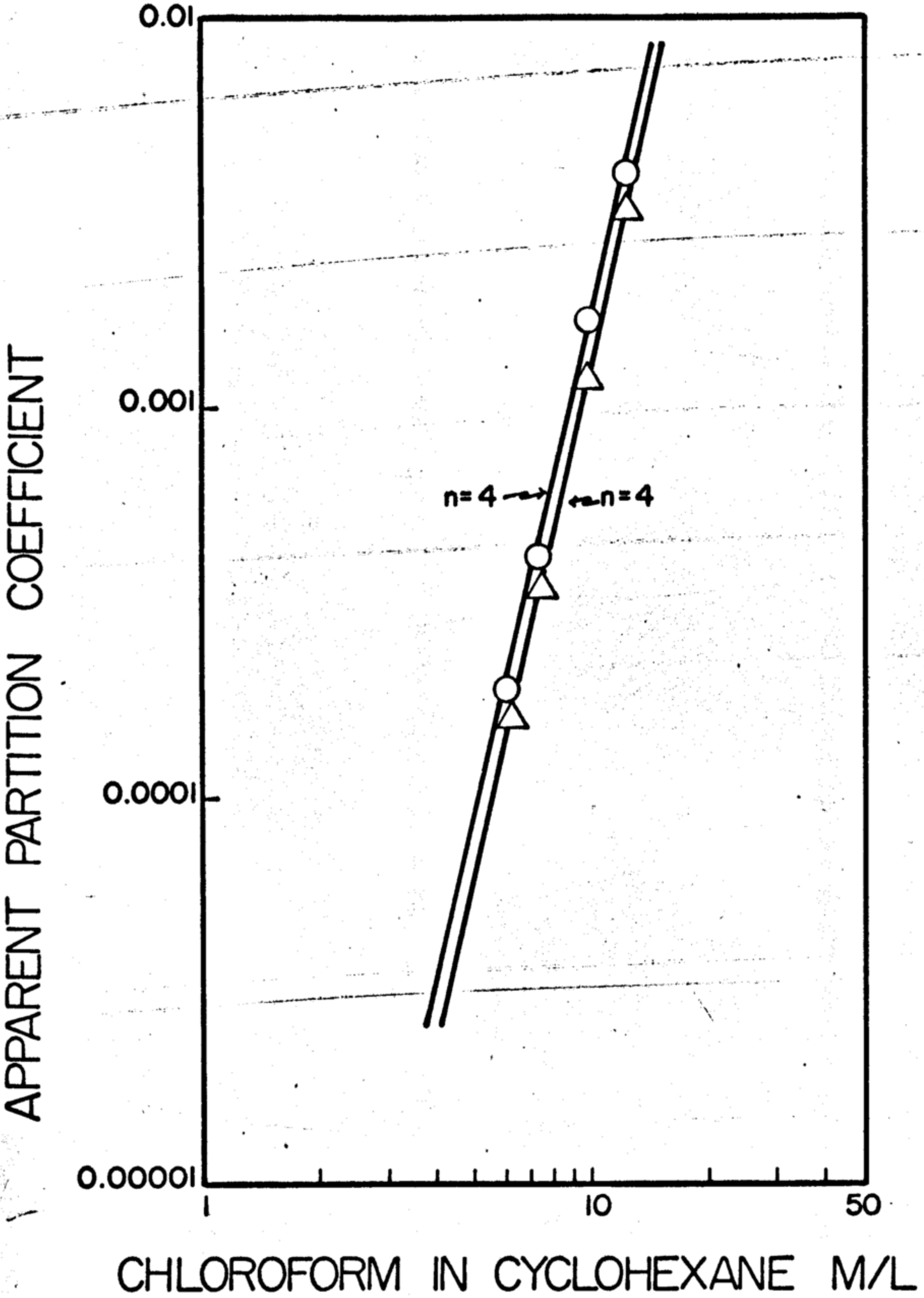

Figure 13. The effect of chloroform on partitioning of d-amphetamine at pH 4.2 (acetate), Na$^+$ conc. = .004 M/L, amine = 5.2×10^{-3} M/L, tropaeolin OO △ = 3.2×10^{-5} M/L, hydroxy tropaeolin OO ○ = 3.65×10^{-5} M/L.

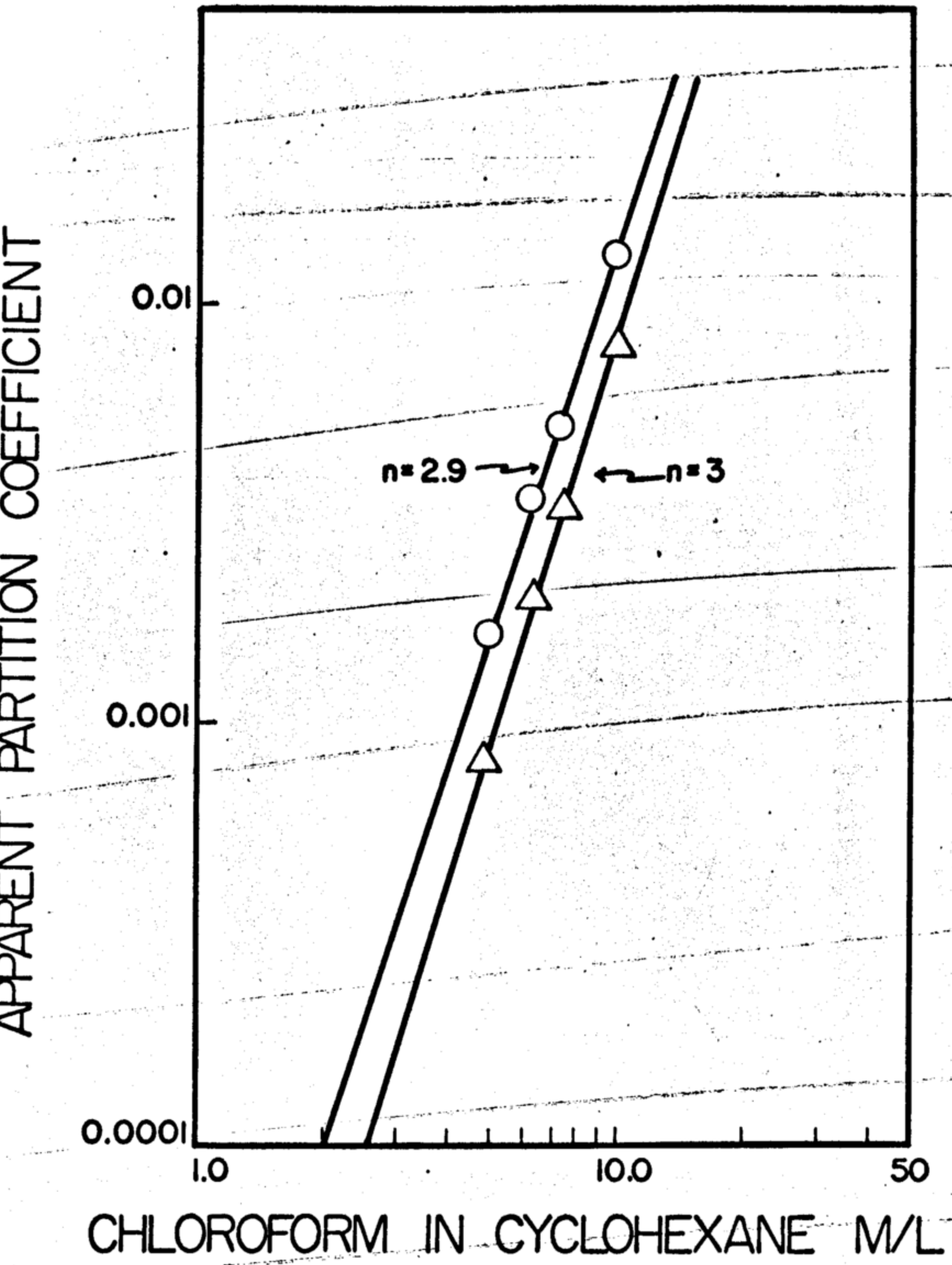

Figure 14. The effect of chloroform on partitioning of n-methyl amphetamine at pH 4.2 acetate, Na^+ conc. .004 M/L, amine conc. 5.2×10^{-3} M/L, tropaeolin OO △ 3.2×10^{-5} M/L, hydroxy tropaeolin OO ○ 3.75×10^{-5} M/L.

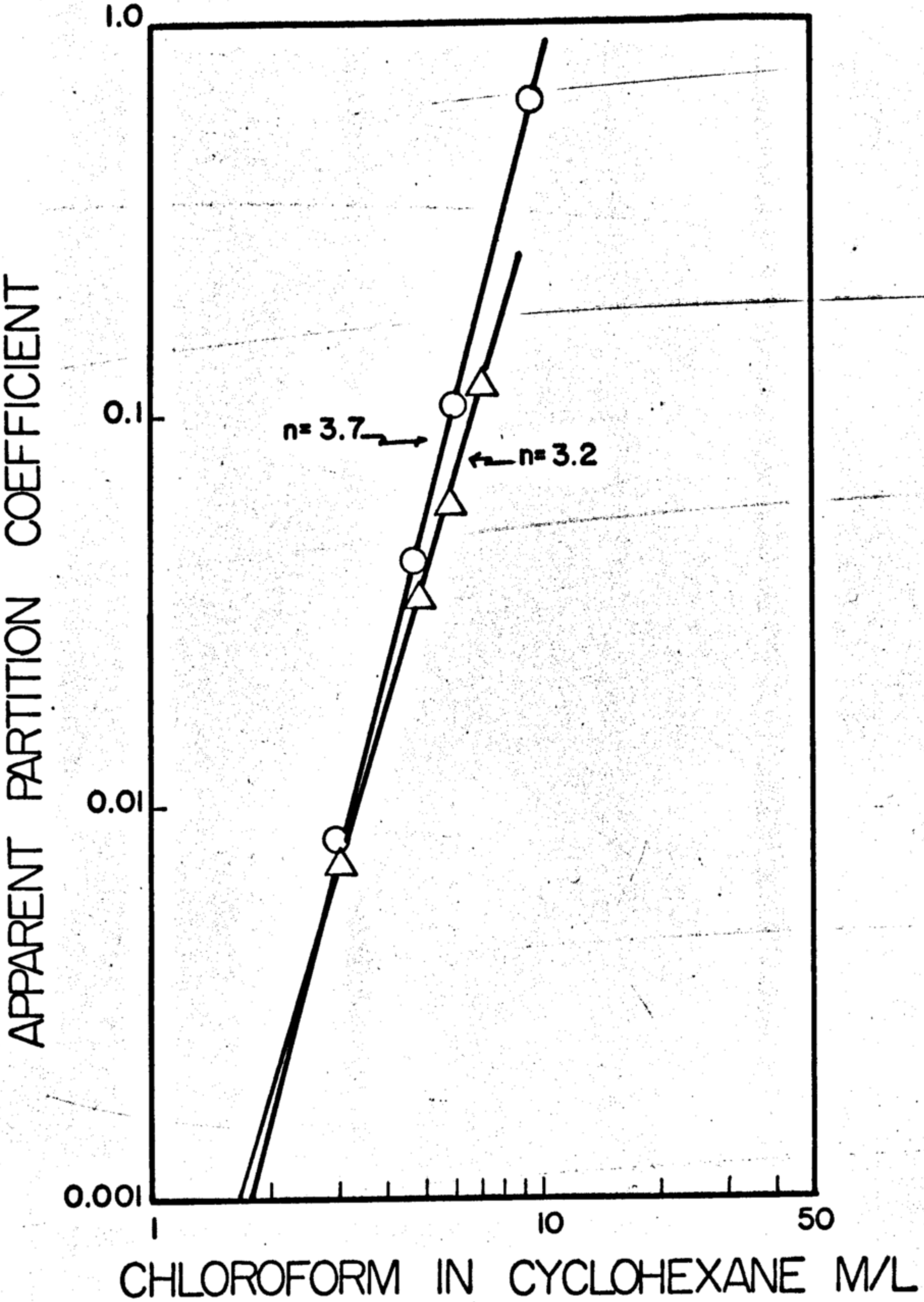

Figure 15. The effect of chloroform on partitioning of dimethyl amphetamine at pH 4.2 acetate, Na^+ .004 M/L, amine 5 x 10^{-5} M/L, tropaeolin OO △ 3.75 x 10^{-5} M/L, hydroxy tropaeolin OO ○ 3.75 x 10^{-5} M/L.

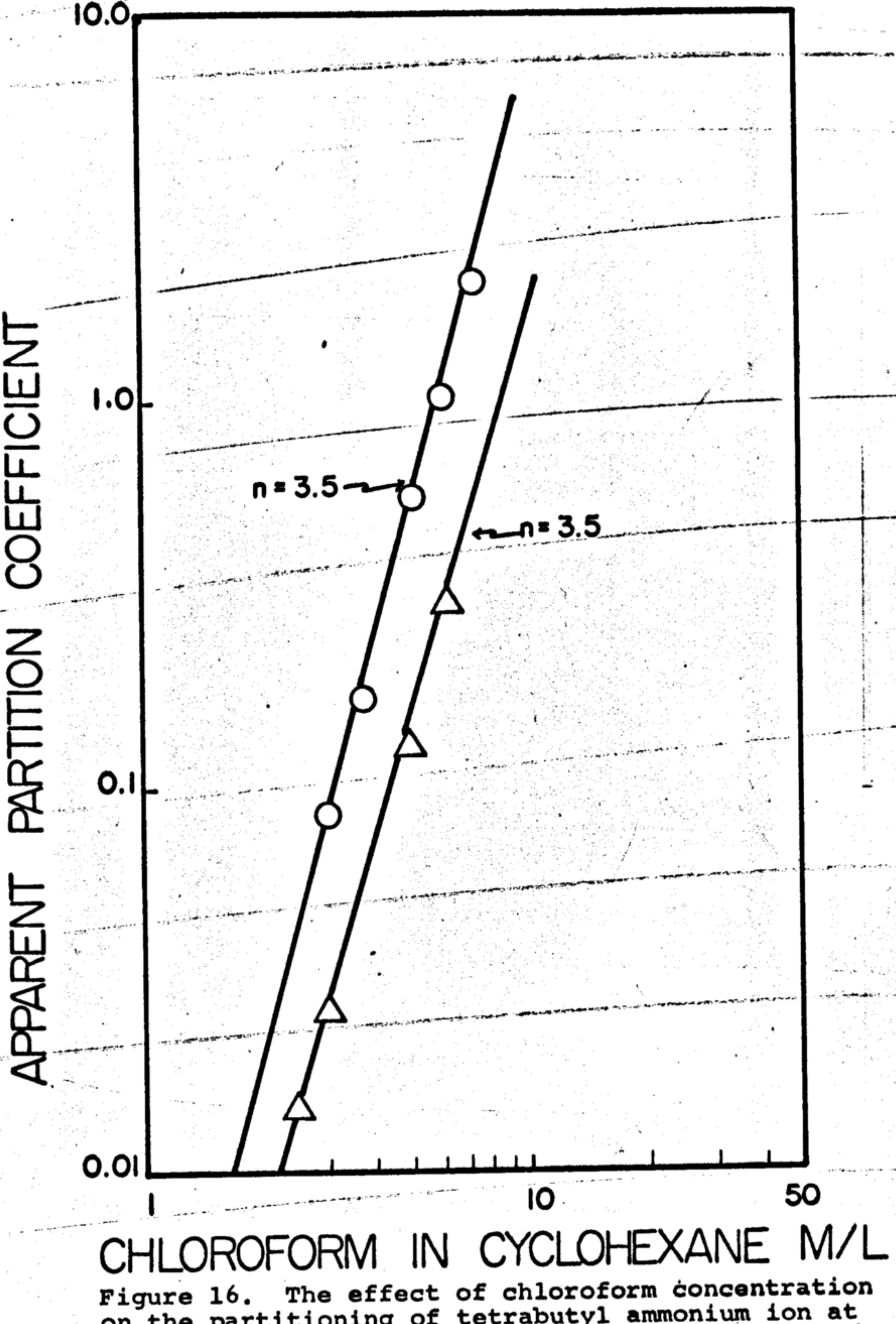

Figure 16. The effect of chloroform concentration on the partitioning of tetrabutyl ammonium ion at pH = 4.2 acetate buffer, amine conc. = 5 x 10^{-5} M/L, tropaeolin OO △ 3.2 x 10^{-5} M/L, hydroxy tropaeolin OO ○ 3.75 x 10^{-5}.

SUMMARY

This work is concerned with the determination of various parameters which influence the formation and extraction of ion pairs. A typical azo mono sulfonic acid dye, tropaeolin OO and its ortho hydroxy analogs were utilized in these studies. The factors investigated were: (1) the effect of dye concentration on the partition coefficient; (2) the effect of buffer concentration on the extraction of the dye amine; (3) the effect of pH on the partitioning of the dye; (4) the effect of concentration and nature of inorganic and organic cations present on the partitioning; (5) the effect of organic masking or solvating agent in the non-aqueous phase.

It was observed that the partition coefficient of the dye was independent of concentration when it was partitioned between an aqueous solution and an organic phase consisting of 16% W/V n-amyl alcohol and chloroform. The linearity of the partition coefficient in the concentration range used (up to 5 x 10^{-5} M/L) indicated that the dye did not undergo appreciable association and that, therefore, partition coefficient could be calculated directly using the analytical concentrations. Similarly, the buffer concentration had no effect on the observed partition coefficient, providing that the cation concentration was kept constant.

Further work on the effect of pH on the partitioning of the dye showed a dramatic effect due to variation of hydrogen ion concentration. The observed partition coefficient was found to be independent of pH in a region above pH 5. Below this there was a marked increase in partition coefficient reaching a maximum of approximately pH 1.2. At a pH of less than one there appeared to be a slight decrease in partitioning which might be attributed to the protonation of the dye. The profile would indicate that the dissociated form of the dye does not partition significantly whereas the undissociated form contributes mainly to the observed partitioning.

Studies dealing with the effect of inorganic cations indicated that under conditions of constant pH the addition of sodium markedly increased the partition coefficient of the dye when the dye was present in its dissociated form and had negligible effect at low pH where the predominant specie present was the undissociated acid. This would indicate that at high pH the dye is extracted as a sodium ion pair. This was found to be true for both tropaeolin OO and its hydroxy analog. Previous studies cited suggested that divalent cations did not form extractable ion pairs. Consequently, a study identical to that of the sodium investigation was carried out using Ca^{++} as the inorganic cation. It was found that partitioning was independent of added calcium and was essentially equal to the value which was extrapolated to zero sodium concentration.

Studies of the effect of organic cations were conducted using a series of amines, specifically d-amphetamine, methyl amphetamine, dimethyl amphetamine, and tetrabutyl ammonium chloride. Studies using these compounds were carried out using an organic phase consisting of 1.8% W/V amyl alcohol in chloroform. In the case of the 1^0, 2^0, 3^0 amines the order of extraction was $3^0>2^0>1^0$. The order of extraction does not correlate with the order of the pKa's of the amines which is $2^0>3^0>1^0$, indicating steric effects are more influencial than basicity of the nitrogen. The enhanced partition of the quaternary might be attributed to the higher molecular weight and increased number of lipophilic groups around the nitrogen.

The effect of the solvating and masking agent in the organic phase was investigated using various concentrations of chloroform in cyclohexane. Cyclohexane was chosen since it alone was not capable of extracting ion pairs. Consequently, the effect of the chloroform could be determined as an independent variable. It was found that the primary amine required greater solvation or shielding than the other compounds, possibly due to the lack of inherent alkyl groups on the amino function.

From these studies, it can be suggested that this technique may be utilized for separating rather similar structures and that the separation can be modified by consideration of the variables studied. The technique offers a rather sensitive method for analysis of various amino compounds.

REFERENCES

1. Higuchi, T., and Bodin, J. I., *Pharmaceutical Analysis*, Higuchi, T., and Brochman-Hanssen, E., Eds., Interscience Publishers, New York, N. Y., 1961, p. 413.

2. Bjerrum, N., *Kgl. Danskeividensk. Selskab*, *7*, No. 9 (1926).

3. Fuoss, R. M., and Kraus, C. A., *J. Am. Chem. Soc.*, *55*, 1019 (1933).

4. Fuoss, R. M., *Trans. Farad. Soc.*, *30*, 967 (1934).

5. Fuoss, R. M., *J. Am. Chem. Soc.*, *80*, 5051 (1958).

6. Reiss, H., *J. Chem. Phys.*, *25*, 400 (1956).

7. Batson, F. M., and Kraus, C. A., *J. Am. Chem. Soc.*, *56*, 2017 (1934).

8. Mukerjee, P., *Anal. Chem.*, *28*, 870 (1956).

9. Mukerjee, P., and Mysels, K. J., *J. Am. Chem. Soc.*, *77*, 2937 (1955).

10. McClellan, B. E., and Freiser, H., *Anal. Chem.*, *36*, 2262 (1964).

11. Zolotov, Yu. A., *Dokl. Akad. Nauk SSSR*, *162*, 577 (1965).

12. Kraus, E. A., *J. Phys. Chem.*, *60*, 129 (1956).

13. Morrison, G. H., and Freiser, H., *Solvent Extraction, Analytical Chemistry*, John Wiley and Sons, Inc., New York, N. Y., 1957.

14. Morrison, G. H., and Freiser, H., *Anal. Chem.*, *30*, 632 (1958).

15. *Ibid.*, *93R* (1964).

16. *Ibid.*, *131R* (1966).

17. Prudhomme, R. O., *Bull. Soc. Path. Exotique*, *31*, 929 (1938).

18. Auerbach, M. E., *Ind. and Eng. Chem.*, *Am. Ed.*, *15*, 192 (1943).

19. Brodie, B. B., Udenfriend, S., and Dill, W., *J. Biol. Chem.*, *168*, 335 (1947).

20. Durick, F., King, J. S., Ware, P. A., and Ronheim, G., J. A. Ph. A., Sci. Ed., 39, 680 (1950).

21. Ballard, C. W., Isaac, J., and Scott, P. C. W., J. Pharm. and Pharmacol., 6, 971 (1954).

22. Thomis, G. N., and Kotonis, A. Z., Anal. Chim. Acta., 14, 11 (1956).

23. Ibid., 14, 457 (1956).

24. Ibid., 16, 261 (1958).

25. Helgren, P. F., Theivagt, J. G., and Campbell, D. J., J. A. Ph. A., Sci., Ed., 46, 630 (1957).

26. Divatia, G. J., and Biles, J. A., J. Pharm. Sci., 50, 916 (1961).

27. Higuchi, T., and Roubal, E. J., Am. Chem. Soc., Abstracts of Papers, 149th Meeting, 28B, April, 1965.

28. Biles, J. A., and Hull, R. L., J. Pharm. Sci., 53, 869 (1964).

29. Biles, J. A., Plakogiannis, F. M., Wong, B. J., and Biles, P. M., J. Pharm. Sci., 55, 909 (1966).

30. Schill, G., and Marsh, M., Svensk. Farm. Tidskr., 67, 385 (1963).

31. Schill, G., Acta. Pharm. Suec., 1, 101 (1964).

32. Ibid., 1, 169 (1964).

33. Ibid., 2, 19 (1965).

34. Ibid., 2, 99 (1965).

35. Ibid., 2, 109 (1965).

36. Higuchi, T., Michaelis, A., Tan, T., and Hurwitz, A., Anal. Chem., 39, 974 (1967).

37. Tan, T., Master's Thesis, University of Wisconsin, 1966.

38. Michaelis, A., Ph.D. Thesis, University of Wisconsin, 1967.

39. Michaelis, A., and Higuchi, T., Anal. Chem., 40, 1925 (1968).

40. Roubal, E. J., Ph.D. Thesis, University of Wisconsin, 1968.

41. Miller, A. L., Mosher, H. S., Gram, F. W., and Whitmore, F. C., J. Am. Chem. Soc., 71, 3559 (1949).

42. Fierz-Davis, H. E., and Blangey, L., Fundamental Processes of Dye Chemistry, English Translation by Vittum, P. W., Interscience Publishers, Inc., New York, N. Y., 1949, p. 273.

43. Handbook of Chemistry and Physics, C. D. Hodgman, Ed., 39th Ed., The Chemical Rubber Company, Cleveland, Ohio, p. 1642.

44. Higuchi, T., and Kato, K., J. Pharm. Sci., 55, 1080 (1966).

45. Schill, G., Modin, R., and Persson, R., Acta. Pharm. Suec., 2, 119 (1965).