CETYLTRIMETHYLAMMONIUM BROMIDE CATALYZED REACTION OF 1-FLUORO-2,4-DINITROBENZENE WITH AMINES : KINETIC STUDIES AND ANALYTICAL APPLICATIONS

BY

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A thesis submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

(Pharmacy)

at the

UNIVERSITY OF WISCONSIN-MADISON

1981

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Professor Kenneth A. Connors for his patience and interest in the development of my scientific career. It has been a valued privilage to be associated with Professor Connors. I shall always admire his remarkable enthusiasm, dedication and gift for scientific insight.

I also like to thank Professor Pasupati Mukerjee and Dr. Noriaki Funasaki for their helpful comments and criticisms. Many thanks are also due to the members of my doctoral committee.

The technical assistance of Professor William Porter, Mr. Shude Yang, Mr. Robert Aylesworth, and Mr. Kenneth Berg are gratefully appreciated.

To Mr. Philip Wang and Miss Jane Lo, goes gratitude for their assistance in typing this thesis.

Finally, my sincere appreciation and gratitude are extended to my friends and colleagues without whom my experience as a graduate student would have been much less enriching and enjoyable.

To the Lord

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Michelle Pik-Han Wong

Under the supervision of Professor Kenneth A. Connors

1-Fluoro-2,4-dinitrobenzene undergoes aromatic nucleophilic substitution by amines to give arylated amines. This reaction was employed to study the amino acid sequence of insulin, and it has since been adapted to the analysis of other amines and polyamines. The reaction is carried out in an alkaline medium, and the product is measured spectrophotometrically. For the determination of many amines and amino acids, the reaction was essentially complete (within 5% of its maximum) for most samples in 10 minutes at 65° ; some compounds required $\geqslant$ 20 minutes at this temperature.

Several surfactants, notably the cationic cetyltrimethylammonium bromide, catalyze substitution reactions between fluorodinitrobenzene and amine nucleophiles. Therefore this reaction appeared to provide

an appropriate system for testing the proposal that micellar catalysis may be analytically useful. We described the use of micellar catalysis to increase the derivative formation rate prior to spectrophotometric measurement of a product. [K. A. Connors and M. P. Wong, J. Pharm. Sci., 68, 1470 (1979)]. This thesis reports the further development of this system to the quantitative determination of many amines, amino-acids and peptides. All rate enhancements are analytically useful, and this method can be applied with the reaction carried out at room temperature. An additional rate enhancement factor of 3 was obtained by increasing the temperature to 45 °C. The concentration-response curves are linear at 25° and 45° C, and the slopes of these curves are identical.

The observed rate constant dependence on surfactant concentration can be fitted by Berezin's model, and by an equivalent "binding-partitioning" model derived in this work. These models allow the separation of catalysis into intrinsic rate effects and concentration effects. Investigation of the mechanism of the micellar catalyzed reaction of 1-fluoro-2,4-dinitrobenzene with five amines, namely alanine, phenylalanine, aniline, p-methylaniline and p-methoxyaniline, reveals that catalysis is mainly

a result of the concentration of reactants in the micelle. The factor which represents the acceleration produced by the localization of the reactants in the micelle is highest for phenylalanine among the five amines studied. Phenylalanine possesses a hydrophobic moiety and a negatively charged carboxyl end. These two factors contribute to the rate enhancement : the possession of a negative charge, and the possession of a nonpolar moiety. When both features are present, as in phenylalanine, very high rate enhancement can occur.

APPROVED _____

(Kenneth A. Connors)

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I. INTRODUCTION

Ai) Micelles in Analysis

Possible analytical applications of micellar catalysis are conveniently considered in terms of reactivity and selectivity. In focussing on reactivity changes induced by micelles we seek to take advantage of changes in absolute rates. Usually this means an increase in reaction rate; but one can imagine problems in which an inhibition might be analytically useful, for example to reduce an interference.

The general type of analysis under consideration is that in which the sample substance is made to undergo one or more chemical reactions, followed by detection, usually quantitative, of a product or reactant. There is no doubt that for an extended period of time modern analytical chemistry did not greatly concern itself with the study of such methods, concentrating instead on purely instrumental techniques. More recently a return in research interest to the reaction-based methods is evident. This is particularly obvious in analytical methods culminating in chromatographic separations,

when to achieve desirable separation properties (in gas chromatography) or detection characteristics (in liquid chromatography) the sample is subjected to a chemical reaction (derivatization). Biochemical analytical problems also find derivative formation useful, for example, to develop a fluorophor.

It is in this context that catalysts and catalytic phenomena are of analytical importance. Many analytical reactions are relatively slow, in the sense that the reaction component of the analysis limits the overall speed of analysis. The nature, as well as the magnitude, of the catalytic effect is important; for example, if the reaction conditions can be made milder, side effects and interferences may be reduced.

Thus the development of catalysts for analytical reactions in order to reduce reaction times and achieve milder reaction conditions is the approach to be taken. In the course of such investigations opportunities to study mechanisms of the phenomena can be pursued, because such understanding may lead to better control of analytical sensitivity and specificity.

A micelle is a unique kind of catalyst. Micellar systems possess certain features and properties that

should be very useful in helping to overcome many of the problems commonly encountered in the development of new analytical methods. These can include some of the following: solubility problems of the required analytical reagents; slowness of the analytical reaction; unfavorable equilibrium position for methods based on an equilibrium reaction; problems due to undesirable side reactions; inadequate sensitivity or selectivity; spectral overlap problems in spectral methods of analysis; and instability of the analytical species upon which the analytical method is based. In addition, since only very small amounts of surfactants are required for micelle formation, there would be essentially no loss in sensitivity due to dilution. Surprisingly, in spite of the many useful and well documented properties of micellar systems, only a relatively few analytical applications have been reported. The use of surfactants and micellar systems in analysis was recently reviewed by Hinze (1).

The majority of the analytical work done using surfactant and micellar systems concern their application to ultraviolet visible absorption spectral methods, particularly those involving metal ion determinations. Some metal ions react with an appropriate chelometric indicator or ligand to form binary metal-chelate complexes that are not soluble

in water. Thus the complex must be extracted into a suitable organic solvent prior to measurement of the absorbance. The step is time-consuming and is expensive because of the high cost of the purified organic solvent required for the extractions. However, in some instances it has been reported that addition of a surfactant to the aqueous system renders the metal complex water-soluble. This is due to formation of an aqueous micellar system which solubilizes the metal-chelate complex. As can be seen from Table I, nonionic micellar systems such as that formed from the surfactant polyoxyethylene p-1,1,3,3-tetramethylbutylphenol (Triton X-100 or TX-100) are typically employed in these methods. There has been no extensive study of the exact solubilization sites of the metal-chelate complexes in micellar TX-100.

Table I. Analytical Parameters of Some Spectrophotometric Methods for the Determination of Metal Ions Using Nonionic Micellar Systems.

Surfactant	Metal Ion	Complexing Agent	λ_{max} (nm)	ϵ ($\times 10^{-4}$) $M^{-1}cm^{-1}$	Ref.
TX-100	Ni^{2+}	TAN	595	4.0	2
TX-100	Ni^{2+}	TAM	560	6.5	3
TX-100	Cu^{2+}	Dithizone	671	2.8	4
TX-100	Mg^{2+}	XB	515	---	5
TX-100	Zn^{2+}	PAN	555	5.6	6
POESLE	Fe^{3+}	ECR	---	---	7

TAN = 1-(2-thiazolylazo)-2-naphthol

TAM = 2-(2-thiazolylazo)-5-dimethylaminophenol

XB = sodium [1-azo-2-hydroxy-3-(2,4-dimethylcarboxyanalido)-naphthalene-1'-(2-hydroxybenzene-5-sulfonate] or Xylidyl Blue

ECR = Eriochrome Cyanine

PAN = 1-(2-pyridylazo)-2-naphthol

POESLE = Polyoxyethylene sorbitane laurylester

The addition of metal ions to solutions of the micellar solubilized chelometric indicators usually results in the formation of intensely colored complexes over a wide pH range. These complexes have different characteristics from those formed in aqueous solutions in the absence of micelles. This forms the basis for the micellar improved methods. Table II shows a comparison of the spectral parameters of some of the metal-chelate complexes formed in the absence and presence of micellar systems. In the micellar system, there is a greater wavelength separation between the excess chelating agents' absorption and that of the metal-chelate complex formed. Hence the spectral overlap problem is eliminated or greatly reduced.

Table II. Comparison of Spectral Parameters for Reagents and Metal Complexes formed in the Absence and Presence of Micellar System.

System (Conditions)	λ_{max}, nm		ϵ ($\times 10^{-4}$), $M^{-1}cm^{-1}$		Ref.
	Blank Reagents	Metal Complex	Blank Reagents	Metal Complex	
Gallein, La^{3+}					
(no micelles)	540	595	0.20	1.4	8
(with CTAC micelles)	543	630	0.03	2.8	
DSPF, Ti^{4+}					
(no micelles)	500	570	----	10.8	9
(with CPB micelles)	470	620	----	12.0	
Gallein, Er^{3+}					
(no micelles)	540	595	0.02	1.8	8
(with CTAC micelles)	543	615	0.17	2.2	
BDAS, Fe^{3+}					
(no micelles)	---	550	----	4.5	10
(with CPC micelles)	---	565	----	6.7	

Table II. (cont.)

System (Conditions)	λ max, nm		ϵ ($\times 10^{-4}$), $M^{-1}cm^{-1}$		Ref.
	Blank Reagents	Metal Complex	Blank Reagents	Metal Complex	
CAS, Fe^{3+}					
(no micelle)	---	570	----	4.2	11
(with CTAC micelles)	---	630	----	14.7	

CTAC = cetyltrimethylammonium chloride

DSPF = disulfophenylfluorone

CPB = cetylpyridinium bromide

CPC = cetylpyridinium chloride

BDAS = sodium 2-bromo-4,5-dihydroxyazobenzene-4'-sulfonate

CAS = Chromazurol S

The stability of the metal-chelate complexes is enhanced in the presence of cationic surfactant. This is due to favorable interactions between the anionic metal-chelate complexes and the positively charged head groups of the cationic surfactant molecules of the micellar system. Table III presents some data concerning the thermodynamic stability of such complexes in micellar systems. For the Eriochrome Azurol B metal ion system, the complexes are about one hundred times more stable in the presence of micelles than in their absence (12). The procedures in the absence of micelles sometimes showed poor adherence to Beer's Law because of simultaneous formation of two or more complexes between the metal and the chelometric indicator (13). In the micellar systems, the preferred formation and stability of one form greatly reduces this problem.

Table III. Summary of Stability Constants for the Formation of Metal-Chelate Complexes in Presence and Absence of Micellar Systems.

Surfactant	Metal Ion	Complexing Agent	Log $K_{stability}$ no micelle	Log $K_{stability}$ micelle	Ref.
CTAC	Be^{2+}	CBG		10.6	14
CTAB	Sc^{3+}	EAB	8.1	10.1	11
	Y^{3+}		8.1	10.1	
	La^{3+}		8.1	10.1	
	Pr^{3+}		8.1	10.1	
CPB	Ni^{2+}	XO		12.2	15,16
	La^{3+}			13.6	
	Ce(IV)			14.8	
	Rare Earths			13.6-14.8	

CTAC = cetyltrimethylammonium chloride

CTAB = cetyltrimethylammonium bromide

CPB = cetylpyridinium bromide

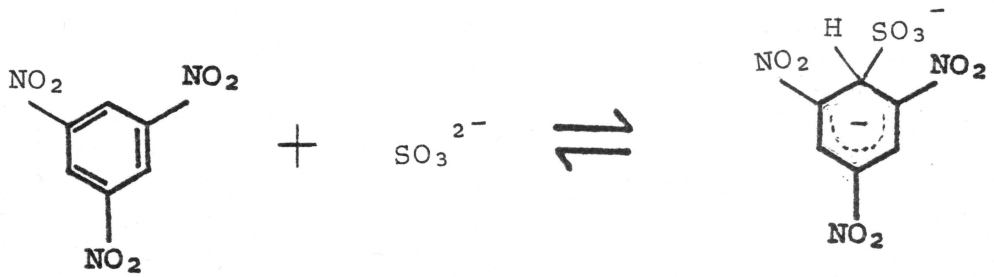
CBG = sodium 2"-chloro-4"-nitro-4'hydroxy-3,3'-dimethylfuchsone-5,5-dicarboxylate (Chrome Blue G)

EAB = Eriochrome Azurol B

Table III. (cont.)

XO = 3,3'-bis/N,N-di(carboxymethyl)aminomethyl-/-O-cresol-sulfonphthalein (Xylenol Orange or XO)

Another type of application of micellar systems to ultraviolet-visible spectroscopy uses the ability of micelles to shift the position of an equilibrium. A determination of sulfite ion is based upon the equilibrium interaction of sulfite ion with polynitroaromatic compounds such as 1,3,5-trinitrobenzene, which results in formation of a colored anionic complex as shown:



The absorption by the colored Meisenheimer complex formed is proportional to the sulfite ion concentration. The position of this equilibrium can be quantitatively shifted to the desired product side via the use of the cationic cetyltrimethylammonium bromide (CTAB) micellar system. Thus, a convenient, sensitive, and rapid method for sulfite ion determination was developed (17). This effect is mainly the result of favorable electrostatic interactions.

Micellar systems cause the fluorescence intensity of

many probes to be enhanced (2-100 fold) relative to the bulk solvent system. This has been observed for probes such as 8-anilinonaphthalene-1-sulfonate (18), perylene (18) pyrene (19,20), 8-toluidinyl-1-naphthalene sulfonate (21), Rhodamine B (22), and dansyl chloride (1-dimethylamino-naphthalene-5sulfonyl-glycine (23). The enhanced quantum yields are usually attributable to micellar solubilization of the fluorescent probe so that it is protected from collisions with molecules which might produce external quenching. In spite of this body of information, there has been only a few reports concerning the application of micellar systems to fluorescence analytical determinations (24,25). The first application in this area concerned the improvement of fluorometric methods for the determination of aluminium (24) and gallium (25) using an azo dye, lumogallion [4-chloro-6-(2,4-dihydroxyphenyl-azo)-1-hydroxybenzene-2-sulfonic acid] in the presence of nonionic surfactants such as polyethylene glycol monolauryl ether. The analytical procedures are based upon the reaction of the metal ion with lumogallion, which forms the fluorescent metal-chelate product. The addition of the surfactant caused a slight hypsochromic shift (10-17 nm) in the emission maximum and a 6-8 fold increase in the fluorescence intensity of the metal-chelate complex.

Another type of application of micellar system is

based upon their ability to catalyze the desired analytical reactions. The determination of cyanide ion via reaction with 1,4-naphthoquinone-2-sulfonic acid (26), 5,5'-dithiobis-(2-nitrobenzoic acid) (27), and 4,4'-dithiodipyridine (26) has been improved with the use of a cationic CTAB micellar system. The analytical reaction in aqueous solution is slow, requiring one to two hours (28,29). As expected, the presence of a cationic CTAB micellar system catalyzed the nucleophilic reaction.

In view of the great number of publications concerning micellar catalysis of a variety of chemical reactions, it is surprising that there are so few reports of their application to analytical reactions which generate the absorbing chromophore. There may be many existing analytical procedures that could be improved by being made more rapid and convenient with use of an appropriate micellar system. The purpose of this work is to develop further the application of catalytic micellar systems in organic analysis.

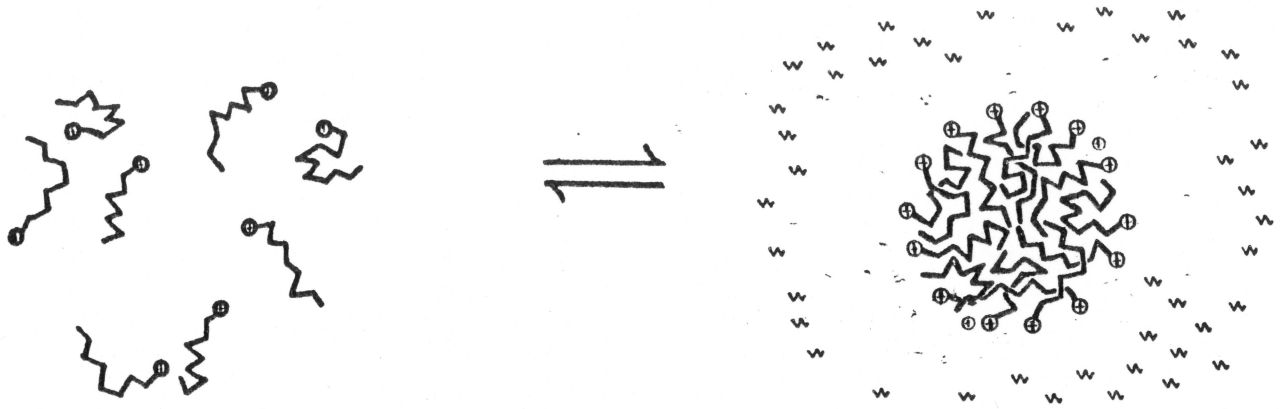
The preceding discussion leads to two general investigative approaches. In one of these a reaction, or reaction type, that has already been shown to undergo catalysis by micelles can be put to analytical use. This method of

attack is valuable in concentrating attention on the potential problems that may arise from using surfactants in analysis, and in establishing the scope and limits of micellar catalysis in typical systems. The second approach is to initiate studies on known analytical reactions in need of greater rates, searching for micellar catalysts. This is a longer-range attack, with a likelihood of some successes and some failures. The present work will focus on the first approach.

ii) Micellar Properties

A schematic two-dimensional representation of the reversible association of a cationic surfactant to form an ionic micelle is shown in Fig. 1. Typically, such micelles have a radius of 12-30 Å depending on the length of the hydrocarbon chain (30). The hydrophobic part of the aggregate forms the core of the micelle whereas the polar head groups are located at the micelle-water interface. Each ionic head group occupies from 40-100 $Å^2$ on the surface of the micelle (31). Micelles are generally spherical but they may be rod-like, when the size is large (32).

Fig. 1. A schematic two-dimensional representation of the reversible association of a cationic surfactant to form an ionic micelle



a) Nature of the monomer-micelle equilibrium

The association of monomers to form micelle becomes appreciable at or about a certain concentration known as the critical micelle concentration (C.M.C.). Above this concentration essentially all of the added monomer participates in micelle formation. The monomer activity increases only slightly above the C.M.C. (33). This change from monomeric to micellar solutions is sharp but nearly all precise determinations show that this change occurs over a narrow range of concentrations rather than at a single concentration (34).

The question of the polydispersity of micelles has been discussed by several authors (35,36). Mukerjee in 1972 presented an analysis of turbidity data which suggested that the size distribution of micelles is narrow if the micelle is small and spheroidal (37). However, the size distribution is expectecd to be broad if the micelle is large.

b) Forces involved in the formation of micelles

The so-called hydrophobic interactions are the primary cause for micelle formation. In a review of the subject of micelle formation, Mukerjee (38) has evaluated the role of the hydrocarbon chain in terms of a model

system that involves the complete transfer from an aqueous to a hydrocarbon environment. His results show that the free energy of transfer from an aqueous environment to the micelle per CH_2 group is less than that for complete transfer to a hydrocarbon environment. This suggested that a part of the hydrocarbon chain must remain exposed to water. This implies that both the role of the hydrocarbon chain and that of the water are important in micelle formation.

Sufficient data in the literature demonstrated that different head groups and/or associated counterions with the same hydrocarbon chain do yield different values of the C.M.C. Schick (39) has shown that the stability of the n-dodecyl sulfate micelle increased in the order $(CH_3)_4N^+ > Na^+ > Li^+$, which was interpreted to indicate that the stability of the micelle increased as the hydrated radius of the counterion decreased. Mujerkee et al (40) have extended this discussion to include K^+ and Cs^+. These results imply that the role of the counterion is to shield the charges of the head groups on the micellar surface; the stability of the micelle increases as the counterion becomes more effective at shielding this charge.

The changes arising from different ionic head groups

are relatively small compared to the differences that can arise from the substitution of nonionic or zwitterionic groups for the ionic groups in a given hydrocarbon chain. The C.M.C. of n-dodecyl/oxyethylene/12 alcohol ($C_{12}H_{25}$-O-$(CH_2CH_2O)_{12}$-H) is 100 times smaller than the C.M.C. of the ionic micelle of the same chain length. Mukerjee (38) has shown that if log C.M.C. is plotted vs. the molecular weight of the head group, a linear variation is formed when the polyoxyethylenes are compared.

c) Solubilization in Micellar Systems

Solubilization is a term coined by J.W.McBain to describe the property of bringing into solution of otherwise insoluble material by the action of colloidal particles. Nakagawa and Tori (41), using NMR, have shown that the lifetime of benzene molecules solubilized in micelles of sodium lauryl sulfate (NaLS) is very short, less than 10^{-4} sec, suggesting a rapid equilibrium for the solubilized species between the aqueous environment and the micelle. Eriksson (42) and Eriksson and Gilberg (43) have utilized NMR to determine the site of the solubilized species in the micelle. These authors solubilized benzene and some of its derivatives in the micelle of cetyltrimethylammonium bromide (CTAB). By following the shift of the resonance line positions of all of the

identifiable protons [$N-CH_3$, $-CH_2-N$, $-CH_2-$, and $C-CH_3$] as a function of solubilizate concentration, they showed that benzene, N,N-dimethylaniline, and nitrobenzene are absorbed primarily at the micelle-water interface, whereas isopropyl benzene and cyclohexane are located primarily in the hydrocarbon core.

Fender et al.(44) studied the nature of the environment experienced by benzene in CTAB, NaLS, and zwitterionic 3-(dimethyldodecylamino)-propane-1-sulfonate. Their results suggested that the sites of solubilization of benzene were near the micellar surface in the CTAB micelles and in micelles of the zwitterionic surfactant. For micelles of NaLS the site of solubilization was closer to the micellar core. Donbrow and Rhodes (45) concluded that benzoic acid is located at the junction between the hydrocarbon interface and the polyoxyethylene chains in micelles of cetamacrogol, by utilizing NMR and UV spectroscopy. Nemelthy and Ray (46) used UV spectroscopy to investigate the environment of phenol innonionic micelles. They found that phenol is H-bonded in a manner similar to that in methanol or water and therefore, that this solute doen not penetrate the alkyl interior of the micelle. Jacobs et al (47) have studied the solubilization of phenol in NaLS micelles using NMR and concluded that phenol is solubilized

at the micelle-water interface with the hydroxy group oriented towards the aqueous layer.

Mukerjee and Cardinal (49) examined the distribution of benzene derivatives in micelles of cetyltrimethylammonium chloride, sodium dodecyl sulfate, sodium myristyl sulfate and Brij-35. They concluded that the microenvironment polarity for a solubilizate appears to be independent of the charge type of the approximately spherical micelles. The second important result is that the microenvironment becomes less polar in alkyl substituted benzenes as the number or size of alkyl groups increases. The data indicated that there must be a distribution of the solubilized species between at least two sites (loci) of solubilization of different environmental polarity. The measured polarity parameters are average quantities. The fraction of solute in the hydrocarbon core increases progressively as the aliphatic character of the substituted benzenes increases. Mukerjee (49,50) also examined the distribution of some solubilized benzoic acid derivatives between the hydrocarbon core and the mantles composed of polyoxyethylene head groups in nonionic micelles. His results suggested that solubilization occurs at both loci; the relative amounts in the core and mantle depend on the structure of the solubilized molecules. Benzoic acid

favors the hydrocarbon core whereas derivatives with hydrophilic groups, such as p-hydroxy and p-amino benzoic acid, are distributed mainly in the oxyethylene layer. Esterification of these derivatives shifts the distribution back in favor of the micellar core. These differences were explained in terms of H-bonding tendencies.

d) Stabilizing Effect of Surfactants

Significant increases in the stability of certain esters can be obtained by introducing micellar species into their aqueous solutions. Despite the large number of possible reactions leading to drug degradation, many, perhaps most, of those that do occur can be classed as either hydrolysis or oxidation. A general use of surfactants to inhibit hydrolysis of pharmaceuticals would be potentially useful.

Ester groups are found in drugs either as an integral part of the molecule or as an added group which improves some physical or chemical property of the compound. Examples of drugs which contain ester functions are aspirin, and local anesthetics such as benzocaine, procaine and cocaine. Esterification is often the method of choice in a prodrug approach. The purpose of pro-drugs is to create a biologically reversible modification of the

of the parent drug, which reverts to the parent drug in the body. The use of the pro-drug approach has increased since problems such as poor solubility, poor bioavailability due to polarity or the "first pass effect", or the lack of chemical stability were overcome by preparing chemically altered temporary forms of the drug. Esters have been used as the reagent of choice to improve the solubility of a drug. Esterification of a substrate has produced a depot or sustained release effect. The "first pass effect" (metabolism or degradation of a drug prior to general hepatic circulation) was overcome by esterifying l-dopa (51) and tyrosine (52). Esterification of epinephrine and phenylephrine with the pivalyl group increased the lipophilicity of the drug and improved the partitioning across the cornea (53).

In the pharmaceutical industry, formulations are normally required to have no greater than a ten percent loss of active ingredients in two years. The ester linkage is susceptible to hydrolysis in aqueous solutions. A usual approach to inhibit hydrolytic reactions in aqueous solutions is to determine the pH at which the reaction rate is slowest and to formulate the drug at this optimal pH. Higuchi and co-workers have used complexation in aqueous systems as a means of increasing the stability

of benzocaine (54). Partial replacement of water with a solvent of lower dielectric constant generally causes a considerable decrease in the rate of ester hydrolysis (55).

The effect of micelles on the rates of hydrolysis of esters has been studied by many authors interested in their catalytic properties. Considerably less attention has been given to the stabilizing effect of surfactants. Smith, Kennedy and Nairn (56) studied the alkaline hydrolysis of benzocaine (I), n-butylaminobenzoate (II) and ethyl p-n-butylaminobenzoate (III) in the presence of the non-ionic surfactant, polyoxyethylene 24 monocetyl ether. The partition coefficients for the distribution of the esters between the micellar and aqueous phases were determined to be 115 for I, 965 for II and 4912 for III at surfactant concentrations of 5% and 30°C. The hydrolysis rates in the micellar phase were 0.2% for I, and 0.05% for compounds II and III, of the hydrolysis rate in aqueous phase (0.045 M NaOH). It was concluded that benzocaine and its homologs were probably associated with the polyoxyethylene portion of the micelle and that the observed decrease in the rate of hydrolysis could be explained by the rate of the hydrolysis reaction in the aqueous and micellar phases and the fraction of the molecules residing in each phase.

B. General Background for the Catalysis by Micelles

The kinetics of most micelle-catalyzed reactions have been formally described in terms of the following scheme:

$$S + M \underset{k_o}{\overset{K}{\rightleftharpoons}} SM \qquad \text{Eq. 1}$$

$$S \xrightarrow{k_o} P \qquad \text{Eq. 2}$$

$$SM \xrightarrow{k_m} P \qquad \text{Eq. 3}$$

where S represents the substrate (reactant), M is the micelle, SM is a 1:1 complex of substrate and micelle having binding constant K, P is the reaction product(s), k_o is the rate constant for the uncatalyzed (bulk phase) reaction, and k_m is the rate constant for the reaction in the micelle phase. (Micellar catalysis implies $k_m > k_o$; the same scheme can account for micelle inhibition if $k_m < k_o$). This scheme embodies obvious assumptions about the stoichiometry of the binding process; nevertheless it often leads to estimates of K in reasonable agreement with values determined by non-kinetic methods. The formal analogy with enzyme kinetics is strengthened by other phenomena such as inhibition of the micelle catalysis by an added inhibitor.

The binding constant for micelle-substrate association

has been obtained from measurements of the substrate solubility as a function of the detergent concentration (57). It is assumed that the increased solubility in the presence of the detergent is caused solely by substrate-micelle complexation in a 1:1 ratio. From the relative solubilities in the presence and absence of detergent, the amount of substrate present in the surfactant can be calculated, which in turn allows the determination of K. It is important to realize, however, that the determination of the substrate-micelle binding constant from solubility data relies entirely on data for saturated solutions and that, in the case of ionic surfactants, differences in the counter-ion interactions with the micelle and the micelle-substrate complex and activity coefficient effects may seriously complicate the results.

Micelle-substrate association constants can also be obtained from nuclear magnetic resonance spectroscopic data (58). Since the micelle-substrate association is rapid on the nmr time scale, a single weight-averaged resonance is generally observed for substrate protons in the micellar and bulk solvent phase. Measurements of the extent to which various concentrations of detergent increase or decrease the rate of passage of a substrate through columns of molecular sieve have also been used to estimate binding constants for the association of substrates

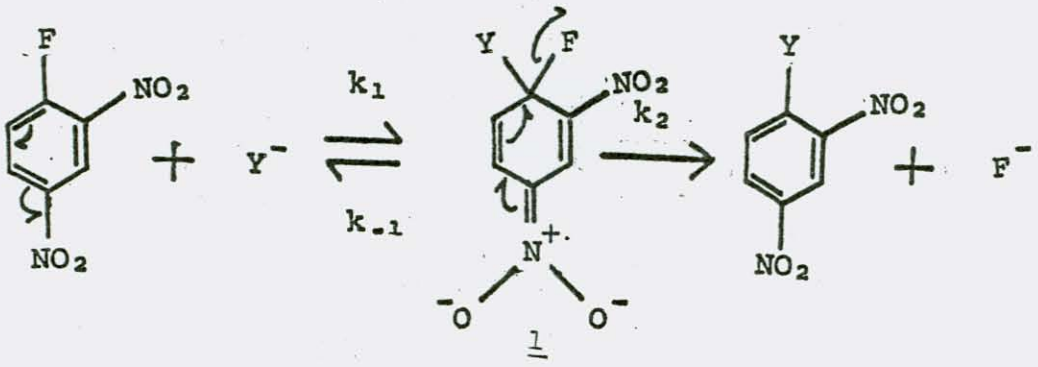
with micelles (59). The magnitude of the substrate-micelle association constant K can fall in a very wide range of approximately 10^{1} to 10^{11} M^{-1}, and the large value of K in some systems is a further similarity to enzyme-substrate systems. The rate enhancement caused by micelles is however usually much smaller than with enzymes; in micelle-catalysed reactions a catalytic factor of the order 10^{2} or less is usually observed.

With the postulate that catalysis by surfactants occurs because the substrate binds onto the micellar surface, simple electrostatic considerations lead to testable predictions. Thus we anticipate that reactions of anionic species would be facilitated by cationic micelles and inhibited by anionic micelles. Though exceptions are known, these predictions are found to be successful rough generalizations (30). The structures of micelles lead to another expectation, namely the more hydrophobic the substrate, the greater the catalytic effect of a given type of micelle. This can be viewed as a consequence of a more favorable partitioning equilibrium between the polar (aqueous) bulk phase and the hydrophobic micelle interior.

C. Mechanisms of Aromatic Nucleophilic Substitution

Since aromatic nucleophilic substitution often involves the formation or the destruction of charged species, these reactions should serve as fertile ground for studying the effects of micelles on rates.

The majority of aromatic nucleophilic substitution reactions appear to proceed via an addition-elimination pathway. An intermediate 1 is formed.



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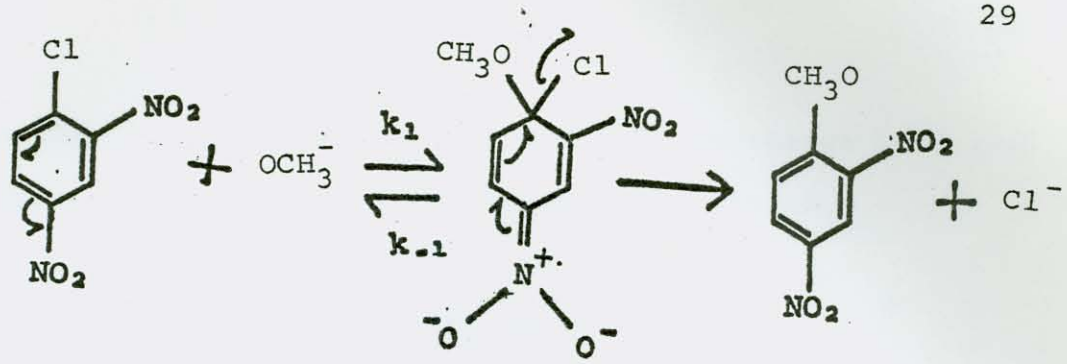
Y^- is a neutral or negatively charged nucleophile. Strongly nucleophilic reagents, "good" or weakly nucleophilic leaving groups and substituents on the aromatic substrate that stabilize the intermediate facilitate the reaction. A typical substitution is illustrated by the formation of 2,4-dinitroanisole from 2,4-dinitrochlorobenzene.



2

The electron-withdrawing o and p nitro groups stabilize the intermediate, 2. Several lines of evidence (60) support the existence of an addition intermediate. The intermediate will be relatively stable if electron-withdrawing groups activate the aromatic substrate and the leaving group is strongly nucleophilic. Meisenheimer (61) found that treatment of 2,4,6-trinitroanisole, 3, with potassium ethoxide appeared to give the same salt as did 2,4,6-trinitrophenetole, 5, upon treatment with potassium methoxide. This salt can be formulated as 4, potassium 1-methoxy-1-ethoxy-2,4,6-trinitrocyclohexadienylide.

Bernasconi (62) studied the Meisenheimer complexes formed from several amine nucleophiles with the substrate 1,3,5-trinitrobenzene.

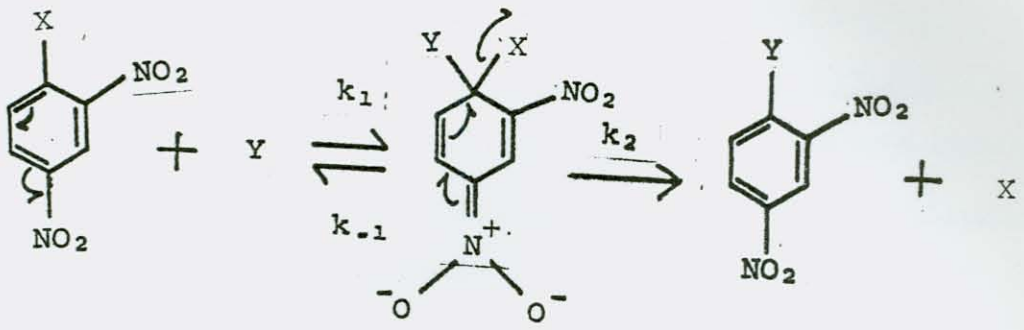
In 10% dioxane - 90% H_2O, at 25 °C, k_1 increases with the increasing basicity of the amine, as expected.

Table IV. Dependence of k_1 on pKa's of amines

	pKa	$10^{-4}k_1$ ($M^{-1}sec^{-1}$)
n-butylamine	10.68	0.0123
piperidine	11.12	3.0
pyrrolidine	11.30	8.1

Synthetic, kinetic and spectroscopic studies on Meisenheimer complexes leave no doubt that addition compounds exist. These studies give further evidence that Meisenheimer complexes may be intermediates in nucleophilic substitution.

The following argument to establish the involvement of an intermediate was used by Bunnett and co-workers (63). When the aromatic substrate 6 is substituted by Y, if the C-X bond breaking has made appreciable progress in the transition state, then the rate of the reaction should be markedly dependent on X.



An insensitivity of rate to the element X would reveal the extent of C-X bond breaking in the transition state of the rate-determining step. Table V shows the relative rates of substitution of nine 1-substituted 2,4-dinitrobenzene by piperidine.

Table V. Relative Rates of Substitution by Piperidine of 1-X-2,4-Dinitrobenzenes (63).

X	Relative Rate
F	3300
NO_2	890
$OSO_2C_6H_4CH_3$-p	100
SOC_6H_5	4.7
Br	4.3
Cl	4.3
$SO_2C_6H_5$	3.2

Table V. (cont.)

X	Relative Rate
$OC_6H_4NO_2$-p	3.0
I	1.0

The maximum rate variation was fivefold for the last six substituents. The last six substituents represent five different elements. A two-step mechanism is the simplest interpretation; the first step is rate determining and C-X bond breaking is not significant in the transition state. The ease of formation of a bond to the attacking nucleophile should be dependent in part upon the nature of X. This may account for the minor variation in rate for the last six substituents as shown in Table V. All of the substituents for the first three substrates are very electronegative. They should facilitate attack by the nucleophile.

Recently, Bernasconi and co-workers (64) studied the kinetics of the reversible Meisenheimer complex formation between 1,3,5-trinitrobenzene and methylamine or dimethylamine in 10% dioxane-90% water (v/v). They also reinvestigated the reactions of piperidine, pyrrolidine,

and n-butylamine in 30% dimethylsulfoxide - 70% water (v/v). Their results suggested that at low pH and low amine concentration, proton transfer between the zwitterionic and the anionic Meisenheimer complex is rate limiting while at high pH and/or high amine concentration, nucleophilic attack by the amine is rate-determining.

Although other kinds of intermediates can be proposed, the Meisenheimer complex can best account for the available data on nucleophilic substitutions of activated aromatic substrates. Most kinetic investigations of these substitutions have dealt with systems in which the steady-state condition applies to the intermediate. The two-step mechanism can be schematically written as

$$Y + Ar\text{-}X \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ArYX \xrightarrow{k_2} Ar\text{-}Y + X$$

Applying the steady-state approximation to the intermediate gives

$$V = \frac{k_1 k_2 [Ar\text{-}X][Y]}{k_{-1} + k_2} \qquad \text{Eq. 4}$$

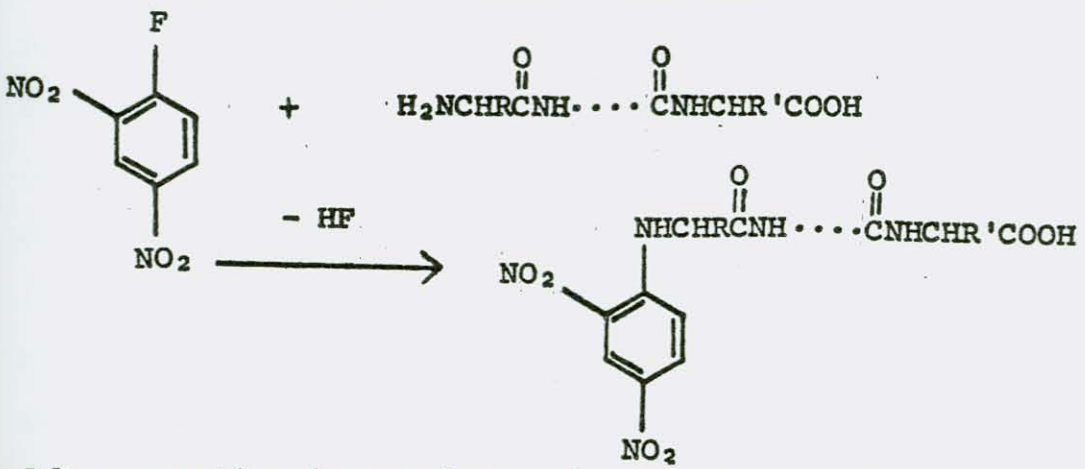
where V is the rate of the reaction. This equation shows the bimolecular nature of the kinetics. The experimental second-order rate equation is therefore

$$k = \frac{k_1 k_2}{k_1 + k_2} \qquad \text{Eq. 5}$$

Now, if $k_2 >> k_{-1}$, Eq 5 becomes simply $k = k_1$; the formation of the intermediate would be rate-determining. The data in Table V for the substitution of several 1-substituted-2,4-dinitrobenzene by piperidine demonstrate this kinetic behavior.

D. Reaction of 1-Fluoro-2,4-dintrobenzene with Amines

1-Fluoro-2,4-dinitrobenzene (DFB) was first employed by Sanger in 1945 to determine the amino acid sequence of insulin (65). On reaction with a protein in alkaline solution, DFB arylates terminal amino groups as well as exposed ϵ- amino groups (lysine), imidazoles (histidine), phenols (tyrosine), and thiols (cysteine).



After reaction is complete, the excess reagent (and dinitrophenol produced by concurrent hydrolysis of DFB)

is extracted and the dinitrophenylated protein is hydrolyzed with 6N HCl into its constituent amino acids. Upon extraction with ether the N-terminal DNP-amino acids (dinitrophenylamino acids) pass into the organic phase, they can be separated, identified, and determined by chromatographic procedures. The aqueous phase retains any ϵ-DNP-lysine, o-DNP-tyrosine, and Im-DNP-histidine, and the amino acids that did not react with DFB. In this way the N-terminal amino acids of a protein can be determined . Besides its use in end group analysis, DFB has been employed as a reagent for active site labeling of enzymes and for studying protein tertiary structure. (66)

McIntire et al (67) in 1953 developed a colorimetric method for the determination of amines based on their dinitrophenylation with DFB. A bicarbonate solution of DFB and the amine is heated to speed the reaction, then excess DFB is hydrolyzed to dinitrophenol with strong alkali. The DNP-amines are extracted into cyclohexane for spectrophotometric measurement. Dubin in 1960 (68) increased the sensitivity and simplified the procedure by acidification prior to spectrophotometric measurement, the extraction step being eliminated.

Bunton and Robinson (69) reported that several surfactants, most notably the cationic cetyltrimethyl-

ammonium bromide (CTAB), catalyze substitution reactions between DFB and amine nucleophiles. They found rate enhancements of approximately 5 to 30-fold. We proposed to apply these catalytic effects to the relatively slow analytical reaction, and to study the nature of the catalysis.

II. EXPERIMENTAL

A. Materials

All inorganic chemicals were analytical reagent grade and were used without further purification.

Cetyltrimethylammonium Bromide, Baker Analysed Reagent, was purified according to the procedure of P. Mukerjee and K.J.Mysels (70). p-Anisidine obtained from the Aldrich Chemical Co. was distilled under pressure, b.p. 98°/2 mm. m.p. 56.5-57°; lit. 57° (71); p-Toluidine (Aldrich) was distilled under reduced pressure, b.p. 61.5°/2 mm, m.p. 44.5-45°, lit. 44.8° (72). Aniline, analytical reagent grade, obtained from Mallinckrodt Chemical Works, was distilled b.p. 181°, lit. 184° (73). 1-Fluoro-2,4-dinitrobenzene was distilled under reduced pressure.

The following chemicals were obtained from the Aldrich Chemical Co. and were used directly as received: glycine, glycylglycine, glycylglycylglycine, alanine, phenylalanine, tryptophan, glutamic acid, tyrosine 11-aminoundecanoic acid, p-aminobenzoic acid, dioxane (spectrophotometric grade), and dinitrophenol. 2,4-Dinitrophenyl-alanine was obtained from Sigma Chemical

Company.

Standard buffers were prepared according to Bates (74) using freshly boiled , purified water. Water was purified by passage of double-distilled water through an ion-exchange column (Continental Deionized WaterService). pH 9.2 borate buffer, in which arylation of amines were run, was prepared to be 6.5×10^{-2} M.

B. Apparatus

UV/VIS Spectral measurements were made with either a Perkin-Elmer 559 spectrophotometer or a Cary model 14 or model 16 spectrophotometer fitted with thermostated cell compartments that maintained temperature constant to $\pm 0.1^{\circ}$.

IR spectra were measured with the Perkin-Elmer 599B Infra-Red Spectrophotometer.

pH measurements at 25.0 °C were made with an Orion Research model 701A pH meter equipped with an Orion combination electrode #91-03.

Water bath temperatures at 25.0° were maintained to ± 0.1 with a "Temptrol 151" water bath (Precision Scientific Co.) or with a "Dual-Purpose" water bath system equipped with a relay, heater, stirrer and

circulator (American Instrument Co.) and regulated by a mercury column thermoregulator (Branwill Scientific Co.)

Melting points were determined on a Thomas-Hoover Capillary melting point apparatus.

Thermometers for use at 25.0° C were calibrated against a thermometer carrying a National Bureau of Standards calibration certificate.

Pre-coated thin layer plates, 0.25 mm thickness (Silica Gel 13181) with and without fluorescent indicator 6060 were obtained from Eastman Kodak.

C. Procedures

1. Dinitrophenylation Time Course Studies

Reaction mixtures were prepared in 25-ml volumetric flasks by mixing appropriate volumes of cetyltrimethylammonium bromide and substrate amine solutions. pH of the solutions were adjusted with appropriate buffers. Temperatures of the solutions were adjusted to 25° by placing them in a water bath. Typical concentrations in the final solutions were 5×10^{-4} M amine solution, 3.5×10^{-2} M surfactant concentration in pH 9.2 borate buffer. Reactions were initiated by adding 1.0 ml of 1.3% v/v 1-fluoro-2,4-dinitrobenzene in acetone. Samples were

removed at recorded times by pipetting a 1.0 ml aliquot into 9.0 ml of a 1:100 dilution of concentrated hydrochloric acid in dioxane. The HCl-dioxane solution serves two purposes here: it stops the reaction, and it adds a proton to the dinitrophenoxide formed as a result of hydrolysis of the reagent 1-fluoro-2,4-dinitrobenzene. Dinitrophenol in the protonated form does not absorb in the visible . Therefore the rate of formation of dinitrophenylamine can be followed spectrophotometrically by measuring the absorbance at the absorption maximum against a reagent blank carried through the same procedure. Plots of absorbance against time are subsequently prepared.

The method described cannot follow very fast reactions because it is not possible to pipette many samples within a short time. Equations were therefore developed to allow the reaction to be followed directly in a spectrophotometer. To a 25-ml volumetric flask were added appropriate amounts of CTAB, 10^{-2} M amine and appropiate buffer. The reaction vessel was placed in a 25° water bath and reaction was initiated by adding 1.0 ml of 10^{-3} M 1-fluoro-2,4-dinitrobenzene in acetone to the reaction mixture. Absorbances were recorded as a function of time in 1 cm cells on the Cary model 14 or Perkin-Elmer 559 spectrophotometer fitted with thermostated cell compartments that maintained

temperature constant to $\pm$ 0.1°.

2. Proposed Analytical Method

To a 25-ml volumetric flask are added 0.255 g of cetyltrimethylammonium bromide, and enough of the amine substrate such that its concentration in the diluted solution will be about 10^{-4} M. The solution is diluted to volume with pH 9.2 borate buffer. The reaction vessel is placed in a 25° to 45° water bath and reaction is initiated by adding 1.0 ml of 1.3% v/v 1-fluoro-2,4-dinitrobenzene in acetone. After about five half-lives at the experimental temperature, a 1.0 ml aliquot is pipetted into a 1:100 dilution of concentrated hydrochloric acid in dioxane. The absorbance is measured immediately at the absorption maximum against a reagent blank carried through the same procedure. A standard curve is prepared by subjecting known concentrations, bracketing the unknown concentration, of the same amine substrate to the procedure.

3. Product Identification Studies

A dinitrophenylamine and dinitrophenol are expected to be the products when 1-fluoro-2,4-dinitrobenzene reacts with amines. IR and UV/VIS spectroscopy and thin layer chromatography were used in product identification studies

for the reaction of alanine and 1-fluoro-2,4-dinitrobenzene in the presence of the surfactant cetyltrimethylammonium bromide.

Two drops of 1.2 N hydrochloric acid were added to the reagent blank and to the dinitrophenol standard. 5 ml of ethyl acetate were used to extract each of the acidified solution. Both extracts were allowed to evaporate to dryness in a desiccator under reduced pressure. The residues from both solutions were dissolved in methanol. 2 to 10 μg of the residue was applied to the silica-gel plates with 2-μl micropipetter (Drummond Microscopes). The sample spots were air-dried. A solvent system consisting of 40 ml chloroform : 10 ml methanol : 0.5 ml acetic acid was used to elute the sample spots. Each of the spots corresponding to dinitrophenol was scraped from the silica-gel plates into a centrifuge tube. Methanol was added to each centrifuge tube before they were centrifuged. The supernatant was vacuum-dried overnight, The dried product was then mixed with KBr. The mixture was compressed to a pellet before an infra-red spectrum was taken. A UV/VIS spectrum of the product in methanol was also taken.

The solution from the reaction of 1-fluoro-2,4-dinitrobenzene with alanine was acidified with concentrated

HCl until the pH was below 3. The acidified solution was then extracted with anhydrous ether. The upper layer containing dinitrophenylalanine and dinitrophenol was vacuum-dried and redissolved in anhydrous ether before being spotted on the silica gel thin-layer chromatography plates. The following solvent system was used to elute the sample spots: 100 ml ether : 3 ml acetic acid : 3 ml water. The product 2,4-dinitrophenylalanine was recrystallized from 50% ethanol and the recrystallized product was vacuum-dried. The infra-red spectrum of the product in KBr was measured for comparison with the spectrum of an authentic sample.

III. RESULTS

A. Arylation of Amines with 1-Fluoro-2,4-dinitrobenzene

The following conditions were initially chosen: 3.5×10^{-2} M cetyltrimethylammonium bromide (CTAB); 5×10^{-4} M amine substrate in pH 9.2 borate buffer. Reaction was initiated by adding 1.0 ml of 1.3% v/v 1-fluoro-2,4-dinitrobenzene in acetone. Samples were removed at recorded times by pipetting a 1.0 ml aliquot into 9.0 ml of a 1:100 concentrated HCl-dioxane solution. The rate of formation of dinitrophenylamine was followed spectrophotometrically by measuring the absorbance at the absorption maximum against a reagent blank carried through the same procedure. Plots of absorbance against time in the presence and absence of 0.035 M cetyltrimethylammonium bromide were subsequently prepared.

Fig. 2 shows the time course for the arylation of alanine with 1-fluoro-2,4-dinitrobenzene in the presence and absence of 0.035 M surfactant. The reaction in the presence of CTAB is complete in about 20 minutes but it takes approximately 400 minutes to complete the reaction in the absence of surfactant. Fig. 3 shows that the log

$(A_\infty - A_t)$ vs. time plot is linear for at least two half-lives. The $t_{1/2}$ for the reaction of glycine in the absence of surfactant is 36.0 minutes and it is 2 minutes in the presence of CTAB. This gives a rate enhancement factor of 18.0.

Fig. 2 : Change of absorbance with time for arylation of alanine at 23° : 5×10^{-4} M alanine, 6.98×10^{-2} M 1-fluoro-2,4-dinitrobenzene, borate buffer pH 9,2. Absorbance measurements were made at 350 nm. Arylation with 1-fluoro-2,4-dinitrobenzene was done both in the absence and presence of 0.035 M cetyltrimethylammonium bromide.

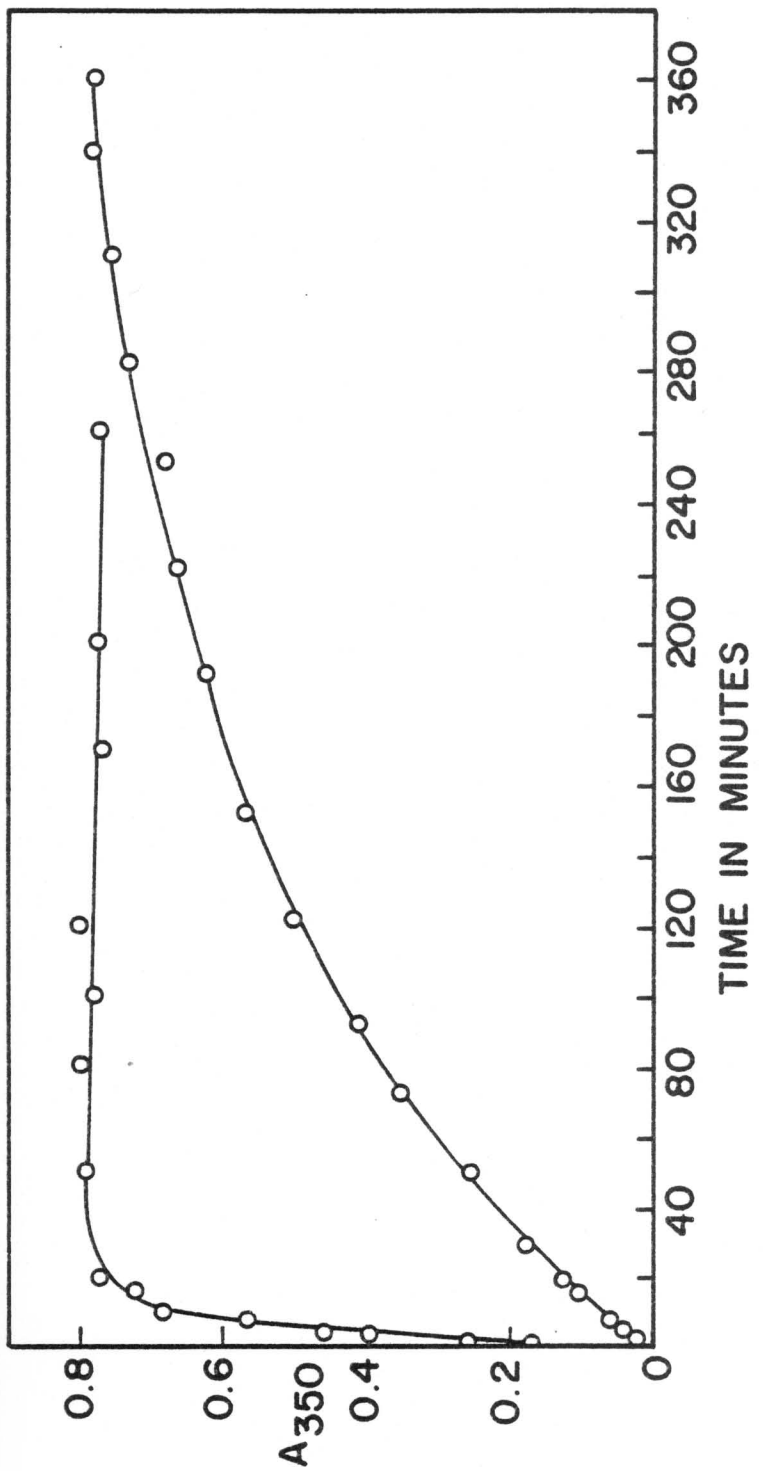
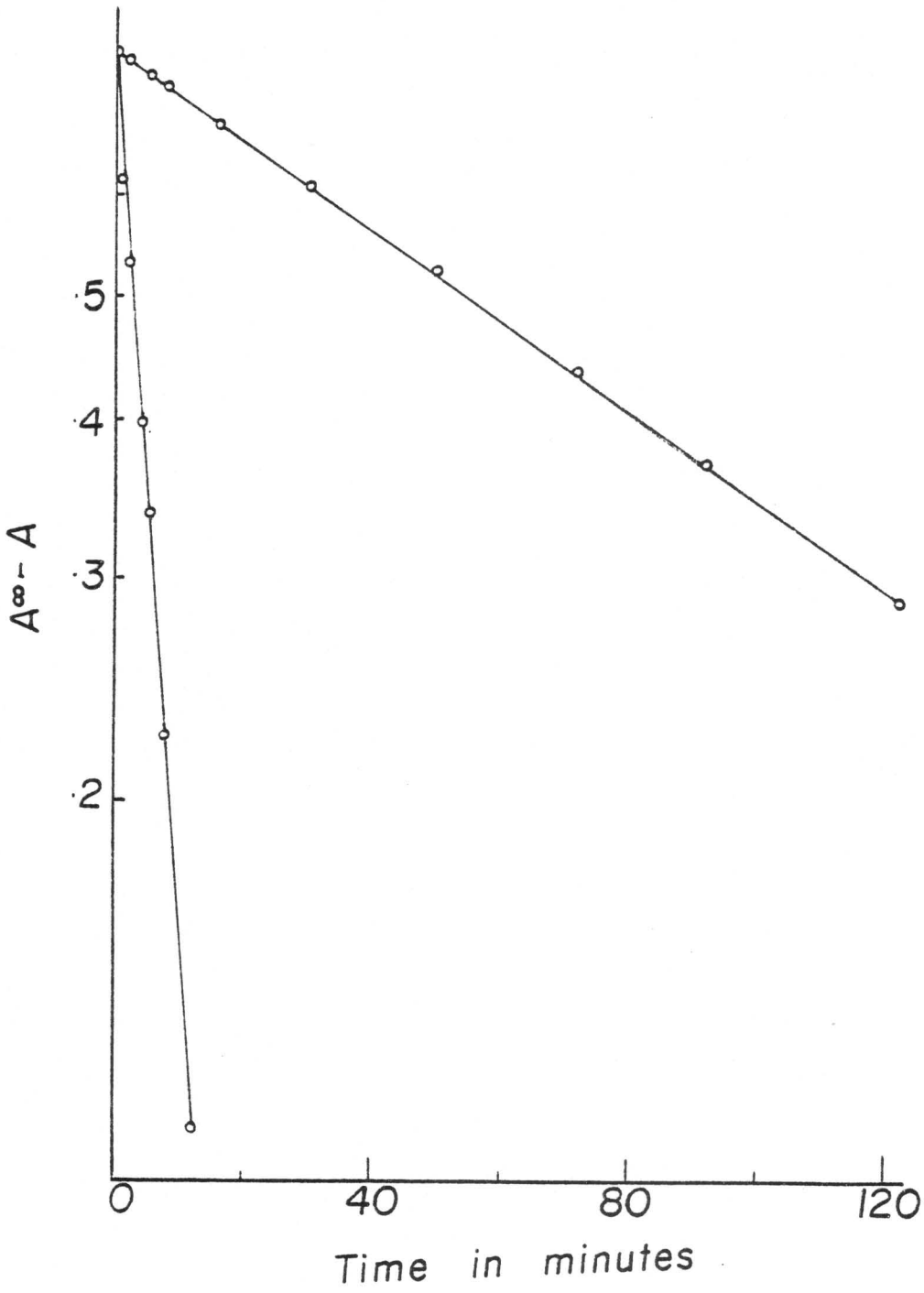

0.8
0.6
A_{350}
0.4
0.2
0
40
80
120
160
200
240
280
320
360
TIME IN MINUTES

Fig. 3 : log $(A_\infty - A_t)$ vs. time for arylation of glycine at 23°: 5×10^{-4} M glycine, 6.98×10^{-2} M 1-fluoro-2,4-dinitrobenzene, borate buffer pH 9.2. Absorbance measurements were made at 350 nm. First-order plots for the arylation in the absence and presence of 0.035 M cetyltrimethylammonium bromide were both prepared.


A∞ - A
.5
.4
.3
.2
0
40
80
120
Time in minutes

This procedure was applied to many different aliphatic amino-acids, aromatic amino-acids and some anilines. Table VI shows the half-lives of the reaction in the presence and absence of surfactant.

Table VI. Micellar Catalysis of Amine-Dinitroflurobenzene Reactions by Cetyltrimethylammonium Bromide.

Amine	Half-Life, min		Ratio
	No Surfactant	0.035 M Surfactant	
Glycine	36.0	2.0	18.0
Alanine	87.4	4.7	18.6
Phenylalanine	32.0	< 0.3	> 94
Tyrosine	22.0	< 0.5	> 44
Tryptophan	9.0	< 0.1	> 90
Glycylglycine	63.5	5.4	11.8
Glycylglycylglycine	94.0	7.5	12.5
Glutamic acid	72.0	< 0.3	> 240
p-Aminobenzoic acid	137.5	3.0	45.8
11-Aminoundecanoic acid	133.0	< 0.1	> 1330
Aniline	60.0	7.5	8.0

Rate enhancements for aliphatic amino-acids range from 10 to 20, whereas rate enhancements for aromatic amino-acids are much larger. (Phenylalanine and tyrosine are two typical aromatic amino-acids). This suggests that the nonpolar portion of the amine can assist the reaction perhaps by anchoring the nonpolar portion of the amino-acid to the micelle. Aniline gave a rate enhancement factor of 8. This suggests that the negative charge of the carboxyl group in amino acids can be attracted to the positively charged micelle surface, thus assisting the reaction. This might explain why glutamic acid, which is an aliphatic amino-acid with two carboxyl groups, gave such a high rate enhancement.

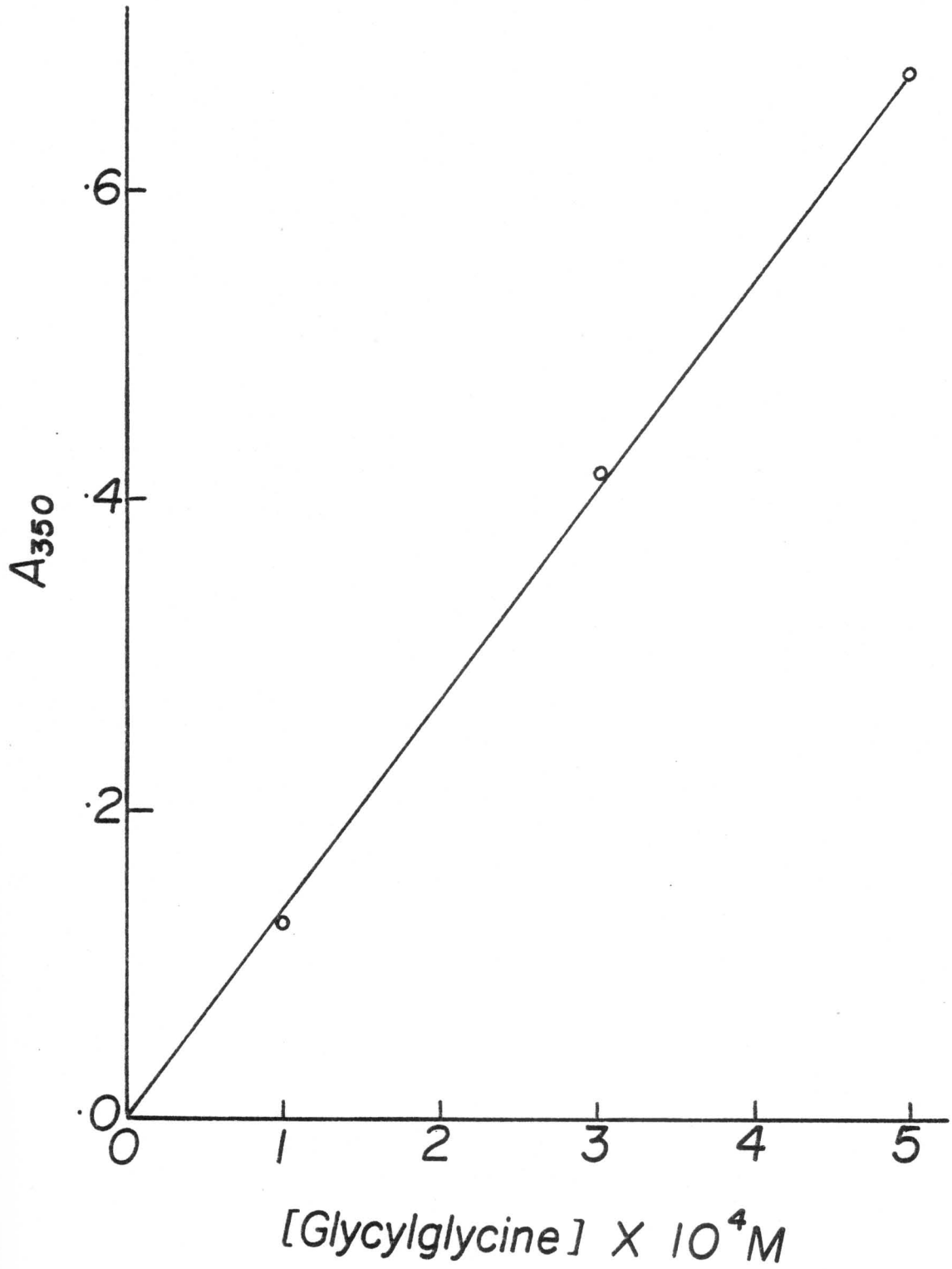
Thus there appear to be two factors contributing in the rate enhancement: one is the possession of a hydrophobic portion, the other is the possession of a negative charge. When both features are present, very high rate enhancements occur, as in 11-aminoundecanoic acid, for which a rate enhancement of greater than 1330 was observed.

B. Proposed Analytical Method

To a 25-ml volumetric flask were added 0.25 g of

cetyltrimethylammonium bromide, 1.0 ml of 1.3% (v/v) 1-fluoro-2,4-dinitrobenzene in acetone, 9.0 ml of 2.5% sodium borate inwater, and 10.0 ml of an aqueous amine solution (1×10^{-4} M - 1×10^{-3} M). After about five half-lives (Table VI) at room temperature, 1.0 ml of the solution was pipetted into 9.0 ml of a 1:100 dilution of concentrated hydrochloric acid in dioxane. The absorbance was read in a 1-cm cell at the absorption maximum against a reagent blank carried through the same precedure. Fig. 4 is a typical calibration plot to show the linearity. An additional rate enhancement factor of 3 is produced by increasing the temperature from 25°C to 45°C. The slopes of the concentration-response curves are identical at 25°C and 45°C.

Fig. 4 : Concentration-response curve for the arylation of glycylglycine at 23° ; 3.5×10^{-2} M cetyltrimethylammonium bromide, 6.98×10^{-2} M 1-fluoro-2,4-dinitrobenzene, borate buffer pH 9.2, 10 minutes reaction time; 1.0 ml of the solution pipetted into 9.0 ml of a 1:100 HCl-dioxane solution. Absorbance was measured at 350 nm.


·6
·4
·2
·0
A_{350}
0
1
2
3
4
5
[Glycylglycine] X 10^{4}M

C. Product Identification Studies

The products of the reaction of 1-fluoro-2,4-dinitrobenzene with alanine in the presence of 0.035 M cetyltrimethylammonium bromide were studied. Thin layer chromatographic separations of 1-fluoro-2,4-dinitrobenzene, dinitrophenol and dinitrophenylalanine are shown in Fig. 5 using the following solvent system:

ether	:	acetic acid	:	H_2O
100 ml		3 ml		3 ml

Products of the reagent blank were applied to TLC silica gel plate IV. The spots were eluted with the solvent system.

chloroform	:	methanol	:	acetic acid
40 ml		10 ml		0.5 ml

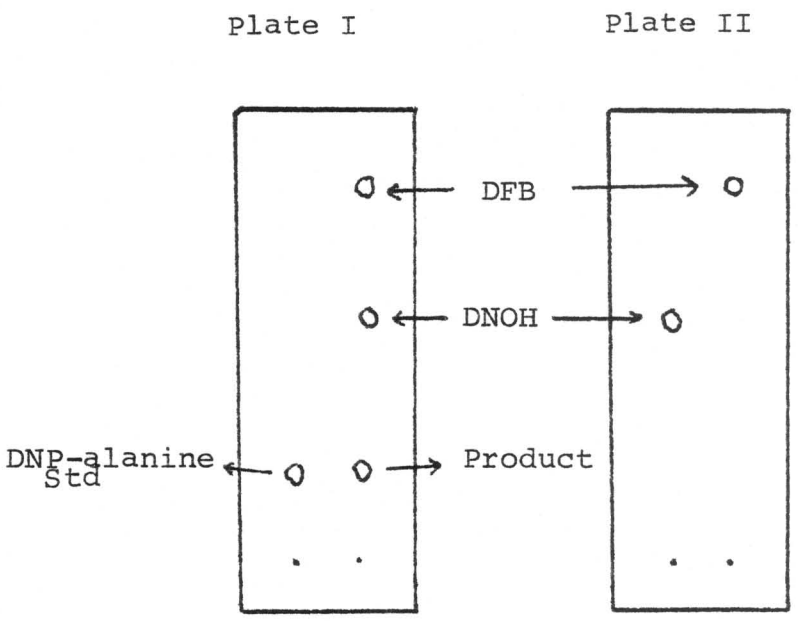
Fig. 6 shows that the R_f value of the reagent product is the same as that of the dinitrophenol standard.

The spot sorresponding to dinitrophenol was scraped from plate IV. It was mixed with methanol, centrifuged and dried. A KBr pellet of dinitrophenol was made. The infra-red spectra of dinitrophenol product and that of the standard were both recorded as shown in Fig. 7. The dinitrophenylalanine product from the TLC plate I was recrystallized from 50% ethanol. The infra-red spectra

of dinitrophenylalanine product and that of the standard are shown in Fig. 8. UV-VIS spectra of dinitrophenol are given in Fig. 9. Combining TLC, IR and UV/VIS spectroscopy, the products of the reaction of 1-fluoro-2,4-dinitrobenzene with alanine in the presence of cetyltrimethylammonium bromide were determined to be 2,4-dinitrophenylalanine and 2,4-dinitrophenol.

Fig. 5 : Silica gel TLC plates I and II indicating the separation of 1-fluoro-2,4-dinitrobenzene, dinitrophenol and dinitrophenylalanine. The solvent system used was:

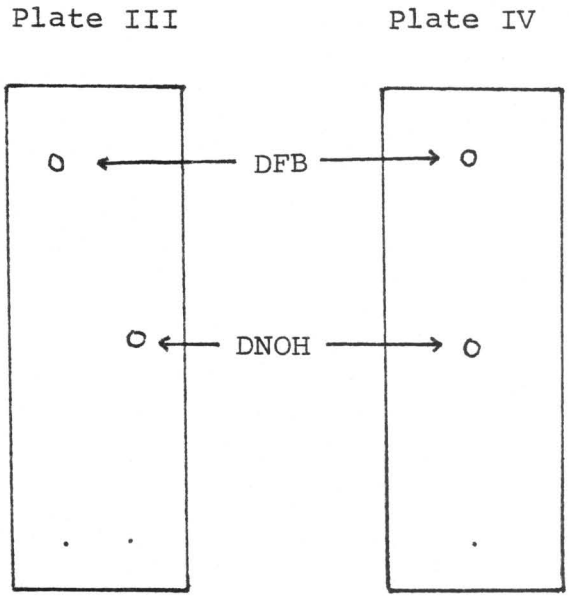
Ether	:	Acetic Acid	:	Water
100	:	3	:	3



Solvent Ether: HOAc: H_2O

100 : 3 : 3

Fig. 6 : Silica gel TLC plates III and IV showing the R_f value of dinitrophenol standard is the same as that of the reagent product. The solvent system developed consist of :
40 ml chloroform : 10 ml methanol : 0.5 ml acetic acid.



Solvent	CHCl	:	CH_3OH	:	HOAc
	40	:	10	:	0.5

Fig. 7 : The top half is an infra-red spectrum of dinitrophenol product as a result of hydrolysis of 1-fluoro-2,4-dinitrobenzene. The bottom half is an infra-red spectrum of dinitrophenol standard scraped from the silica gel TLC plates.

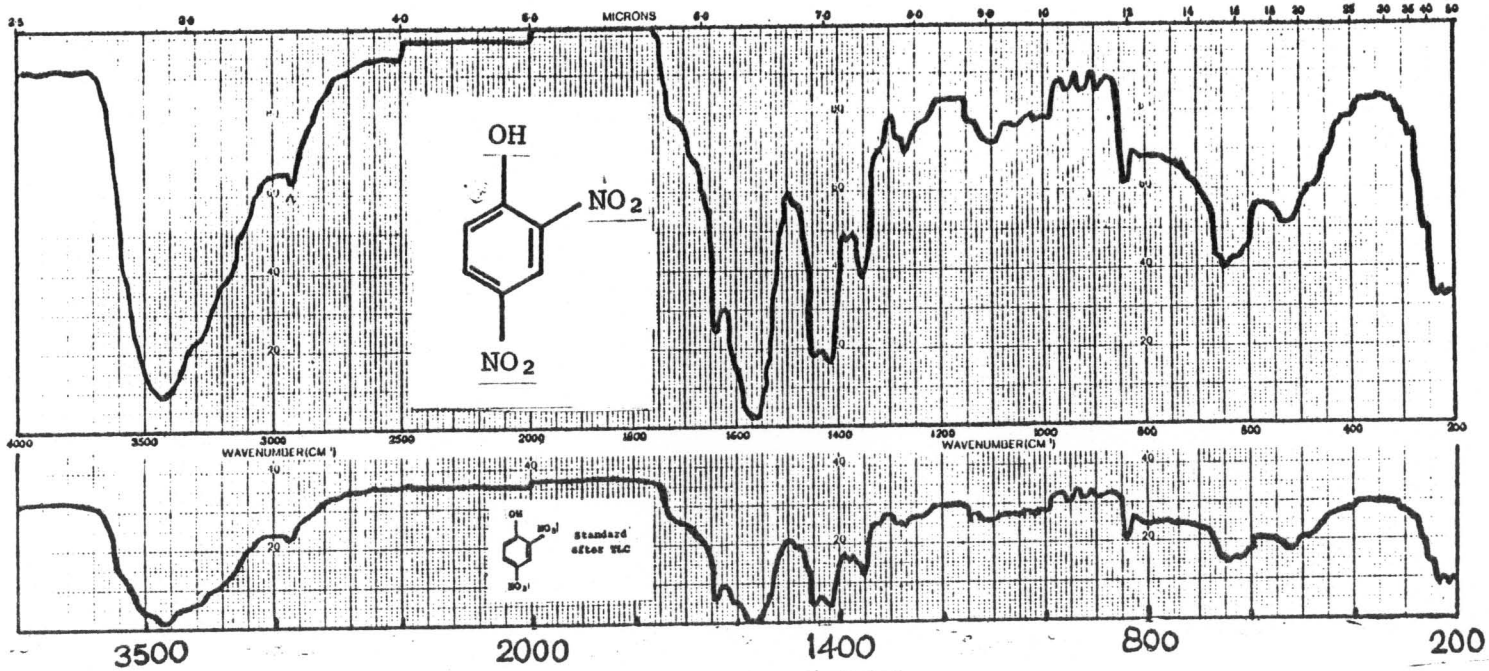

MICRONS
OH
NO2
NO2
WAVENUMBER(CM-1)
Standard after TLC
3500
2000
1400
800
200
WAVENUMBER(CM-1)

Fig. 8 : The top half is an infra-red spectrum of dinitrophenylalanine standard in KBr. The bottom half is an infra-red spectrum of the product dinitrophenylalanine after recrystallization from 50 % ethanol.

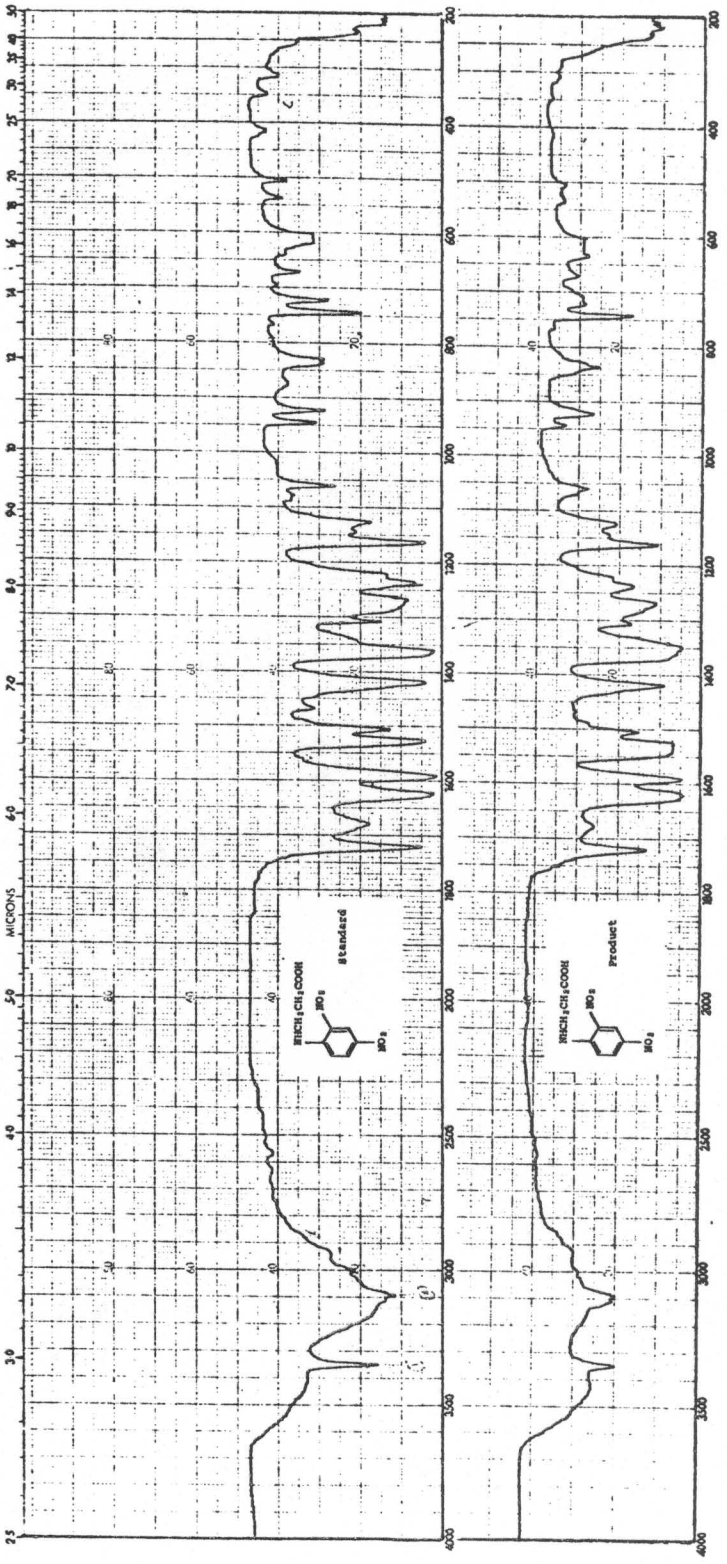
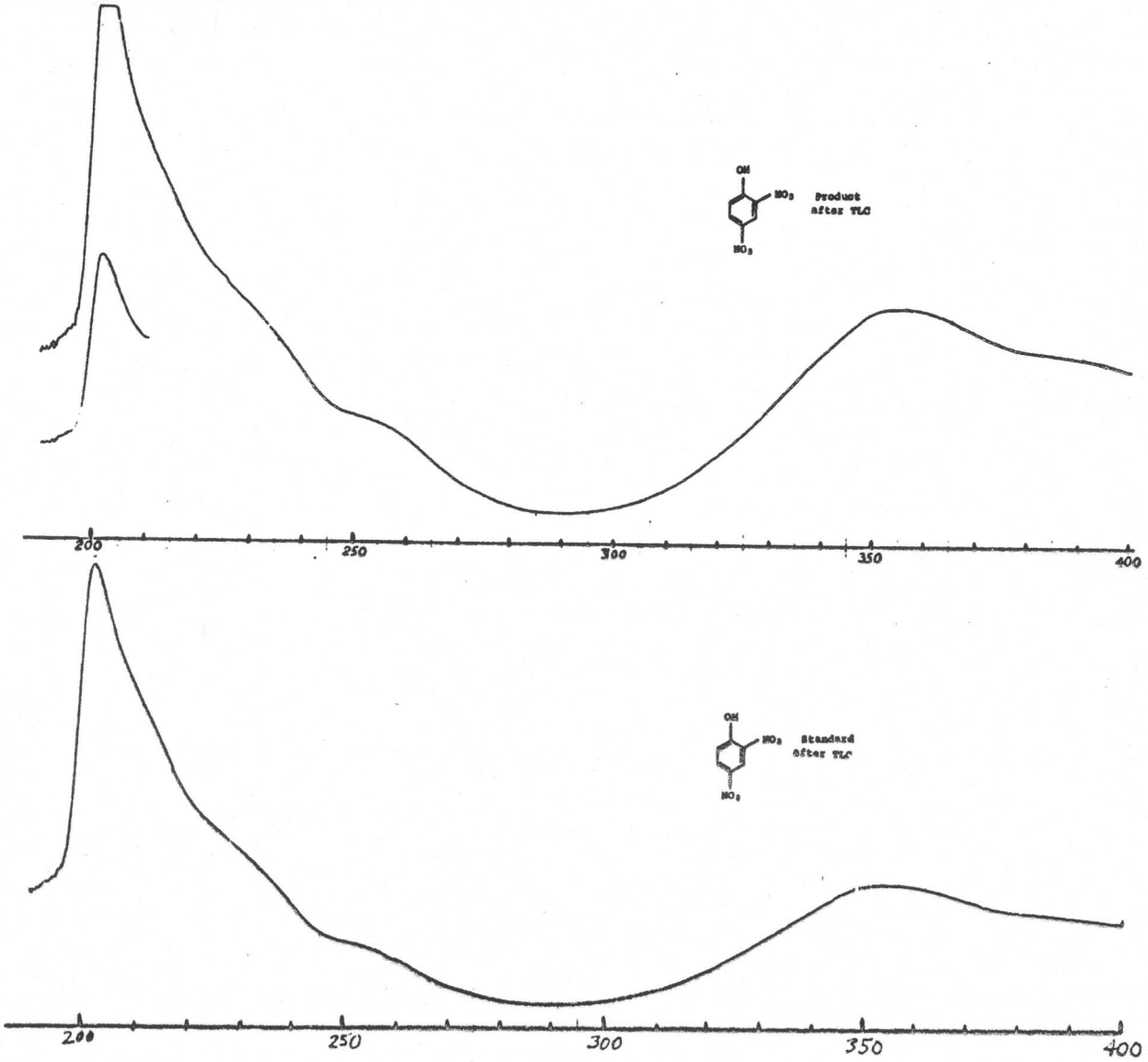

MICRONS
standard
product

Fig. 9 : UV-VIS spectra of the dinitrophenol product and standard in methanol.

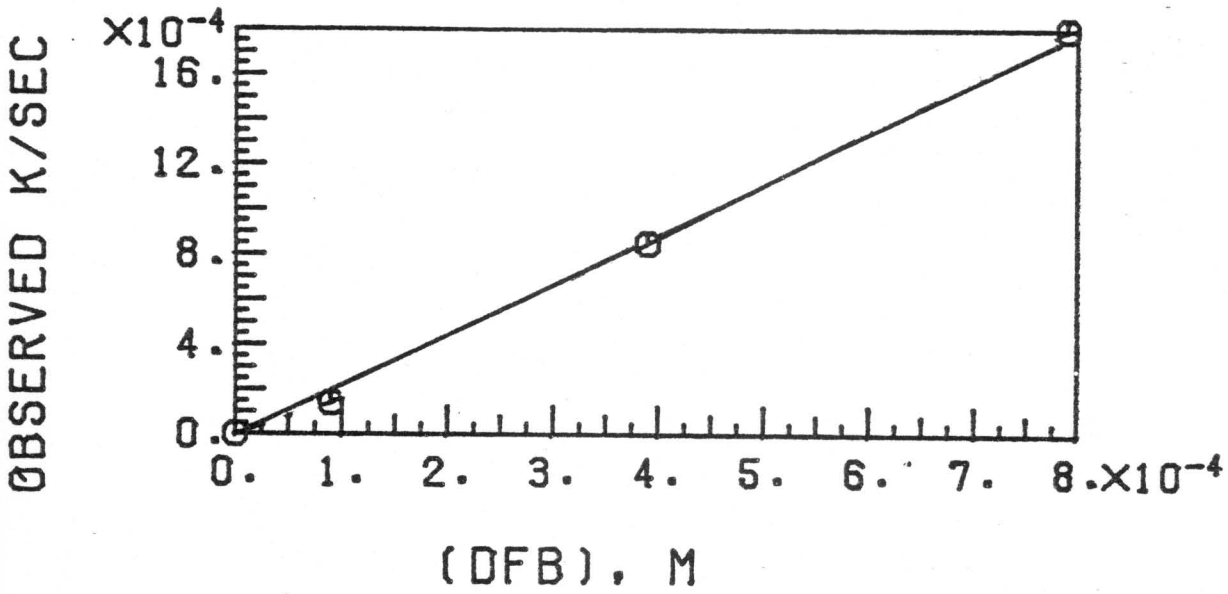

OH
NO2
NO2
Product
after TLC
200
250
300
350
400
OH
NO2
NO2
Standard
after TLC
200
250
300
350
400

D. Dependence of k_{obs} on Concentration of 1-fluoro-2,4-dinitrobenzene and Amine.

10^{-2} M alanine at pH 9.30 was subjected to arylation with different concentrations of 1-fluoro-2,4-dinitrobenzene in the presence of 3.5×10^{-2} M cetyltrimethylammonium bromide. Fig. 10 reports the observed rate constant as a function of 1-fluoro-2,4-dinitrobenzene concentration. The reaction appears to be first-order in dinitrofluorobenzene.

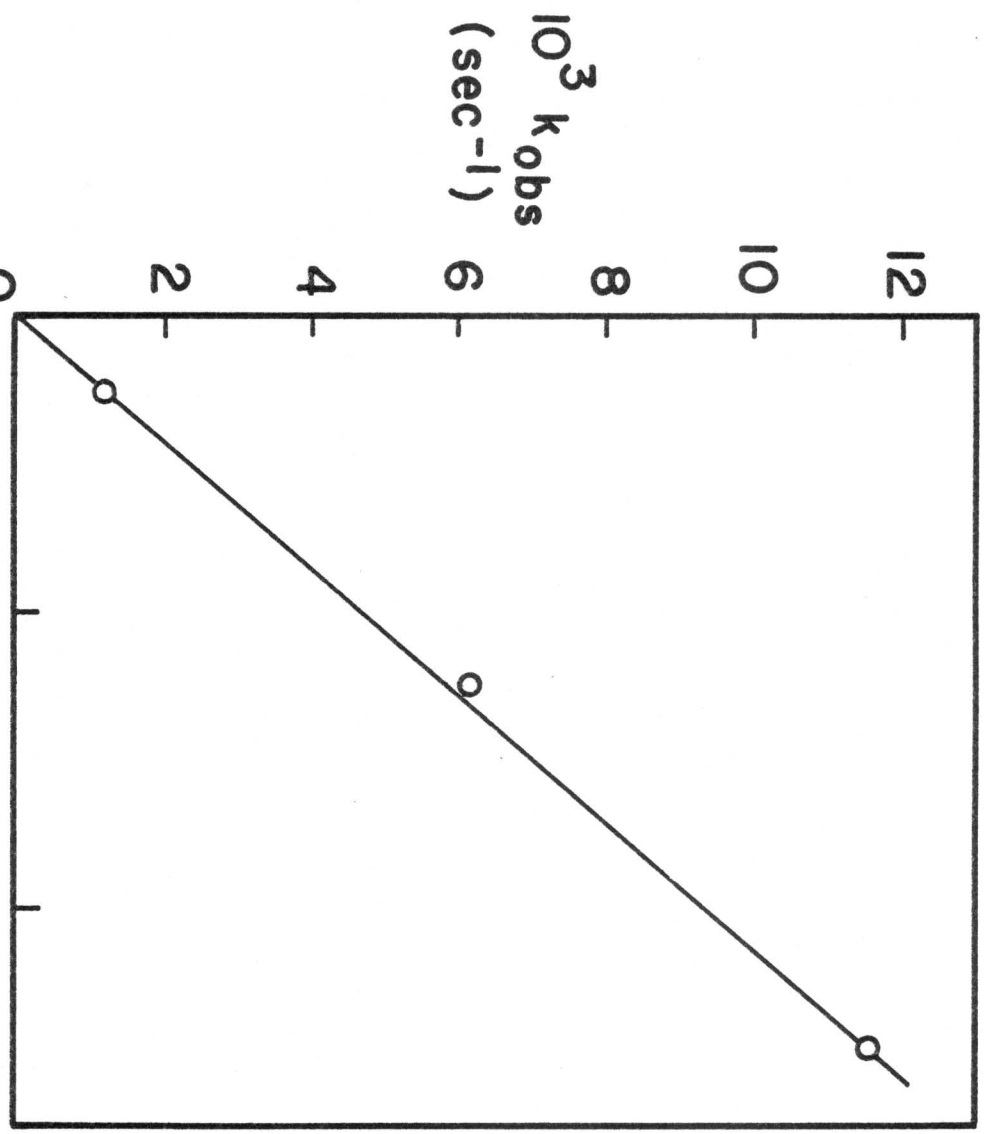
Fig. 10 : Dependence of the observed rate constant on 1-fluoro-2,4-dinitrobenzene concentration at 25° : 10^{-2} M alanine, 3.5 x 10^{-2} M cetyltrimethylammonium bromide, borate buffer pH 9.30.

REACTION OF ALANINE WITH DFB AT PH 9.30



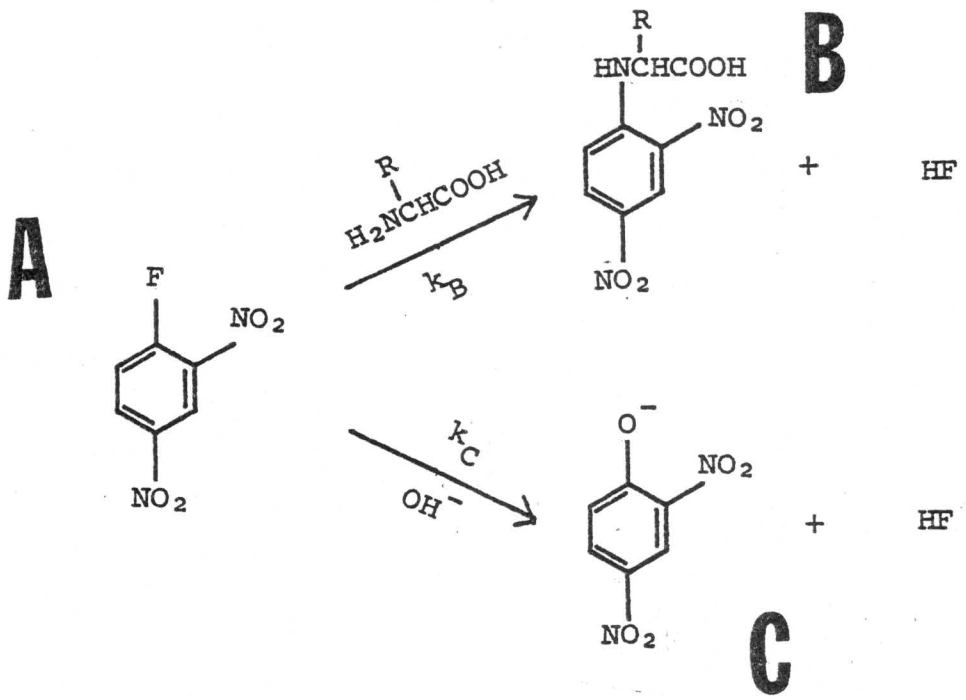
10^{-5} M 1-fluoro-2,4-dinitrobenzene was used to arylate various concentrations of alanine in the presence of 3.5×10^{-2} M CTAB and borate buffer pH 9.2. Fig. 11 shows the observed rate constant as a function of alanine concentration. The reaction appears to be first order with respect to the amine concentration. Figures 10 and 11 demonstrates the bimolecular nature of the reaction of 1-fluoro-2,4-dinitrobenzene with amines.

Fig. 11 : Dependence of the observed rate constant on alanine concentration at 25° : 6.3×10^{-5} M 1-fluoro-2,4-dinitrobenzene, borate buffer pH 9.2.


10^3 k_{obs} (sec^{-1})
0
2
4
6
8
10
12
[Alanine] x 10^2 M
1
2

E. Equations Developed for Following Reactions Directly in a Spectrophotometer.

1-fluoro-2,4-dinitrobenzene undergoes hydrolysis and arylate amines as depicted by the following scheme.



k_B is the pseudo-first order rate constant for the reaction of amine or amino-acid with the reagent 1-fluoro-2,4-dinitrobenzene and k_C is the pseudo-first order rate constant for the hydrolysis of 1-fluoro-2,4-dinitrobenzene . Both B and C absorb in the visible region.

In the preceding kinetic procedure (Part A), solutions containing excess reagent were sampled into

an acid medium at different times, so that C does not absorb, to measure the rate of formation of B. (1-Fluoro-2,4-dinitrobenzene was in excess in part A because it serves as an analytical reagent). This is time consuming and error could arise due to the time lag in pipetting. It is also extremely difficult if not impossible to follow very fast reactions. To study the mechanism of the reaction, we can take the substrate in excess. The following equations were developed to allow the reaction to be followed directly in a spectrophotometer.

The rate of loss of A can be written as $(k_B + k_C)(A)$ or $k_{obs}(A)$ or $k(A)$.

$$-\frac{d(A)}{dt} = (k_B + k_C)(A) = k(A) \qquad \text{Eq. 6}$$

The rate of formation of B is $k_B(A)$, or

$$\frac{d(B)}{dt} = k_B(A) = k_B(A)_0 e^{-kt} \qquad \text{Eq. 7}$$

Integrating $\frac{d(B)}{dt}$ and using the initial conditions that $(B) = 0$ at time $t = 0$, we arrive at Eq. 8.

$$(B) = \frac{-k_B(A)_0}{k} e^{-kt} + \frac{k_B(A)_0}{k} \qquad \text{Eq. 8}$$

Similarly, we can obtain Eq. 9.

$$(C) = \frac{-k_C(A)_0}{k} e^{-kt} + \frac{k_C(A)_0}{k} \qquad \text{Eq. 9}$$

Let D density or absorbance of solution at time t

$$D = \epsilon_A(A) + \epsilon_B(B) + \epsilon_C(C)$$

where ϵ_i is the molar absorptivity of species i.

Substituting (A), (B), and (C) from the preceding equations, we get Eq.10.

$$D = (\epsilon_A - \frac{\epsilon_B k_B}{k} - \frac{\epsilon_C k_C}{k})(A)_0 e^{-kt} + (A_0)(\frac{\epsilon_B k_B}{k} + \frac{\epsilon_C k_C}{k}) \qquad \text{Eq.10}$$

Equation 10 can be simplified if we choose a wavelength where the molar absorptivity of B is equal to that of C. Then Eq. 11 can be obtained :

If $\epsilon_B = \epsilon_C = \epsilon'$

$$(A)_0\epsilon' - D = (\epsilon' - \epsilon_A)(A)_0 e^{-kt} \qquad \text{Eq. 11}$$

A plot of $\ln[(A)_0\epsilon' - D]$ vs. t would be linear with slope equal to -k or $-(k_B + k_C)$. Since $(A)_0\epsilon'$ is equal to the initial concentration of 1-fluoro-2,4-dinitrobenzene times either ϵ_B or ϵ_C, $(A)_0\epsilon' = D_\infty$, where D_∞ is the final absorbance. Thus a plot of $\ln(D_\infty - D)$ vs. t should also be linear with the slope giving $-(k_B + k_C)$. k_C can be determined separately by following the reaction of

1-fluoro-2,4-dinitrobenzene in the presence of surfactant but without added amine. These equations allow us to follow the reaction directly in a spectrophotometer. The only condition is that the reaction must be followed at a wavelength where the molar absorptivity of B is identical to that of C. Figure 12 is a plot of the logarithm of the molar absorptivity of A, B, and C as a function of wavelength. 1-fluoro-2,4-dinitrobenzene is represented by curve A. The logϵ of A is negligible above 300 nm. Figure 12 suggested that kinetics could be followed at 350 nm, since $\epsilon_B = \epsilon_C$ at this wavelength. Kinetics were followed at 350 nm; ln $(A_\infty - A_t)$ was found to be linear with time for over three half-lives as shown in Fig. 13.

Fig. 12 : Logarithm of the molar absorptivity of 1-fluoro-2,4-dinitrobenzene (A), dinitrophenylalanine (B), and dinitrophenol (C) from 450 nm to 230 nm.

DN-0H IN B0RATE - BLACK, DFB - GREEN,
DNP-ALANINE STD - RED

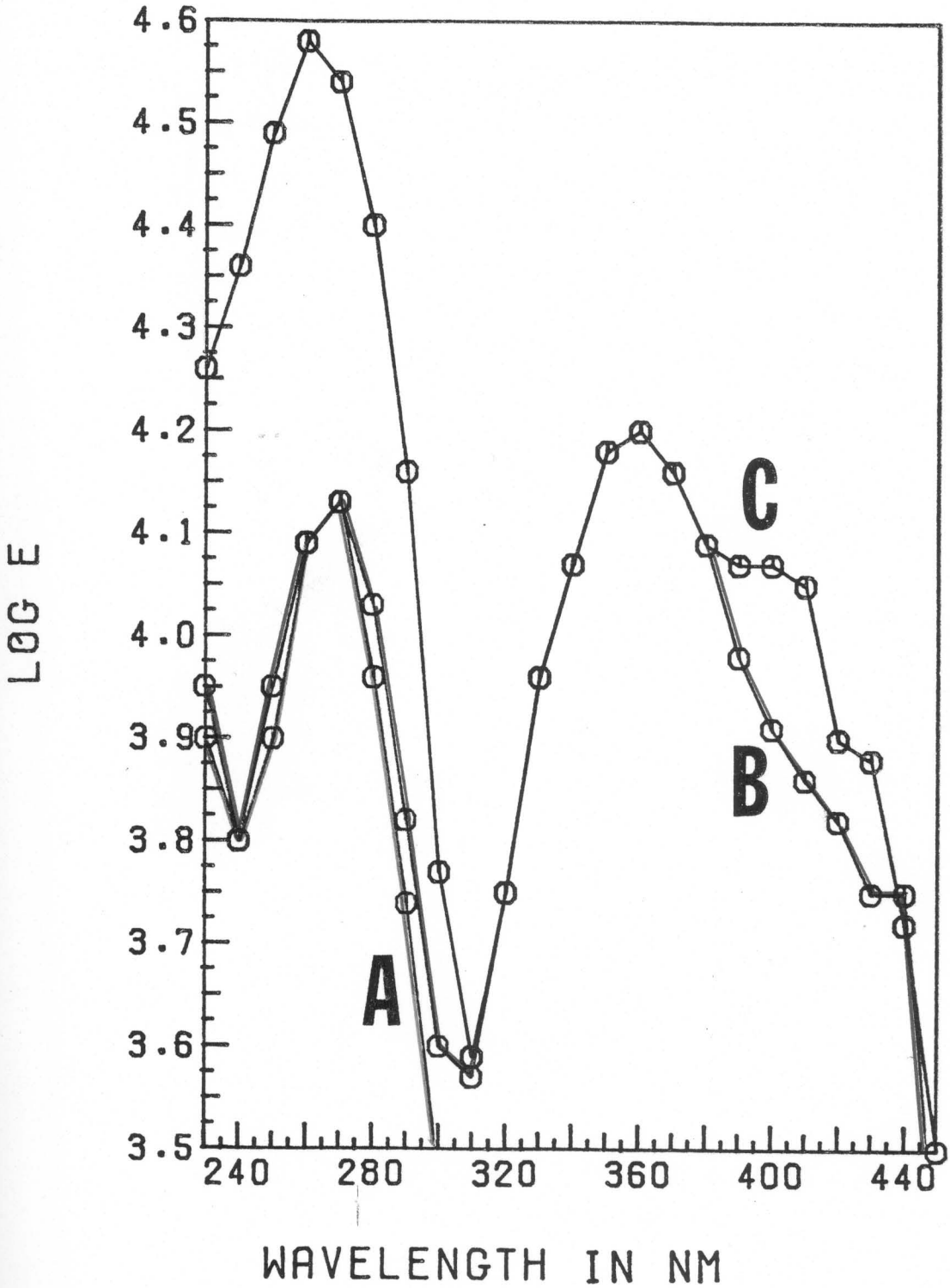
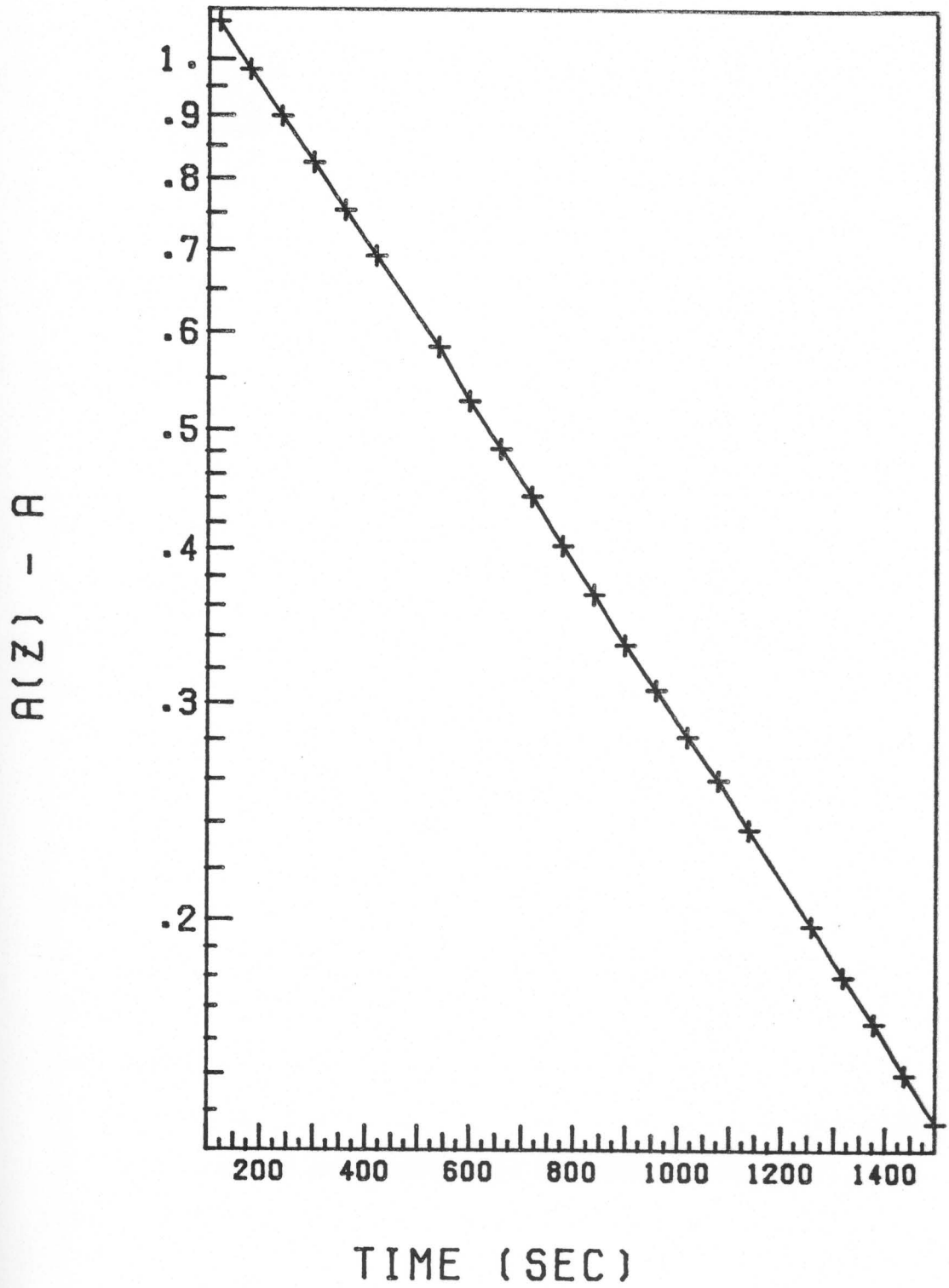


Fig. 13 : Arylation of 0.1004 M alanine in presence of 3.5×10^{-2} M cetyltrimethylammonium bromide at 25° . The rate of the reaction of 1-fluoro-2,4-dinitrobenzene with alanine was followed directly in a spectrophotometer. Absorbance measurements were made at 350 nm.

(ALANINE) = 0.1004 M



F. Temperature Study

One of the goals of this work was to develop an improved analytical procedure for the determination of amines, amino acids and peptides. Cetyltrimethylammonium bromide increases the reaction rate 5 to over 1000 fold. It would be interesting to see if an increase in temperature could reduce reaction or analysis time by another factor.

To a 25-ml volumetric flask were added cetyltrimethylammonium bromide such that its final concentration was 1.75×10^{-2} M, alanine final concentration was 2.5×10^{-3} M in borate buffer pH 9.20. The reaction mixture was allowed to equilibrate at 15°C. Reaction was initiated by adding 1.0 ml of 1.26×10^{-3} M 1-flouro-2,4-dinitrobenzene in acetone. The rate of the reaction of 1-fluoro-2,4-dinitrobenzene with alanine was followed directly in a spectrophotometer. Absorbance measurements were made at 350 nm. This procedure was repeated at 25°C, 35°C, and 45°C. The reaction in the absence of surfactant was also followed at 15°C, 25°C, 35°C and 45°C. The final alanine concentration was 2.5×10^{-2} M. Reaction was initiated by adding 1.0ml of 1.26×10^{-3} M 1-fluoro-2,4-dinitrobenzene in acetone.

Arrhenius plots for the reaction in the presence and absence of surfactant are shown in Figures 14 and 15.

The activation enthalpy $\Delta H^{\neq}$ and activation entropy $\Delta S^{\neq}$ for the reaction of alanine with 1-fluoro-2,4-dinitrobenzene in the absence and presence of 1.75×10^{-2} M cetyltrimethylammonium bromide were calculated from the Arrhenius plots. The calculated values are reported in Table VII.

Table VII. Activation enthalpy $\Delta H^{\neq}$ and activation entropy $\Delta S^{\neq}$ for the reaction of alanine with 1-fluoro-2,4-dinitrobenzene.

	With CTAB	Without CTAB
$\Delta H^{\neq}$, Kcal/mole	13.7 $\pm$ 0.4	16.1 $\pm$ 0.5
$\Delta S^{\neq}$, e.u.	-14.6 $\pm$ 1.3	-11.2 $\pm$ 0.5

Fig. 14 : Dependence of k_{obs} on temperature. 2.5×10^{-3} M alanine, 6.32×10^{-5} M 1-fluoro-2,4-dinitrobenzene and 1.75×10^{-2} M cetyltrimethylammonium bromide in borate buffer pH 9.20.

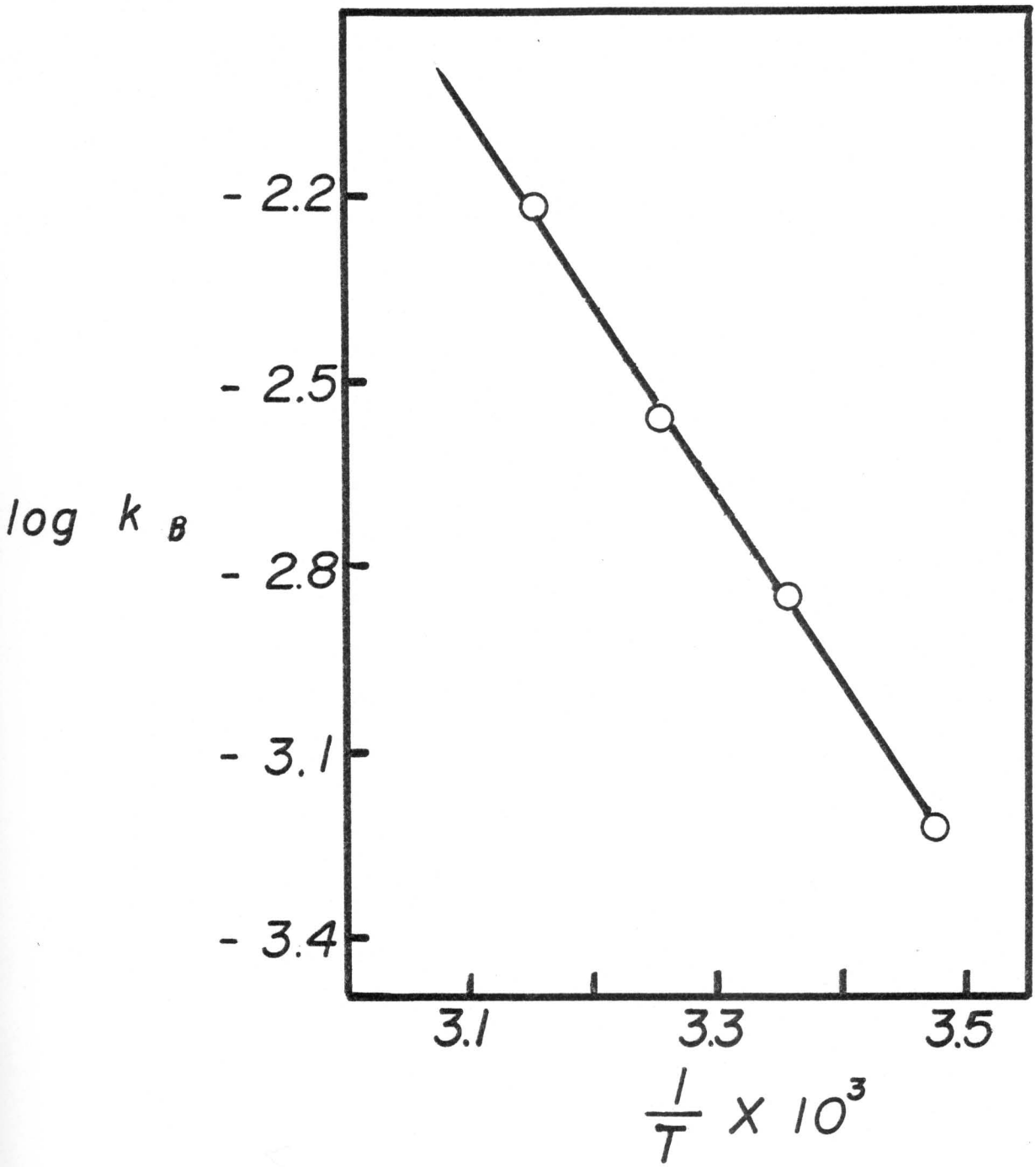
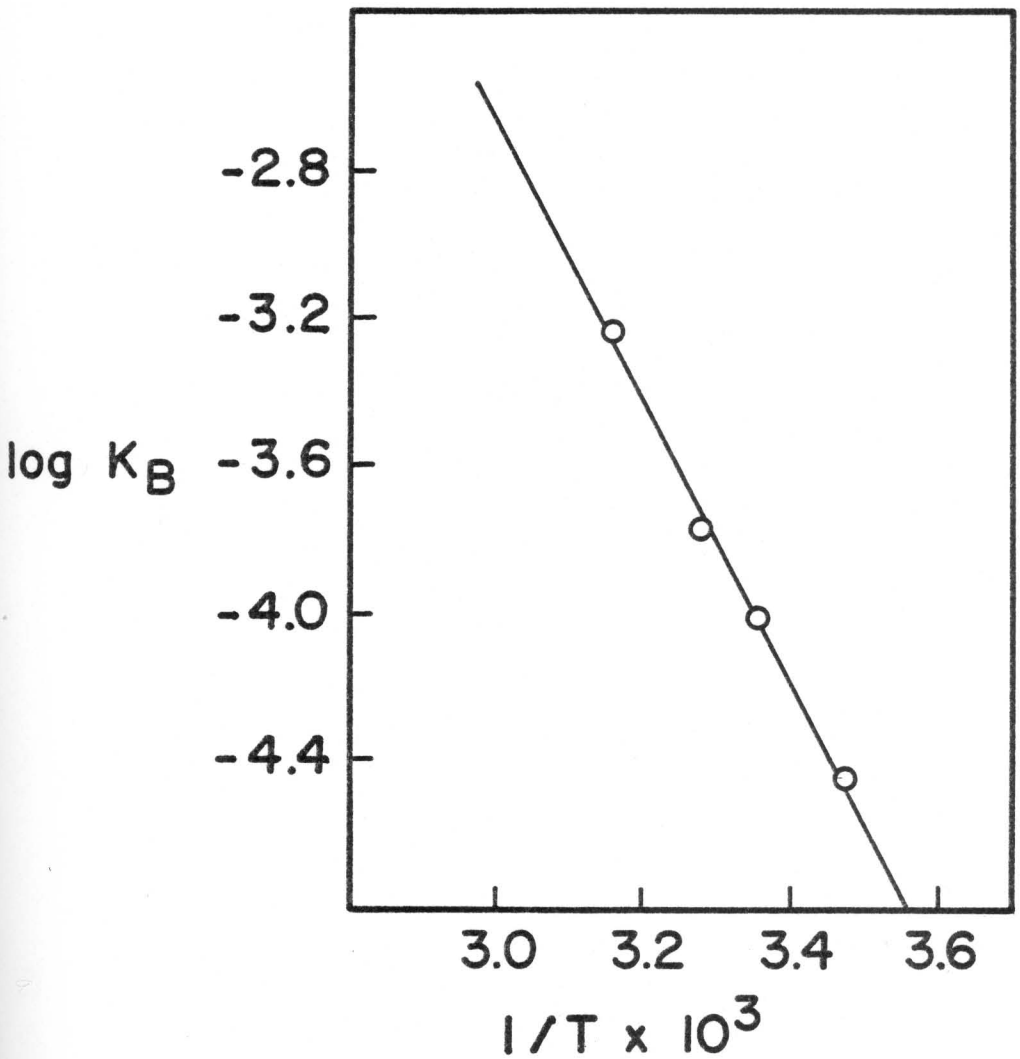

- 2.2
- 2.5
- 2.8
- 3.1
- 3.4
log k_B
3.1
3.3
3.5
$\frac{1}{T} \times 10^3$

Fig. 15 : Dependence of the observed rate constant on temperature. The reaction flask contained 2.5×10^{-2} M alanine and 6.32×10^{-5} M 1-fluoro-2,4-dinitrobenzene in borate buffer pH 9.20.


-2.8
-3.2
log K_B -3.6
-4.0
-4.4
3.0
3.2
3.4
3.6
$1/T \times 10^3$

G. Dependence of k_{obs} on pH

The rate of the reaction of alanine with 1-fluoro-2,4-dinitrobenzene in the presence of 3.5×10^{-2} M cetyltrimethylammonium bromide was studied at pH 7.99, 8.91, 9.67, 10.87 and 12.03. The rate of the reaction was studied with four different total buffer concentrations at each pH. Bicarbonate buffer was used. Attempts were made to keep the ionic strength constant. However, pH was slightly altered when ionic strength was maintained constant, so pH was held constant as higher priority. Ionic strengths for all the reaction solutions were approximately 1.2 M. This high ionic strength is a result of the high buffer concentrations used which was needed to keep the pH constant. The concentrations of alanine and 1-fluoro-2,4-dinitrobenzene were 2.5×10^{-2} M and 6.32×10^{-5} M respectively. The observed rate constant, k_{obs} or $(k_B + k_C)$ extrapolated to zero buffer concentration at each pH could be obtained. The rate constant k_C was obtained by studying the reaction in the presence of 3.5×10^{-2} M cetyltrimethylammonium bromide without added amine. The reaction of alanine with 1-fluoro-2,4-dinitrobenzene in the absence of surfactant was also studied at the above pH's. The observed rate

constants ($k_B + k_C$) and k_C in the absence of the surfactant at four different buffer concentrations for the above pH's were obtained. The observed rate constants ($k_B + k_C$) and k_C in the presence and absence of cetyltrimethylammonium bromide at pH 7.99, 8.91, 9.67, 10.87 and 12.03 are shown in Tables VIII through XII. The data in Tables VIII, IX, X, XI and XII are plotted in Figures 16, 17, 18, 19 and 20 respectively. The pH-rate profile for the reaction of alanine and 1-fluoro-2, 4-dinitrobenzene in the presence of 3.5×10^{-2} M cetyltrimethylammonium bromide, obtained by extrapolating the observed rate constants, ($k_B + k_C$) and k_C to zero buffer concentrations, is depicted in Figure 21. The ($k_B + k_C$) or k_B values shown in Fig. 21 seem to approach a plateau at pH above 11. This suggested that the alanine in the free amine form is the attacking species. The concentration of 2.5×10^{-2} M alanine in the free amine form was calculated with the Henderson-Hasselbalch equation and the pK_a (alanine) value was 9.87 (75).

Table VIII. Dependence of ($k_B + k_C$) and k_C on buffer concentration for the reaction of alanine with 1-fluoro-2, 4-dinitrobenzene in the presence and absence of cetyltrimethylammonium bromide at pH 7.99 with $NaHCO_3$ - HCl buffer; ionic strength ≈ 1.2; temperature 25°.

[Buffer] M	0.035 M Surfactant		No Surfactant	
	$k_B + k_C$ (sec^{-1})	k_C (sec^{-1})	$k_B + k_C$ (sec^{-1})	k_C (sec^{-1})
0.259	6.2×10^{-4}	3.9×10^{-4}	1.4×10^{-4}	1.0×10^{-4}
0.640	5.4×10^{-4}	3.5×10^{-4}	1.4×10^{-4}	0.9×10^{-4}
0.994	5.0×10^{-4}	3.9×10^{-4}	1.1×10^{-4}	0.8×10^{-4}
1.180	4.9×10^{-4}	3.7×10^{-4}	1.3×10^{-4}	0.9×10^{-4}

Fig. 16. Dependence of ($k_B + k_C$) and k_C on buffer concentration for the reaction of alanine with 1-fluoro-2, 4-dinitrobenzene in the presence and absence of 3.5×10^{-2} M cetyltrimethylammonium bromide at pH 7.99. Concentration of alanine and 1-fluoro-2, 4-dinitrobenzene are 2.5×10^{-2} M and 6.32×10^{-5} M respectively.

○ $k_B + k_C$ in the presence of surfactant

● k_C in the presence of surfactant

◑ $k_B + k_C$ in the absence of surfactant

⊖ k_C in the absence of surfactant

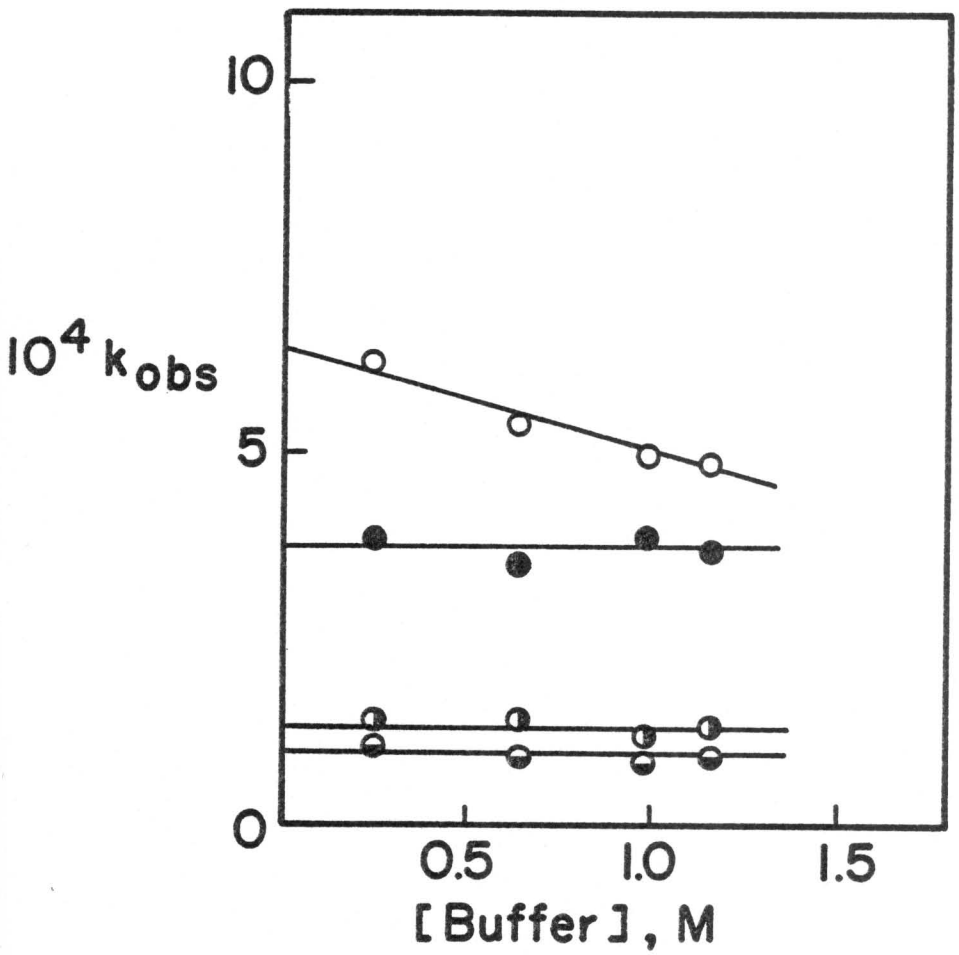

10
5
0
$10^4 k_{obs}$
0.5
1.0
1.5
[Buffer], M

Table IX. Dependence of ($k_B + k_C$) and k_C on buffer concentration for the reaction of alanine with 1-fluoro-2, 4-dinitrobenzene in the presence and absence of cetyltrimethylammonium bromide at pH 8.91 with $NaHCO_3$ - NaOH buffer; ionic strength $\simeq$ 1.2; temperature 25°.

Buffer M	0.035 M Surfactant		No Surfactant	
	$k_B + k_C$ (sec^{-1})	k_C (sec^{-1})	$k_B + k_C$ (sec^{-1})	k_C (sec^{-1})
0.200	1.92×10^{-3}	9.62×10^{-4}	2.20×10^{-4}	2.18×10^{-5}
0.508	1.76×10^{-3}	9.86×10^{-4}	1.81×10^{-4}	2.91×10^{-5}
0.820	1.57×10^{-3}	9.50×10^{-4}	1.82×10^{-4}	4.37×10^{-5}
1.047	1.48×10^{-3}	9.92×10^{-4}	2.08×10^{-4}	6.55×10^{-5}

Fig. 17. Dependence of ($k_B + k_C$) and k_C on buffer concentration for the reaction of alanine with 1-fluoro-2, 4-dinitrobenzene in the presence and absence of 3.5×10^{-2} M cetyltrimethylammonium bromide at pH 8.91. Concentration of alanine and 1-fluoro-2, 4-dinitrobenzene are 2.5×10^{-2} M and 6.32×10^{-5} M respectively.

○ $k_B + k_C$ in the presence of surfactant

● k_C in the presence of surfactant

◑ $k_B + k_C$ in the absence of surfactant

◒ k_C in the absence of surfactant

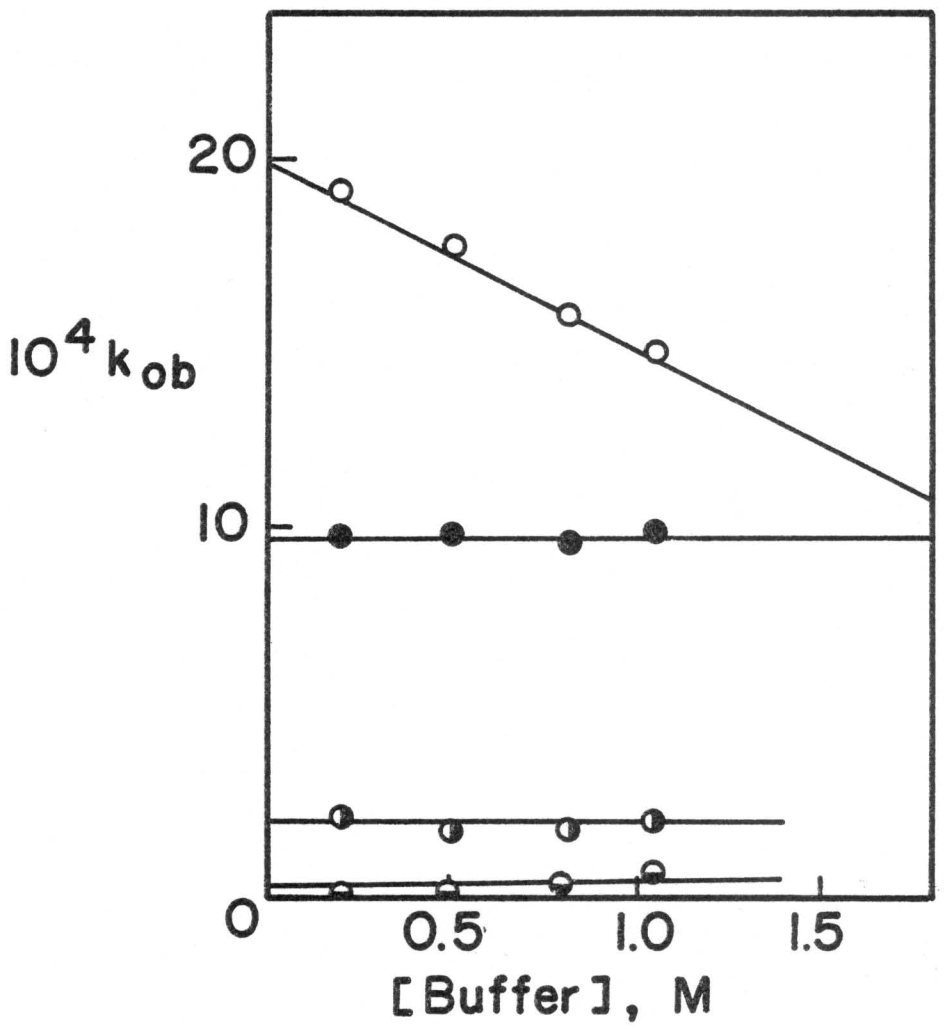

20
10
0
$10^4 k_{ob}$
0.5
1.0
1.5
[Buffer], M

Table X. Dependence of ($k_B + k_C$) on buffer concentration for the reaction of alanine with 1-fluoro-2,4-dinitrobenzene in the presence and absence of cetyltrimethylammonium bromide at pH 9.67 with $NaHCO_3$ - NaOH buffer; ionic strength $\approx$ 1.2; temperature 25°.

[Buffer] M	0.035 M Surfactant		No Surfactant	
	$k_B + k_C$ (sec^{-1})	k_C (sec^{-1})	$k_B + k_C$ (sec^{-1})	k_C (sec^{-1})
0.354	8.31×10^{-3}	4.17×10^{-3}	7.59×10^{-4}	6.89×10^{-5}
0.597	7.66×10^{-3}	4.45×10^{-3}	7.77×10^{-4}	1.30×10^{-4}
0.751	7.50×10^{-3}	4.70×10^{-3}	7.80×10^{-4}	1.50×10^{-4}
0.892	7.17×10^{-3}	5.07×10^{-3}	9.43×10^{-4}	2.27×10^{-4}

Fig. 18. Dependence of ($k_B + k_C$) and k_C on buffer concentration for the reaction of alanine with 1-fluoro-2, 4-dinitrobenzene in the presence and absence of cetyltrimethylammonium bromide at pH 9.67. Concentration of alanine and 1-fluoro-2, 4-dinitrobenzene were 2.5×10^{-2} M and 6.32×10^{-5} M respectively.

○ $k_B + k_C$ in the presence of surfactant

● k_C in the presence of surfactant

◑ $k_B + k_C$ in the absence of surfactant

◒ k_C in the absence of surfactant

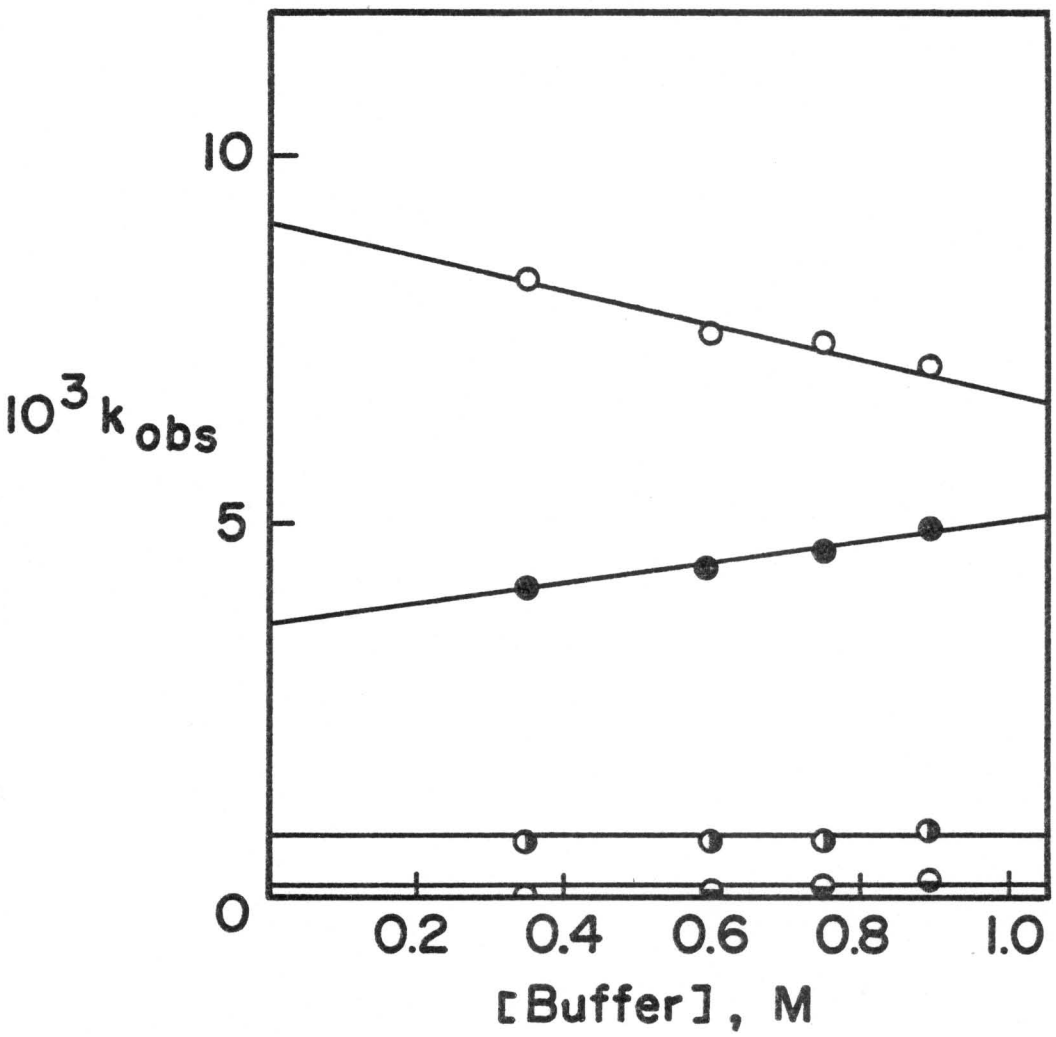

10
5
0
$10^3 k_{obs}$
0.2
0.4
0.6
0.8
1.0
[Buffer], M

Table XI. Dependence of ($k_B + k_C$) and k_C on buffer concentration for the reaction of alanine with 1-fluoro-2, 4-dinitrobenzene in the presence and absence of cetyltrimethylammonium bromide at pH 10.87 with $NaHCO_3$ - NaOH buffer; ionic strength ≃ 1.2; temperature 25°.

[Buffer] M	0.035 M Surfactant		No Surfactant	
	$k_B + k_C$ (sec^{-1})	k_C (sec^{-1})	$k_B + k_C$ (sec^{-1})	k_C (sec^{-1})
0.12	1.78×10^{-2}	8.67×10^{-3}	2.17×10^{-3}	3.0×10^{-4}
0.31	1.83×10^{-2}	9.43×10^{-3}	2.68×10^{-3}	4.2×10^{-4}
0.50	1.93×10^{-2}	1.00×10^{-2}	3.50×10^{-3}	6.0×10^{-4}
0.62	1.96×10^{-2}	1.05×10^{-2}	4.00×10^{-3}	8.5×10^{-4}

Fig. 19. Dependence of ($k_B + k_C$) and k_C on buffer concentration for the reaction of alanine with 1-fluoro-2, 4-dinitrobenzene in the presence and absence of cetyltrimethylammonium bromide at pH 10.87. Concentration of alanine and 1-fluoro-2, 4-dinitrobenzene are 2.5×10^{-2} M and 6.32×10^{-5} M respectively.

○ $k_B + k_C$ in the presence of surfactant

● k_C in the presence of surfactant

◑ $k_B + k_C$ in the absence of surfactant

◒ k_C in the absence of surfactant

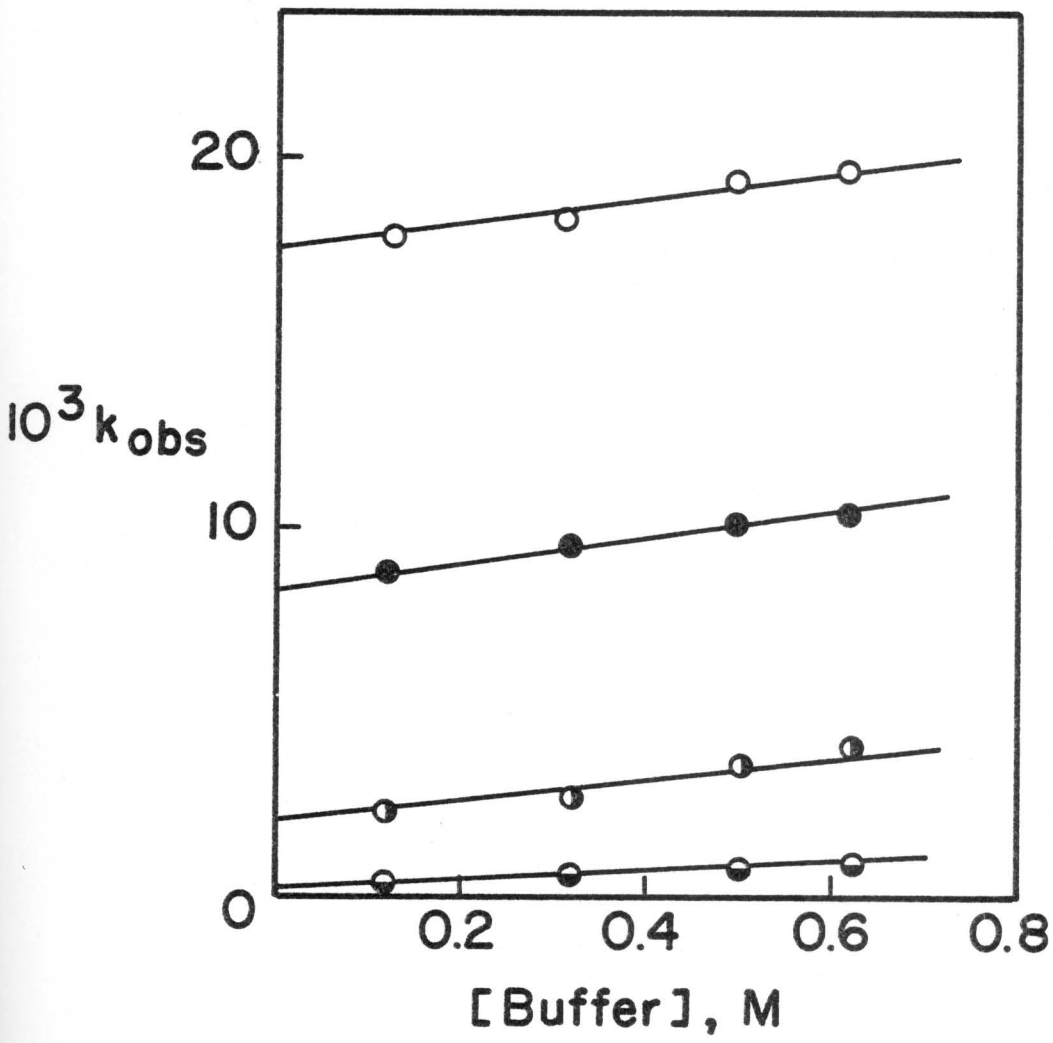

20
10
0
$10^3 k_{obs}$
0.2
0.4
0.6
0.8
[Buffer], M

Table XII. Dependence of ($k_B + k_C$) and k_C on total buffer concentration for the reaction of alanine with 1-fluoro-2, 4-dinitrobenzene in the presence of 0.035 M cetyltrimethylammonium bromide at pH 12.03. Concentrations of alanine and 1-fluoro-2, 4-dinitrobenzene were 2.5×10^{-2} M and 6.32×10^{-5} M respectively. pH 12.03 was made with $NaHCO_3$ - NaOH buffer; ionic strength $\simeq$ 1.2; temperature 25°.

[Buffer] M	$k_B + k_C$ (sec^{-1})	k_C (sec^{-1})
0.16	2.10×10^{-2}	1.29×10^{-2}
0.37	2.40×10^{-2}	1.42×10^{-2}
0.60	2.57×10^{-2}	1.66×10^{-2}
0.75	2.94×10^{-2}	2.04×10^{-2}

Fig. 20. Dependence of ($k_B + k_C$) and k_C on total buffer concentration for the reaction of alanine with 1-fluoro-2, 4-dinitrobenzene in the presence of 0.035 M cetyltrimethylammonium bromide at pH 12.03. Concentrations of alanine and 1-fluoro-2, 4-dinitrobenzene were 2.5×10^{-2} M and 6.32×10^{-5} M respectively.

○ $k_B + k_C$ in the presence of surfactant

● k_C in the presence of surfactant

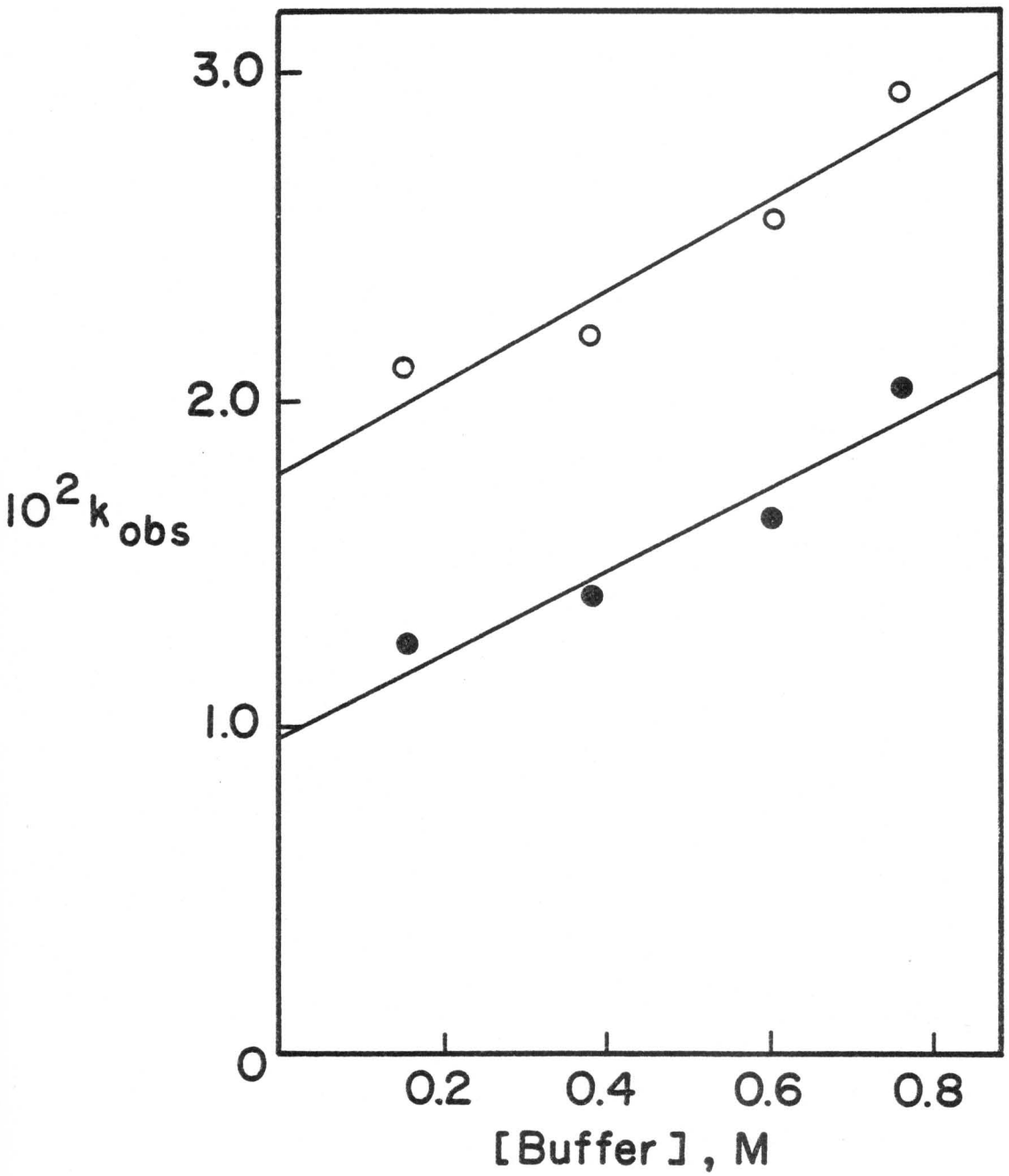
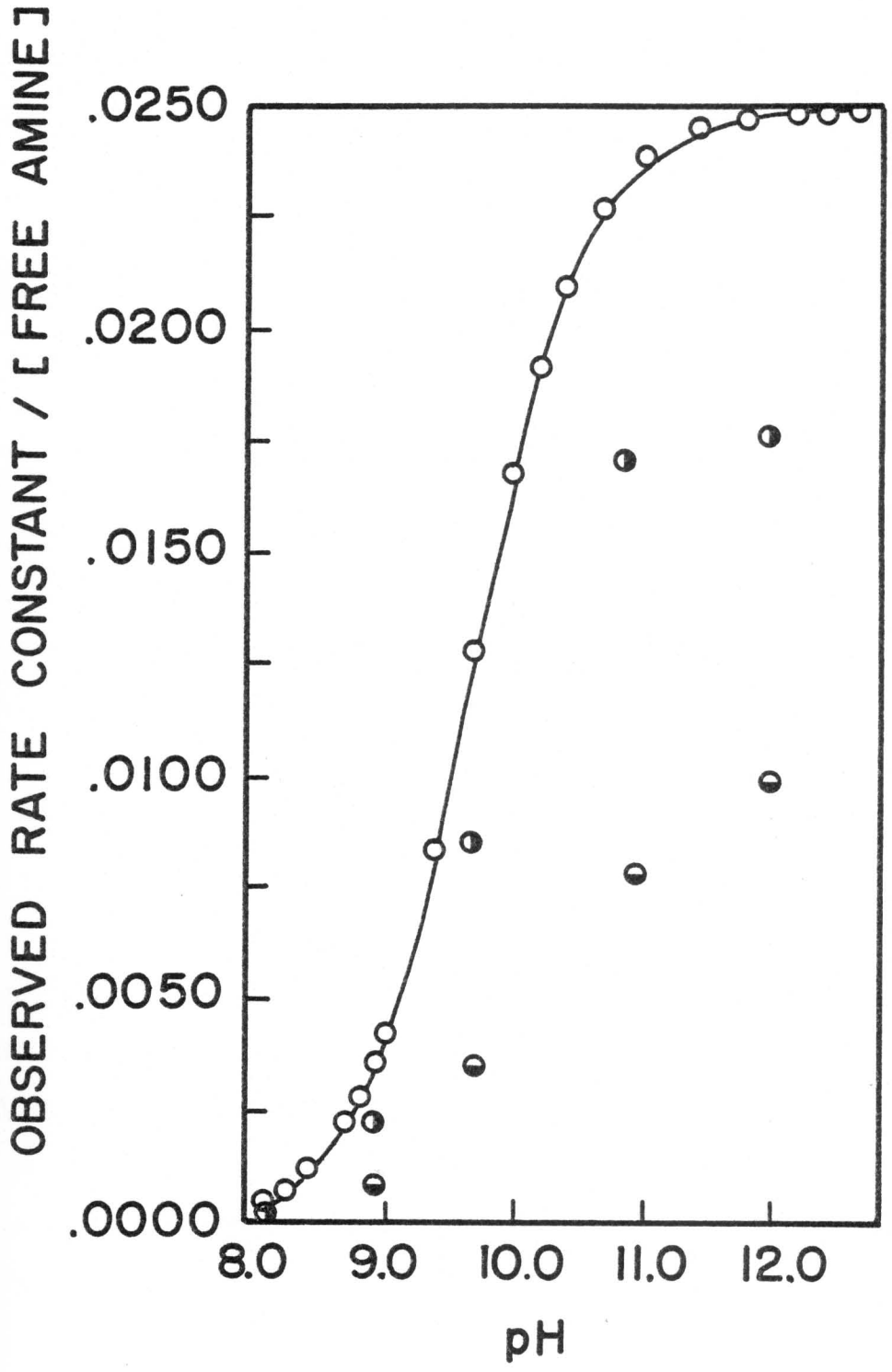

3.0
2.0
1.0
0
$10^2 k_{obs}$
0.2
0.4
0.6
0.8
[Buffer], M

Fig. 21. pH-rate profile for the reaction of alanine with 1-fluoro-2, 4-dinitrobenzene in the presence of 0.035 M cetyltrimethylammonium bromide. Observed rate constants ($k_B + k_C$) or k_C were obtained by extrapolation to zero buffer concentration at each pH. Concentrations of alanine and 1-fluoro-2, 4-dinitrobenzene were 2.5×10^{-2} M and 6.32×10^{-5} M respectively. Concentration of alanine in the free amine form at each pH was calculated with the Henderson - Hasselbalch equation.

○ Concentration of alanine in the free amine form

◑ $k_B + k_C$ in the presence of surfactant

◒ k_C in the presence of surfactant


OBSERVED RATE CONSTANT / [FREE AMINE]
.0250
.0200
.0150
.0100
.0050
.0000
8.0
9.0
10.0
11.0
12.0
pH

H. Dependence of k_{obs} on Concentration of Surfactant (Cetyltrimethylammonium Bromide)

The rate of the reaction of five amines with 1-fluoro-2, 4-dinitrobenzene in the presence of various concentrations of surfactant cetyltrimethylammonium bromide was studied. The five amines were : alanine, phenylalanine, aniline, p-methylaniline and p-methoxyaniline. The amine concentrations were 10^{-3} to 10^{-2} M, the concentration of 1-fluoro-2, 4-dinitrobenzene was 6.32×10^{-5} M, and the reactions were carried out at $25^{o}C$.

The observed rate constant for the reaction of 1-fluoro-2, 4-dinitrobenzene with alanine, phenylalanine, aniline, p-methylaniline and p-methoxyaniline as a function of [CTAB] are reported in Tables XIII, XIV, XV, XVI and XVII respectively. The data for the reaction of 1-fluoro-2, 4-dinitrobenzene with alanine, phenylalanine, aniline, p-methylaniline and p-methoxyaniline are plotted in two consecutive figures for each amine, beginning with Figures 22, 24, 26, 28 and 30 respectively.

A model is proposed later to explain the observed catalytic rate constant dependence on surfactant concentration. The proposed binding-partitioning model is described in section IV B. The dependence of k_{obs} on [CTAB] seems to fit the following equation :

$$k_{obs} - k_w' = \left(\frac{dC}{1 + dC}\right)\left(\frac{e}{1 + K_sC} - k_w\right) (S)_T \quad \text{Eq. 67}$$

where $d = \frac{k_R}{n}$

$$e = k_M P_s$$

Parameters d, e and K_s can be estimated by the following procedure as shown later :

(1) The initial rising slope of the k_{obs} vs [CTAB] plot gives the quantity $d\left[e(S)_T - k_w'\right]$, where $k_w' = k_w(S)_T$.

(2) A plot of the initial slope vs S_T gives the quantity $(de - dk_w)$. Since $e > k_w$, this is mainly controlled by de.

(3) If we ignore the K_s term, we have a Michaelis - Menten form :

$$k_{obs} - k_w' = \frac{dC}{1 + dC}\left[e\,(S)_T - k_w'\right]$$

Thus the maximum value of $k_{obs} - k_w'$ is approximately $\left[e(S)_T - k_w'\right]$. This should give a reasonable first estimate of this quantity.

(4) $$K_s = \frac{1}{d(C_{max})^2}$$

where the maximum value of the observed rate constant occurs at C_{max}.

The d, e, and K_s parameters estimated by the above procedure were then used as initial estimates for curve fitting. The final values of d, e and K_s are determined after several iterations. These parameters are reported in Table XVII.

Table XIII. Dependence of the observed rate constant on surfactant concentration for the reaction of 1-fluoro-2, 4-dinitrobenzene and alanine.

	[Alanine]		
10^3 [Surfactant]	2.5×10^{-2} M	1.25×10^{-2} M	2.67×10^{-3} M
M	$10^3 k_{obs}$ (sec^{-1})	$10^3 k_{obs}$ (sec^{-1})	$10^4 k_{obs}$ (sec^{-1})
No surfactant	0.585	0.33	0.67
0.0962	0.586	—	—
0.134	0.585	—	—
0.150	—	0.32	0.68
0.178	0.626	—	—
0.240	0.699	0.375	0.95
0.385	—	—	1.17
0.467	1.06	—	—
0.550	1.20	—	1.55
0.701	1.35	0.741	—
0.756	1.45	—	—
0.80	—	—	2.10

Table XIII. (cont.)

10^3 Surfactant M	[Alanine] 2.5 x 10^{-2} M $10^3 k_{obs}$ (sec^{-1})	1.25 x 10^{-2} M $10^3 k_{obs}$ (sec^{-1})	2.67 x 10^{-3} M $10^4 k_{obs}$ (sec^{-1})
1.02	1.82	–	2.60
1.05	–	1.03	–
1.13	1.98	–	–
6.00	–	3.51	–
7.50	–	–	8.75
8.22	8.51	4.33	–
17.5	10.8	5.63	11.8
30.5	–	–	11.8
34.4	–	–	11.7
35.0	11.46	6.13	–
49.9	–	–	10.7
50.0	11.3	6.03	10.7
68.6	10.8	5.73	–

Fig. 22. Dependence of the observed rate constant, k_{obs} on surfactant concentration for the reaction of alanine and 1-fluoro-2, 4-dinitrobenzene. Slopes of this plot give the initial estimate for the quantity ($deS_T - dk_w'$). Concentration of 1-fluoro-2, 4-dinitrobenzene was 6.32×10^{-5} M.

○ 2.5×10^{-2} M alanine

◑ 1.25×10^{-2} M alanine

◒ 2.67×10^{-3} M alanine

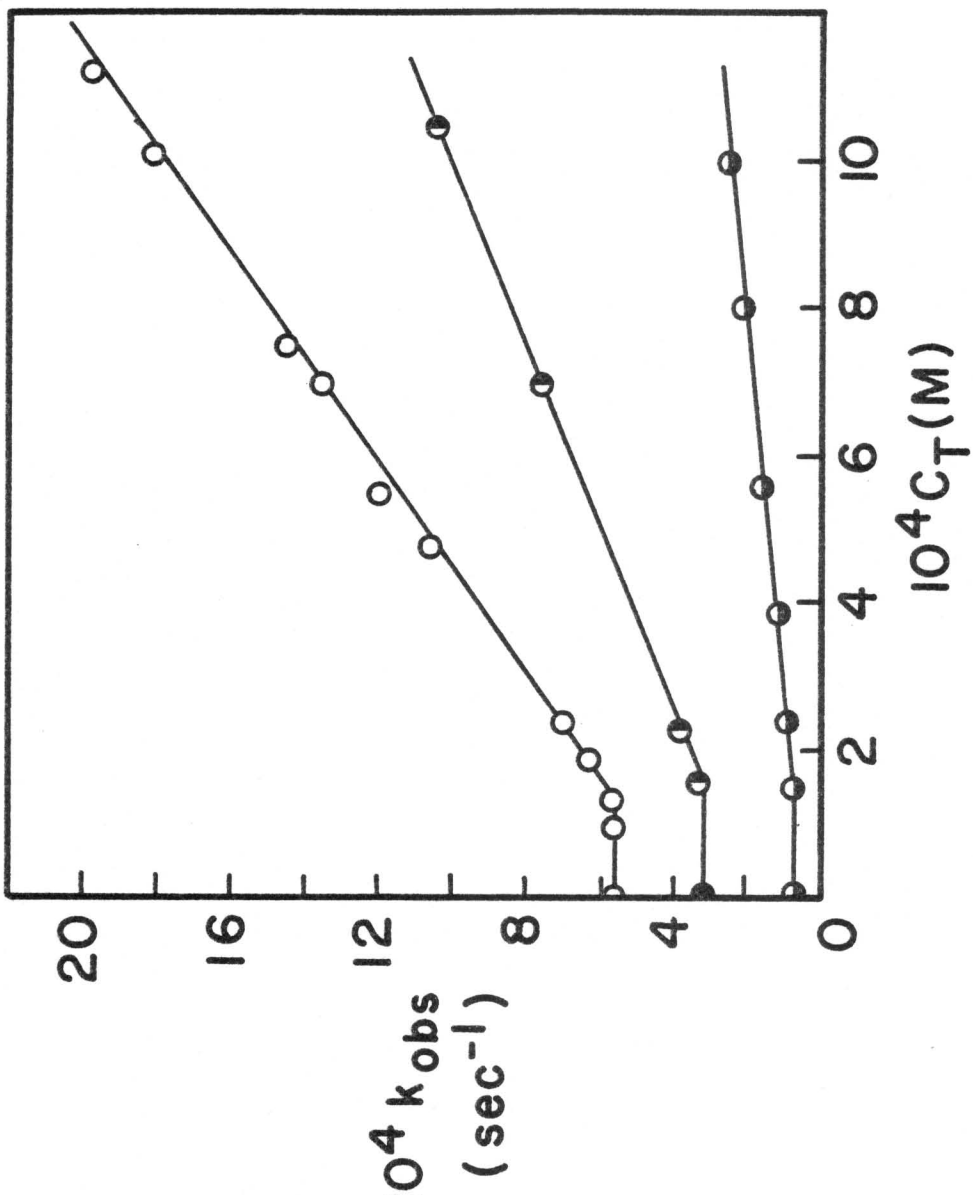

20
16
12
8
4
0
$10^4 k_{obs}$ (sec^{-1})
2
4
6
8
10
$10^4 C_T$ (M)

Fig. 23. Dependence of k_M' on surfactant concentration for the reaction of alanine and 1-fluoro-2, 4-dinitrobenzene. Surfactant concentration range from 10^{-4} M to 10^{-2} M. Concentration of 1-fluoro-2, 4-dinitrobenzene was 6.32×10^{-5} M. $k_M' = k_{obs} - k_w'$

○ 2.5×10^{-2} M alanine

◑ 1.25×10^{-2} M alanine

◒ 2.67×10^{-3} M alanine

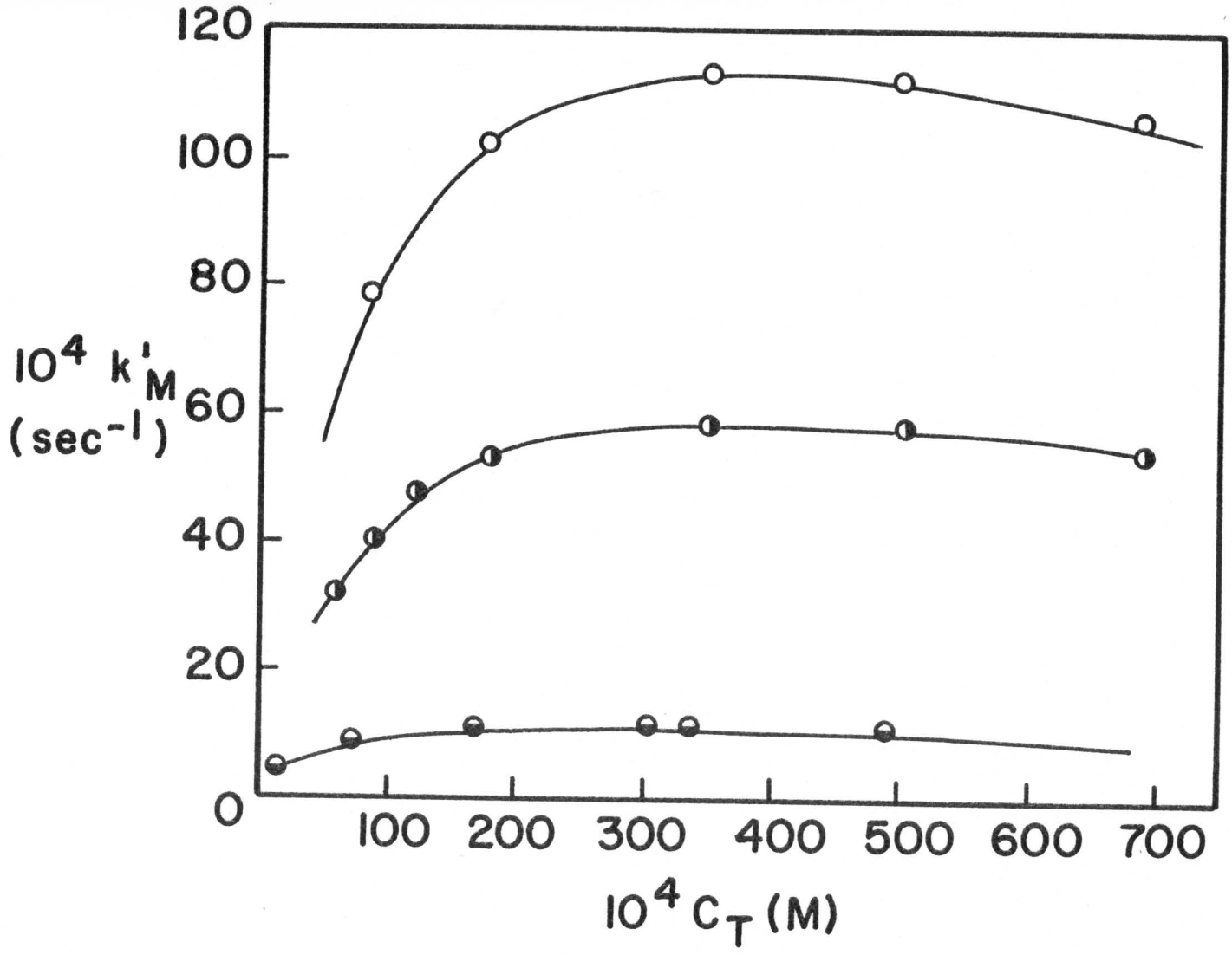

120
100
80
60
40
20
0
10^4 k'M (sec^{-1})
100 200 300 400 500 600 700
10^4 C_T (M)

Table XIV. Dependence of the observed rate constant on surfactant concentration for the reaction of 1-fluoro-2, 4-dinitrobenzene and phenylalanine.

10^3 [Surfactant], M	10^3 k_{obs} (sec^{-1})
No surfactant	0.0969
0.117	0.0960
0.467	0.788
0.783	1.48
1.10	2.036
7.96	10.1
17.5	13.9
34.8	13.8
49.8	12.6
68.3	10.7

Fig. 24. Dependence of the observed rate constant, k_{obs} on surfactant concentration for the reaction of phenylalanine and 1-fluoro-2,4-dinitrobenzene. The slope of this plot gives the initial estimate for the quantity ($deS_T - dk_0'$). Concentration of phenylalanine and 1-fluoro-2,4-dinitrobenzene were 1.038×10^{-3} M and 6.32×10^{-5} M respectively.

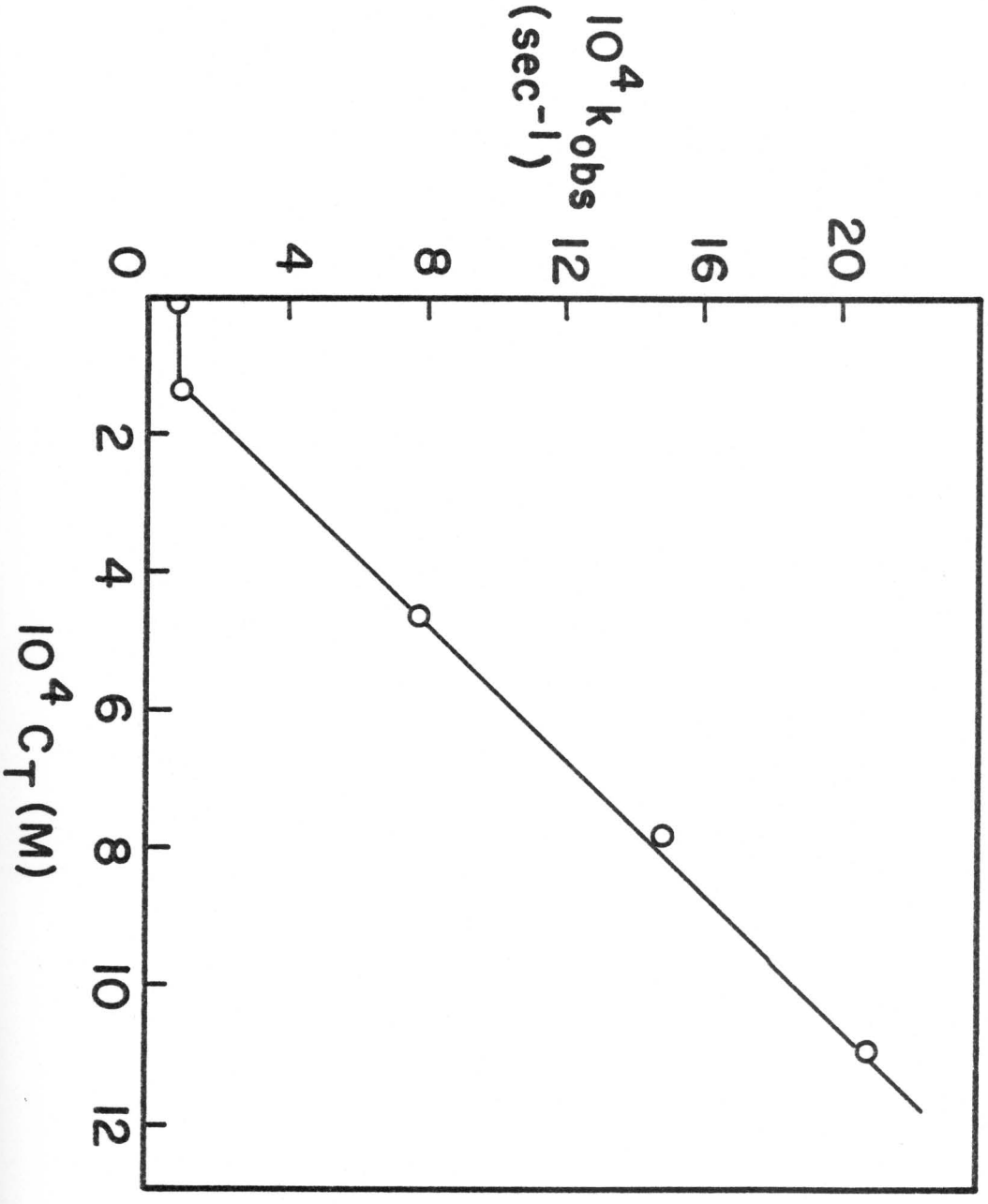

$10^4 k_{obs}$ (sec^{-1})
0
4
8
12
16
20
$10^4 C_T$ (M)
2
4
6
8
10
12

Fig. 25. Dependence of k_M' on surfactant concentration for the reaction of phenylalanine and 1-fluoro-2,4-dinitrobenzene. Surfactant concentration range from 10^{-4} M to 10^{-2} M. Concentration of phenylalanine and 1-fluoro-2,4-dinitrobenzene were 1.038×10^{-3} M and 6.32×10^{-5} M respectively.

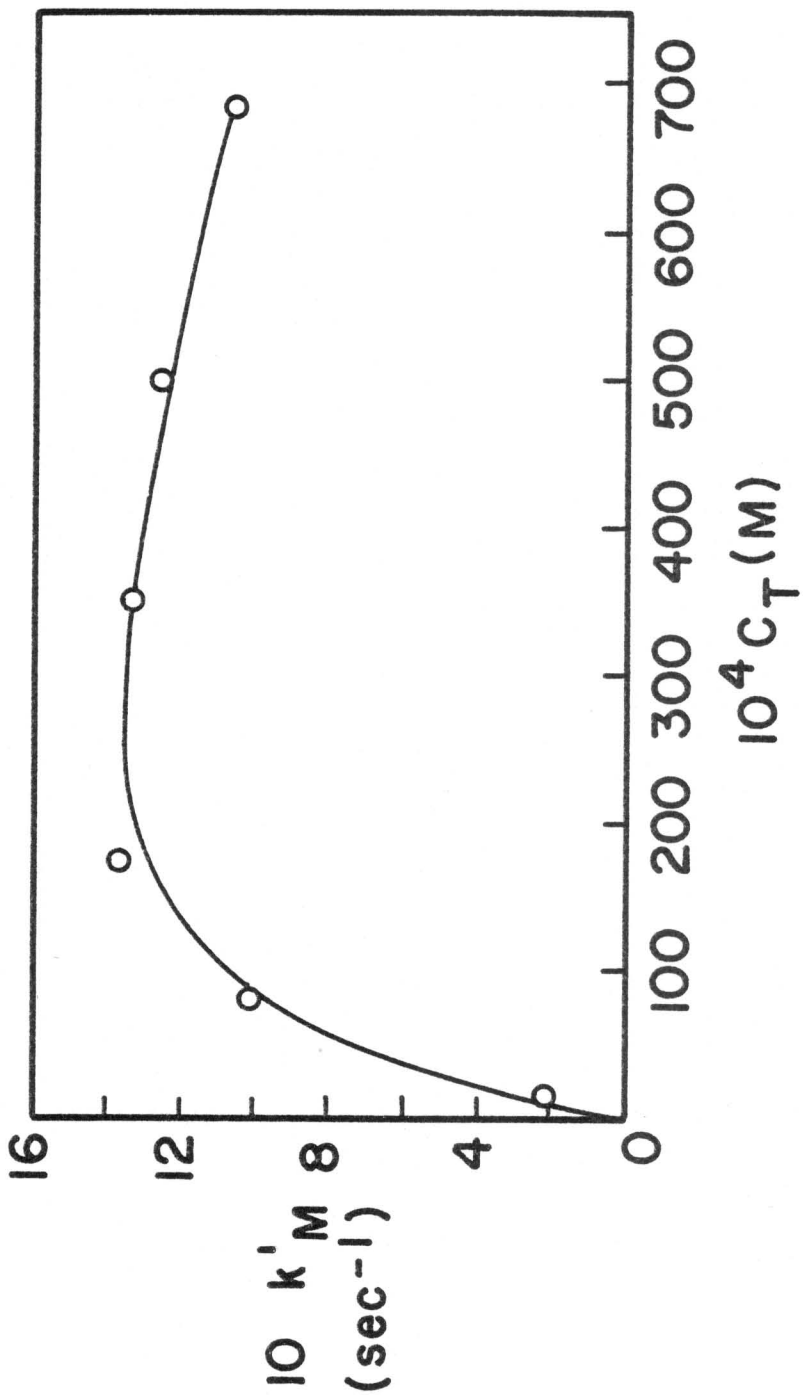

16
12
8
4
0
10 k' M
(sec⁻¹)
100 200 300 400 500 600 700
10⁴ C_T (M)

Table XV. Dependence of the observed rate constant on surfactant concentration for the reaction of 1-fluoro-2,4-dinitrobenzene and aniline. Concentration of aniline and 1-fluoro-2,4-dinitrobenzene were 9.88×10^{-2} M and 6.32×10^{-5} M respectively.

10^3 [Surfactant] , M	$10^3 k_{obs}$ (sec^{-1})
No surfactant	0.493
0.11	0.494
0.485	0.645
0.756	0.794
0.907	0.869
1.22	1.016
1.40	1.127
8.57	3.068
17.3	3.96
35.4	4.26
50.4	3.96
68.6	3.61

Fig. 26. Dependence of the observed rate constant, k_{obs} on surfactant concentration for the reaction of aniline and 1-fluoro-2,4-dinitrobenzene. The slope of this plot gives the initial estimate for the quantity ($deS_T - dk_w'$). Concentration of aniline and 1-fluoro-2,4-dinitrobenzene were 9.88×10^{-2} M and 6.32×10^{-5} M respectively.

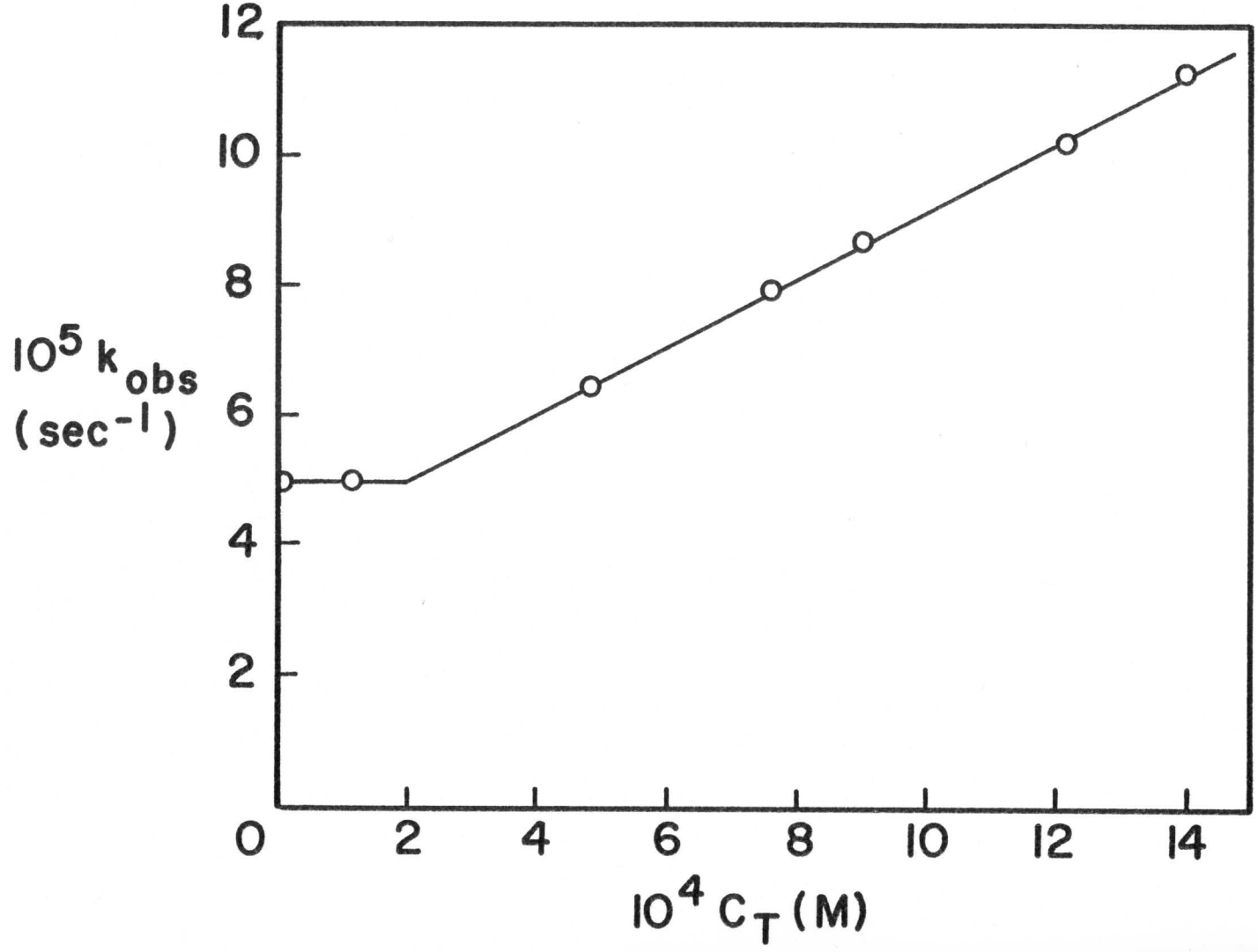

12
10
8
6
4
2
0
2
4
6
8
10
12
14
$10^5 k_{obs}$ (sec^{-1})
$10^4 C_T$ (M)

Fig. 27. Dependence of k_M' on surfactant concentration for the reaction of aniline and 1-fluoro-2,4-dinitrobenzene. Surfactant concentration range from 10^{-4} M to 10^{-2} M. Concentration of 1-fluoro-2,4-dinitrobenzene and aniline were 6.32×10^{-5} M and 9.88×10^{-2} M respectively.

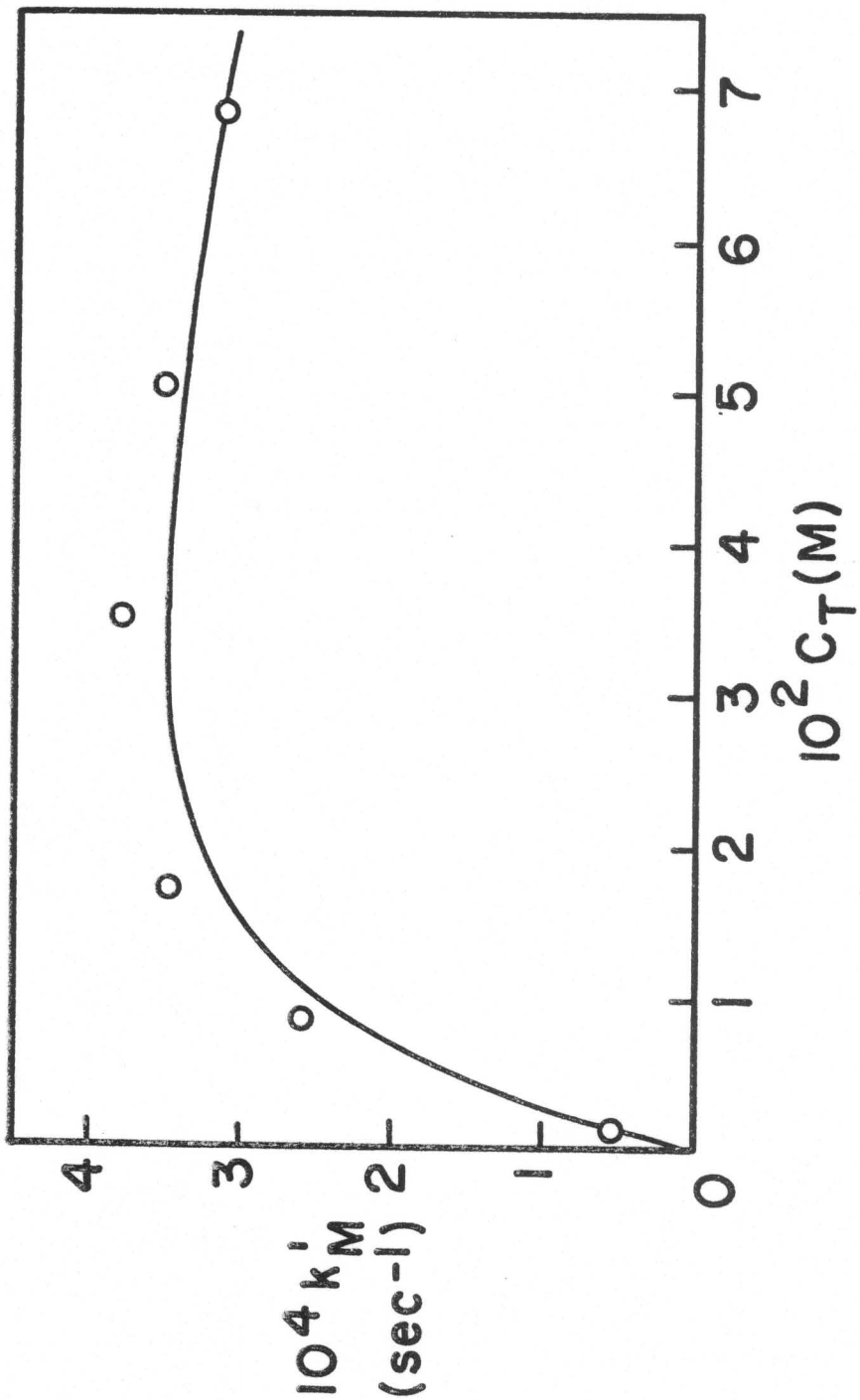

$10^4 k'_M$ (sec^{-1})
0
1
2
3
4
0
1
2
3
4
5
6
7
$10^2 C_T$(M)

Table XVI. Dependence of the observed rate constant, k_{obs} on surfactant concentration for the reaction of 1-fluoro-2,4-dinitrobenzene and p-methylaniline. Concentration of p-methylaniline and 1-fluoro-2,4-dinitrobenzene were 8.07×10^{-3} M and 6.32×10^{-5} M respectively.

10^3 [Surfactant] , M	$10^2 k_{obs}(sec^{-1})$
No surfactant	0.115
0.0824	0.116
0.206	0.154
0.385	0.188
0.394	0.187
0.714	0.245
0.880	0.293
1.15	0.339
10.4	1.34
18.6	1.49
18.8	1.53
25.2	1.58
30.4	1.52
36.1	1.49
36.7	1.49

Table XVI. (cont.)

10^3 Surfactant , M	10^2 k_{obs} (sec^{-1})
49.9	1.36
53.2	1.34

Fig. 28. Dependence of the observed rate constant, k_{obs} on surfactant concentration for the reaction of p-methylaniline and 1-fluoro-2,4-dinitrobenzene. The slope of this plot gives the initial estimate for the quantity ($deS_T - dk_w'$). Concentration of p-methylaniline and 1-fluoro-2,4-dinitrobenzene were 8.07×10^{-3} M and 6.32×10^{-5} M respectively.

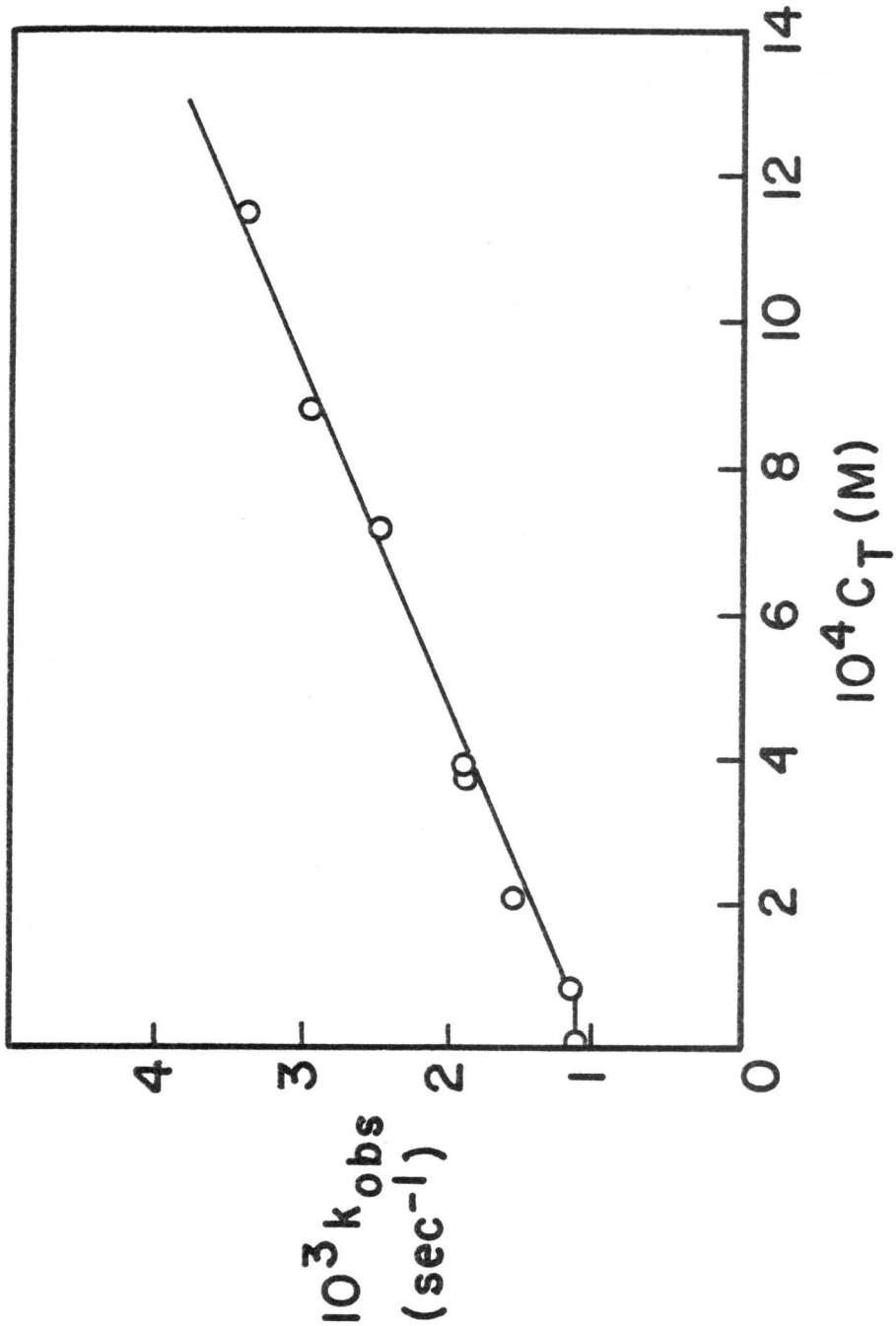

$10^3 k_{obs}$ (sec^{-1})
4
3
2
1
0
2
4
6
8
10
12
14
$10^4 C_T$ (M)

Fig. 29. Dependence of k_M' on surfactant concentration for the reaction of p-methylaniline and 1-fluoro-2,4-dinitrobenzene. Surfactant concentration range from 10^{-4} M to 10^{-2} M. Concentration of 1-fluoro-2,4-dinitrobenzene and p-methylaniline were 6.32×10^{-5} M and 8.07×10^{-3} M respectively.

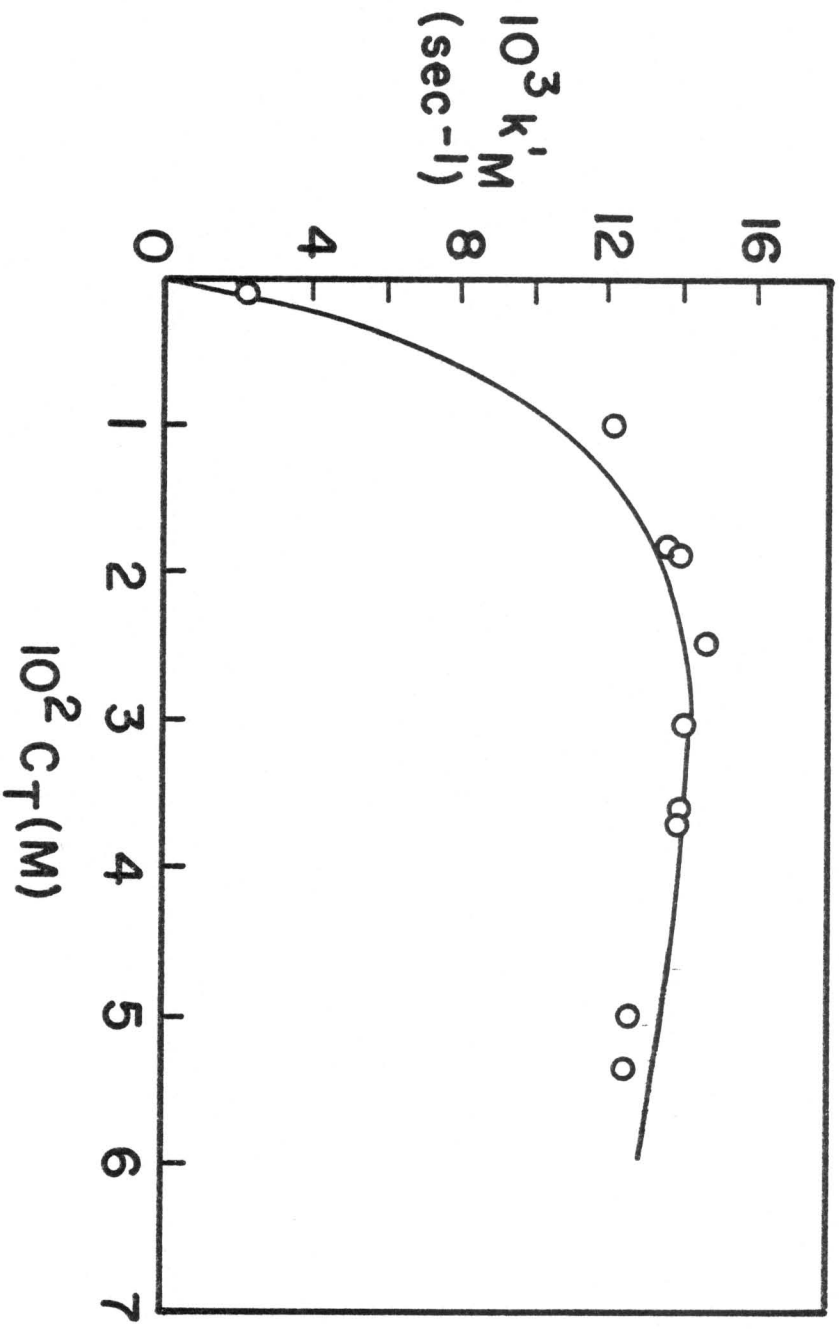

$10^3 k'_M$ (sec^{-1})
0
4
8
12
16
1
2
3
4
5
6
7
$10^2 C_T$(M)

Table XVII. Dependence of the observed rate constant on surfactant concentration for the reaction of 1-fluoro-2,4-dinitrobenzene and p-methoxyaniline. Concentration of 1-fluoro-2,4-dinitrobenzene and p-methoxyaniline were 6.32×10^{-5} M and 2.43×10^{-2} M respectively.

10^3 [Surfactant] , M	$10^3 k_{obs}$ (sec^{-1})
No surfactant	0.543
0.0824	0.543
0.22	0.582
0.412	0.731
0.577	0.815
0.701	0.817
0.904	1.09
1.09	1.12
1.21	1.29
4.84	2.64
8.37	4.09
17.9	5.78
34.8	6.72

Table XVII. (cont.)

10^3 [Surfactant] , M	10^3 k_{obs} (sec^{-1})
49.9	6.72
68.8	6.10

Fig. 30. Dependence of the observed rate constant, k_{obs} on surfactant concentration for the reaction of p-methoxyaniline and 1-fluoro-2,4-dinitrobenzene. The slope of this plot gives the initial estimate for the quantity ($deS_T - dk_w'$). Concentration of 1-fluoro-2,4-dinitrobenzene and p-methoxyaniline were 6.32×10^{-5} M and 2.43×10^{-2} M respectively.

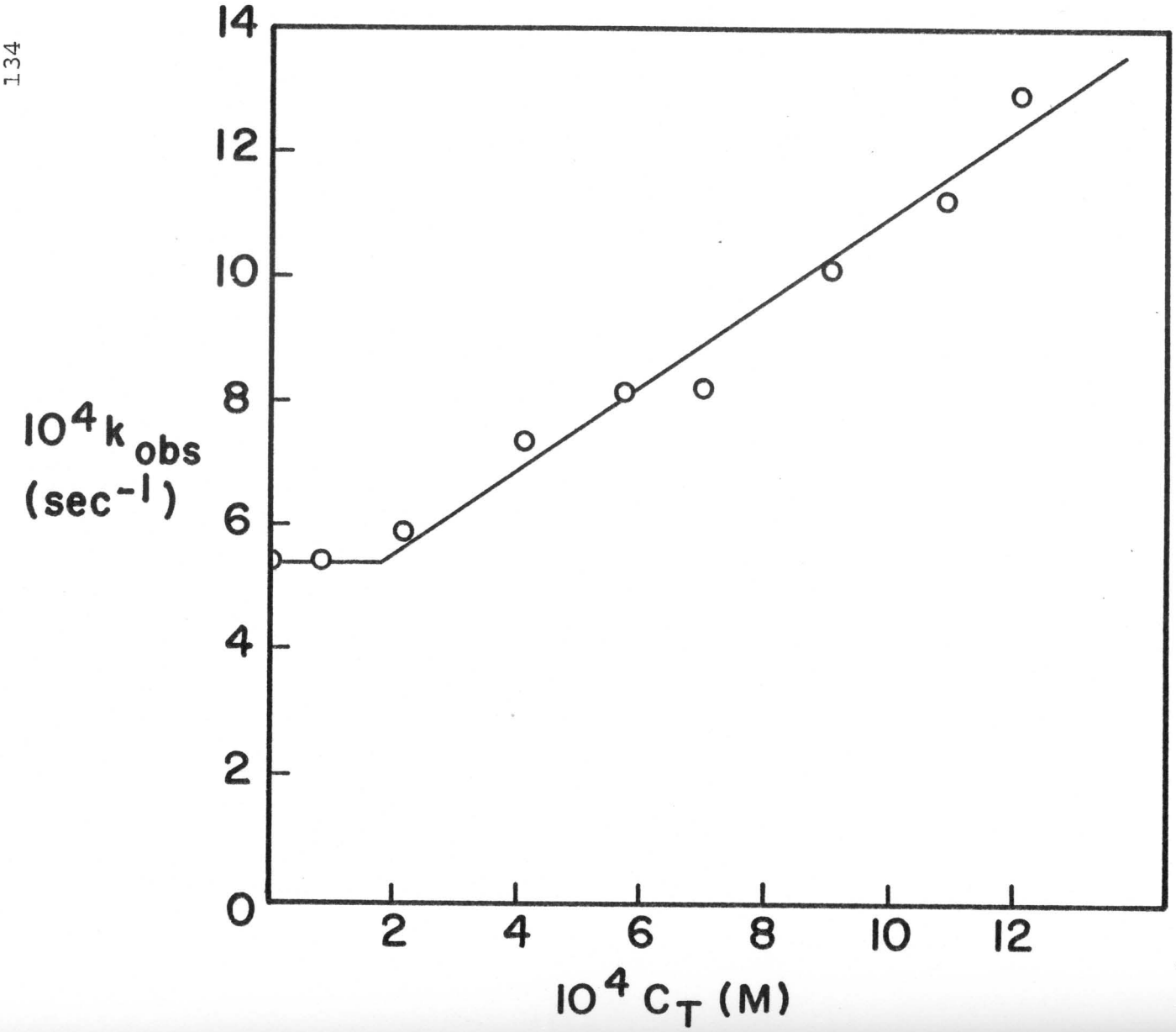

14
12
10
8
6
4
2
0
$10^4 k_{obs}$ (sec^{-1})
2
4
6
8
10
12
$10^4 C_T$ (M)

Fig. 31. Dependence of k_M' on surfactant concentration for the reaction of p-methoxyaniline and 1-fluoro-2,4-dinitrobenzene. Surfactant concentration range from 10^{-4} M to 10^{-2} M. Concentration of 1-fluoro-2,4-dinitrobenzene and p-methoxyaniline were 6.32×10^{-5} M and 2.43×10^{-2} M respectively.

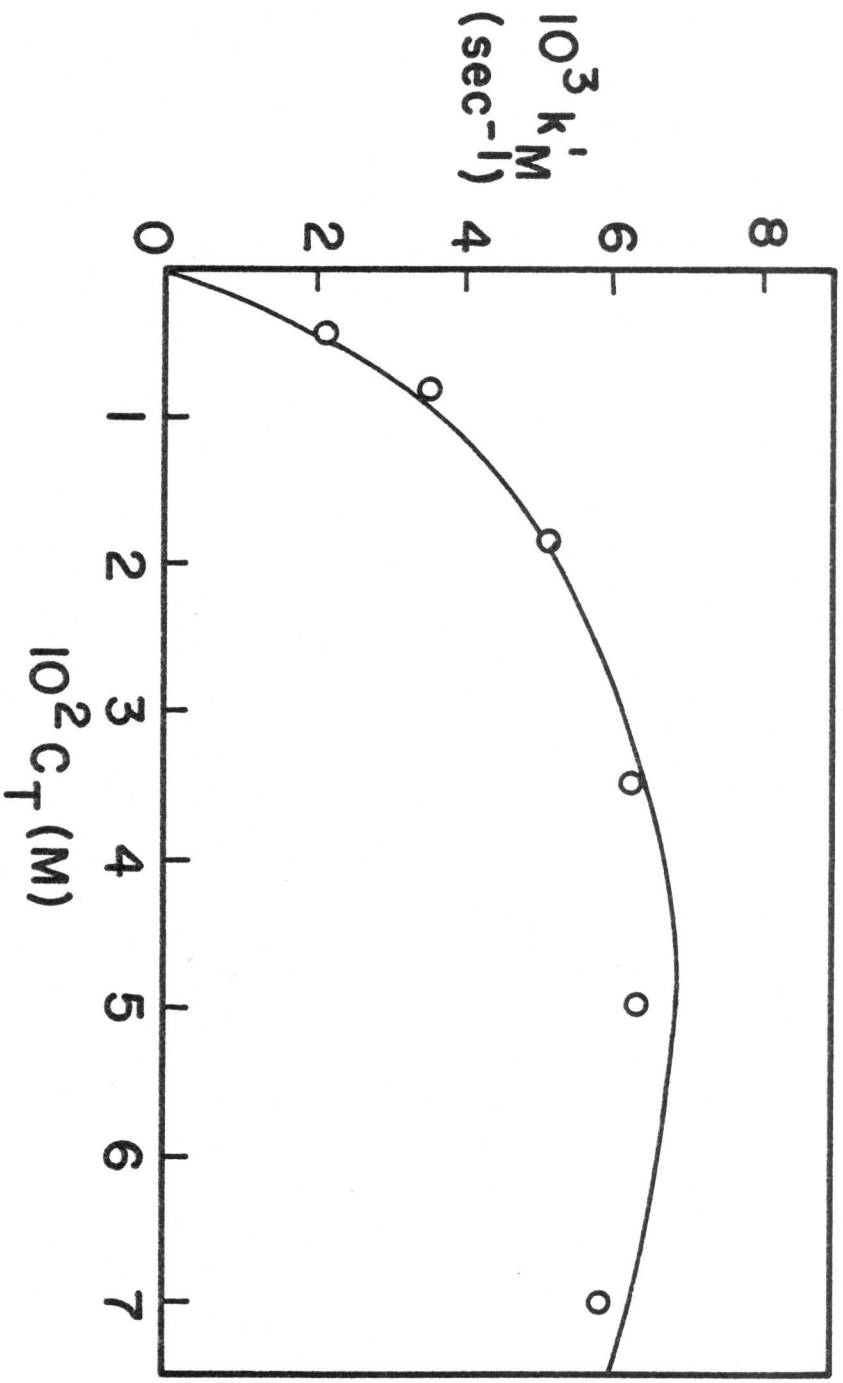
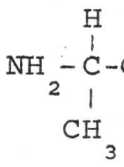
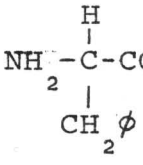

$10^3 k'_M$ (sec^{-1})
0
2
4
6
8
$10^2 C_T$ (M)
1
2
3
4
5
6
7

Table XVIII. Estimates of d, e, and K_s.

Substrate	Parameter[a] d	e	K_s
	90	0.80	6.86
	70	31.41	21.85
$\phi - NH_2$	90	0.00636	7.00
$CH_3-\phi-NH_2$	70	3.766	11.92
$CH_3O-\phi-NH_2$	50	0.553	8.22

$a_d = K_R/n$;

$e = k_M P_s$;

$$K_s = \frac{N_A \nu_M (P_s - 1)}{n} \quad ;$$

or $K_s = V (P_s - 1)$

where V is the molar volume of surfactant.

IV. DISCUSSION

A. Proposed Analytical Procedure

The proposed analytical method for the determination of aqueous solutions of amines, amino-acids and peptides can be found in the Experimental Section. The rate enhancement ranged from 8 to greater than 1330 for the reaction of 1-fluoro-2,4-dinitrobenzene with amines in the presence of surfactant cetyltrimethylammonium bromide as compared to its absence (at 25^{0}). An additional rate enhancement factor of 3 is produced by increasing the temperature from 25^{0} to $45^{0}C$.

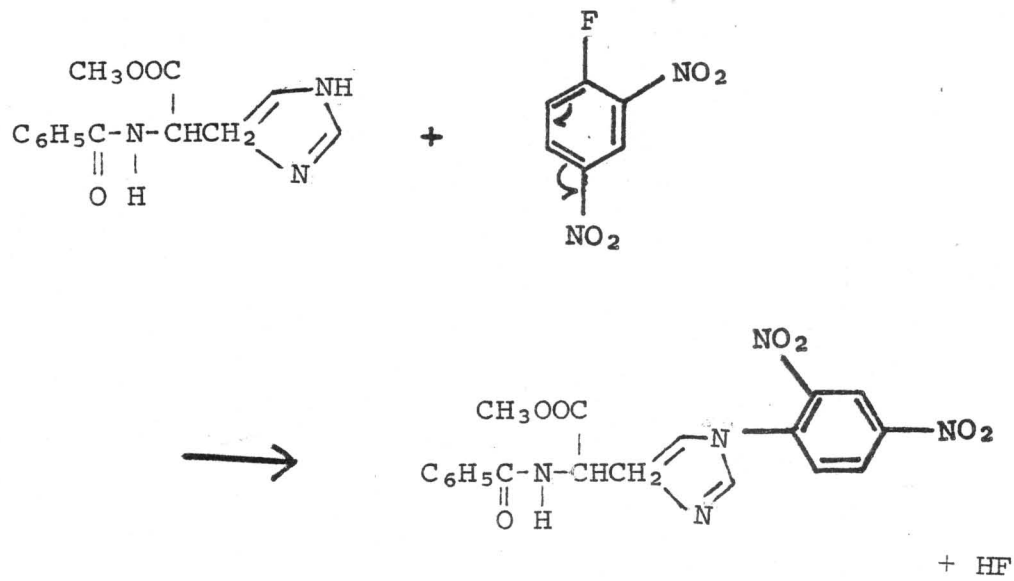
The analytical method is applicable to amine compounds in the range 10^{-4} M to 10^{-3} M. The concentration-response curves are linear in this range. The slopes of the concentration-response curves are identical at $25^{0}C$ and $45^{0}C$.

This modification of the conventional DFB method of amine and amino acid determination by taking advantage of micellar catalysis has resulted in a significant decrease

in the reaction time, which could result in new applications of this reaction. As analytical problems arise in which spectrophotometric detection of amino compounds may be useful (for example, after separations by high-performance liquid chromatography) this faster means for forming a strongly absorbing derivative may provide a useful analytical tool.

B. Berezin's Partitioning-Partitioning Model

Berezin et al. (76) have investigated the mechanism of the acceleration of the aminolysis of 1-fluoro-2,4-dinitrobenzene with N-benzoyl-L-histidine methyl ester in the presence of cetyltrimethylammonium bromide.



They postulated that solutes R (1-fluoro-2,4-dinitro-benzene) and S (amine) are distributed between the micellar and aqueous phases in conformity with the simple distribution law, and they developed a relationship between the observed catalytic rate constant and the CTAB concentration along the following lines. This model may be referred to as the partitioning-partitioning model, since it postulates a partitioning mechanism for the uptake of both solutes.

For species R and S:

$$R_{(aq)} \rightleftharpoons R_{(mic)} \qquad S_{(aq)} \rightleftharpoons S_{(mic)}$$

$$P_R = \frac{(R)_M}{(R)_w} \qquad P_S = \frac{(S)_M}{(R)_w}$$

Eq. 12

where P is a partition coefficient between the aqueous and micellar phases.

Define V_T = total volume of the system

V_M = micellar phase of volume

V_w = water phase volume

Then $V_T = V_M + V_w$

or $1 = \frac{V_M}{V_T} + \frac{V_w}{V_T}$ Eq. 13

Mass balance gives:

$(R)_T V_T = (R)_M V_M + (R)_w V_w$ $\quad$ $(S)_T V_T = (S)_M V_M + (S)_w V_w$ Eq. 14

Solve Eq. 13 for $\frac{V_w}{V_T}$ and substitute into Eq. 14

$$(R)_T = (R)_M \frac{V_M}{V_T} + (R)_w \left[1 - \frac{V_M}{V_T}\right] \qquad (S)_T = (S)_M \frac{V_M}{V_T} + (S)_w \left[1 - \frac{V_M}{V_T}\right] \quad \text{Eq. 15}$$

Now define

$$C = C_T - (CMC) \quad \text{Eq. 16}$$

C_T = total molar surfactant concentration.

(CMC) = critical micelle concentration.

C = molar concentration of surfactant present as micelles.

Define V as the molar volume of surfactant.

Thus C and V have these units:

$$C = \frac{\text{moles of surfactant as micelles}}{\text{liters of solution}}$$

$$V = \frac{\text{liters of surfactant}}{\text{mole of surfactant}}$$

Thus,

$$CV = \frac{\text{liters of surfactant as micelles}}{\text{liters of solution}}$$

So CV is the volume fraction of the micellar phase, or

$$CV = \frac{V_M}{V_T} \qquad \text{Eq. 17}$$

From Eq. 16 and Eq. 17,

$$(R)_T = (R)_M CV + (R)_w(1 - CV) \qquad (S)_T = (S)_M CV + (S)_w(1 - CV) \qquad \text{Eq. 18}$$

Now substitute Eq. 12 into Eq. 18 giving,

after rearrangement:

$$\frac{(R)_T}{(R)_w} = 1 + (P_R - 1)CV \qquad \frac{(S)_T}{(S)_w} = 1 + (P_S - 1)CV \qquad \text{Eq. 19}$$

Define

$$K_R = (P_R - 1)V \qquad K_S = (P_S - 1)V \qquad \text{Eq. 20}$$

Therefore

$$\frac{(R)_T}{(R)_w} = 1 + K_R C \qquad \frac{(S)_T}{(S)_w} = 1 + K_S C \qquad \text{Eq. 21}$$

where K_R and K_S have the units M^{-1}, i.e., they have the character of 1:1 binding constants.

The bimolecular reaction between R and S can occur in both phases:

$$\underline{v}_M = k_M (R)_M (S)_M$$

$$\underline{v}_w = k_w (R)_w (S)_w$$

For the observed velocity of the total system:

$$\underline{v} = \underline{v}_M \begin{pmatrix} \text{fraction system in} \\ \text{micellar phase} \end{pmatrix} + \underline{v}_w \begin{pmatrix} \text{fraction system in} \\ \text{aqueous phase} \end{pmatrix}$$

But from Eq. 17, this becomes

$$\underline{v} = \underline{v}_M CV + \underline{v}_w (1 - CV) \qquad \text{Eq. 22}$$

Experimentally, we can define an observed second-order rate constant according to Eq. 23

$$\underline{v} = k_{exp}(R)_T(S)_T \qquad \text{Eq. 23}$$

Thus:

$$k_{exp}(R)_T(S)_T = k_M(R)_M(S)_M CV + k_w(R)_w(S)_w(1 - CV) \qquad \text{Eq. 24}$$

Substituting from Eq. 12 into Eq. 24 for $(R)_M$ and $(S)_M$ gives

$$k_{exp} = \frac{(R)_w(S)_w\left[k_M P_R P_S CV + k_w(1 - CV)\right]}{(R)_T(S)_T} \qquad \text{Eq. 25}$$

Now putting Eq. 21 into Eq. 25 gives

$$k_{exp} = \frac{k_M P_R P_S CV + k_w(1 - CV)}{(1 + K_R C)(1 + K_S C)} \qquad \text{Eq. 26}$$

When the volume fraction of micelles is small ($CV \ll 1$), this becomes

$$k_{exp} = \frac{k_M P_R P_S CV + k_w}{(1 + K_R C)(1 + K_S C)} \qquad \text{Eq. 27}$$

Now when partitioning into the micelle phase is highly favored, then $P \gg 1$, and from Eq. 20 we can write

$$K_R = P_R V \qquad K_S = P_S V \qquad \text{Eq. 28}$$

In this case, which should be valid for anionic and hydrophobic species, Eq. 28 into Eq. 27 gives

$$k_{exp} = \frac{k_M' K_R K_S C + k_w}{(1 + K_R C)(1 + K_S C)} \qquad \text{Eq. 29}$$

where $k_M' = k_M/V$ Eq. 30

When $k_M' K_R K_S C \gg k_w$, then

$$k_{exp} = \frac{k_M' K_R K_S C}{(1 + K_R C)(1 + K_s C)} \qquad \text{Eq. 31}$$

Berezin proposed the above partitioning-partitioning model to explain the dependence of the experimental rate constant for reaction between 1-fluoro-2,4-dinitrobenzene and the methyl ester of N-benzoyl-L-histidine on surfactant concentration. This dependence shows a maximum in the rate, which can be accounted for by Eq. 31. As Berezin points out, in order to account for this maximum it is necessary to describe the uptake of both solutes, a situation making the model qualitatively different from the usual enzyme (Michaelis-Menton) model of catalysis.

C. Proposed Binding-Partitioning Model

We now propose a formally equivalent binding-partitioning model. The physical picture is that one of

the reactants, the one in large excess, partitions between the micellar and aqueous phases; the other reactant binds to the micelle surface with 1:1 stoichiometry.

Partitioning of S

$$S_w \rightleftharpoons S_M$$

$$P_s = \frac{(S)_M}{(S)_w} \qquad \text{Eq. 32}$$

Binding of R

$$R + MS \rightleftharpoons MSR$$

$$K_R = \frac{(MSR)}{(R)_w(MS)} \qquad \text{Eq. 33}$$

In this formulation, $(S)_M$ is the concentration of S in the micelle, (MS) is the concentration of micelle-containing-S in the solution. There are no micelles not containing S, because we adopted a partitioning hypothesis for S, the micelle being a second phase. However, there can be micelles without R, because we are using a binding hypothesis. This is physically reasonable when $(S)_T$ > (micelle) > $(R)_T$.

The kinetic scheme is :

$$R_{(w)} + S_{(w)} \xrightarrow{k_w} P$$

$$MSR + S_{(M)} \xrightarrow{k_M} P$$

Therefore the hypothetical rate equation is

$$\frac{d(P)}{dt} = k_w(R)_w(S)_w + k_M(MSR)(S)_M \qquad \text{Eq. 34}$$

Combine Eq. 33 and Eq. 34

$$\frac{d(P)}{dt} = k_w(R)_w(S)_w + k_M K_R(MS)(R)_w(S)_M \qquad \text{Eq. 35}$$

$$-\frac{d(R)_T}{dt} = k_w(R)_w(S)_w + k_M K_R P_s(MS)(R)_w(S)_w \qquad \text{Eq. 36}$$

Now consider the mass balance on surfactant

$$C_T = (CMC) + n(MS) + n(MSR) \qquad \text{Eq. 37}$$

or, since

$$C = C_T - (CMC)$$

we have

$$C = n[(MS) + (MSR)] \qquad \text{Eq. 38}$$

The mass balance on R gives

$$(R)_T = (R)_w + (MSR) \qquad \text{Eq. 39}$$

since this is a binding phenomenon, (MSR) is simply the concentration of micelles each containing an R. From Eq. 33

$$(R)_T = (R)_w + K_R(R)_w(MS)$$

or $$(R)_w = \frac{(R)_T}{1 + K_R(MS)} \qquad \text{Eq. 40}$$

Now Eq. 40 is put into Eq. 36

$$-\frac{d(R)_T}{dt} = (R)_T \left[\frac{k_w(S)_w + k_M K_R P_s(S)_w(MS)}{1 + K_R(MS)}\right] \qquad \text{Eq. 41}$$

$$\text{or} \quad k_{obs} = \left[\frac{k_w + k_M K_R P_s (MS)}{1 + K_R (MS)}\right] (S)_w \qquad \text{Eq. 42}$$

where k_{obs} is the pseudo-first-order rate constant, when $(S)_T >>> (R)_T$.

Now we need a mass balance on S, and we need an expression for (MS). From Eq. 38

$$(MS) = \frac{C - n(MSR)}{n}$$

and combining this with Eq. 33 gives

$$(MS) = \frac{C/n}{1 + K_R (R)_w} \qquad \text{Eq. 43}$$

Put this into Eq. 42

$$k_{obs} = \left[\frac{k_w + \dfrac{k_M K_R P_s C}{n\,1 + K_R (R)_w}}{1 + \dfrac{K_R C}{n\,1 + K_R (R)_w}}\right] (S)_w \qquad \text{Eq. 44}$$

Now for the mass balance on S :

Total moles of S = (moles S in H_2O) + (moles S in micelles)

$$\text{or} \quad (S)_T V_T = (S)_w V_w + (S)_M V_M \qquad \text{Eq. 45}$$

In general, also

$$\text{No. of moles} = \frac{\text{No. of molecules}}{N_A} \qquad \text{Eq. 46}$$

where N_A is Avogadro's number.

$$V_M = (\text{no. of micelles})(\text{volume per micelle}) \qquad \text{Eq. 47}$$

Let ν_M = volume of one micelle. Also

$$\left(\begin{matrix}\text{no. of}\\ \text{micelles}\end{matrix}\right) = \frac{\text{no. of surfactant molecules in micelle phase}}{n} \qquad \text{Eq. 48}$$

where n is the aggregation number.

$$\begin{matrix}\text{No. of moles of CTAB}\\ \text{in micelles}\end{matrix} = \begin{matrix}\text{No. of moles of CTAB}\\ \text{in micelles per liter}\end{matrix} \times V_T$$

$$= CV_T \qquad \text{Eq. 49}$$

where V_T is the total volume

$$C = C_T - (CMC)$$

Combining this with Eq. 46

$$\begin{matrix}\text{No. of surfactant molecules}\\ \text{in micellar phase}\end{matrix} = N_A C V_T \qquad \text{Eq. 50}$$

From Eq. 48 and Eq. 50

$$(\text{no. of micelles}) = \frac{N_A C V_T}{n} \qquad \text{Eq. 51}$$

From Eq. 47 and Eq. 51

$$V_M = \frac{N_A C V_T \nu_M}{n} \qquad \text{Eq. 52}$$

since $V_T = V_M + V_w$, Eq. 45 becomes

$$(S)_T V_T = (S)_w (V_T - V_M) + (S)_M V_M \qquad \text{Eq. 53}$$

Substituting Eq. 52 into Eq. 53 :

$$(S)_T = (S)_w \left(1 - \frac{N_A C \nu_M}{n}\right) + (S)_M \frac{N_A C \nu_M}{n} \qquad \text{Eq. 54}$$

Substitute Eq. 32 into Eq. 54

we get finally

$$\frac{(S)_T}{(S)_w} = 1 + \frac{N_A C \nu_M (P_s - 1)}{n} \qquad \text{Eq. 55}$$

or $$\frac{(S)_T}{(S)_w} = 1 + K_s C$$

where $$K_s = \frac{N_A \nu_M (P_s - 1)}{n}$$

which is seen to be equal to Berezin's result, Eq. 20, achieved via a different route.

Therefore

$$(S)_w = \frac{(S)_T}{1 + K_s C} \qquad \text{Eq. 56}$$

Now suppose $K_R(R)_w \lll 1$. Then Eq. 44 becomes

$$k_{obs} = \left[\frac{k_w + \frac{k_M K_R P_s C}{n}}{1 + \frac{K_R C}{n}} \right] (S)_w \qquad \text{Eq. 57}$$

Let $d = \frac{K_R}{n}$

$$k_{obs} = \frac{k_w + k_M d C P_s}{1 + dC} \quad (S)_w \qquad \text{Eq. 58}$$

$$k_{obs} = \frac{k_w' + k_M d C P_s (S)_w}{1 + dC} \qquad \text{Eq. 59}$$

Subtract k_w' from each side

$$k_{obs} - k_w' = k_M' = \frac{k_w' + k_M dC P_s (S)_w}{1 + dC} - k_w' \qquad \text{Eq. 60}$$

$$k_M' = \frac{k_M dC P_s (S)_w - k_w' dC}{1 + dC} \qquad \text{Eq. 61}$$

$$k_M' = \left(\frac{dC}{1 + dC}\right)(k_M P_s (S)_w - k_w') \qquad \text{Eq. 62}$$

Now put Eq. 56 into Eq. 62 :

$$k_M' = \left(\frac{dC}{1 + dC}\right)\left(\frac{k_M P_s (S)_T}{1 + K_s C} - k_w'\right) \qquad \text{Eq. 63}$$

Now define

$$e = k_M P_s \qquad \text{Eq. 64}$$

$$k_M' = \left(\frac{dC}{1 + dC}\right)\left(\frac{e\, S_T}{1 + K_s C} - k_w S_T\right) \qquad \text{Eq. 65}$$

$$k_M' = \left(\frac{dC}{1 + dC}\right)\left(\frac{e}{1 + K_s C} - k_w\right)(S)_T \qquad \text{Eq. 66}$$

or

$$k_{obs} - k_w' = \left(\frac{dC}{1 + dC}\right)\left(\frac{e}{1 + K_s C} - k_w\right)(S)_T \qquad \text{Eq. 67}$$

When $k_w \ll 0$

$$\frac{k_{obs}}{(S)_T} = \left(\frac{dC}{1 + dC}\right)\left(\frac{e}{1 + K_s C}\right) \qquad \text{Eq. 68}$$

which is identical in form with Eq. 31.

Putting in the definitions of d and e gives :

$$\frac{k_{obs}}{(S)_T} = \frac{\frac{k_M K_R P_s}{n} C}{(1 + \frac{K_R}{n} C)(1 + K_s C)} \qquad \text{Eq. 69}$$

or $$k_{exp} = \frac{\frac{k_M K_R P_s}{n} C}{(1 + \frac{K_R}{n} C)(1 + K_s C)} \qquad \text{Eq. 70}$$

The maximum value of k_{exp} occurs at the concentration of surfactant C_{max} where

$$\frac{dk_{exp}}{dC} = 0$$

$$\frac{dk_{exp}}{dC} = (1 + \frac{K_R}{n} C_{max})(1 + K_s C_{max}) \frac{k_M K_R P_s}{n} -$$

$$\frac{k_M K_R P_s}{n} C_{max} \left[\frac{K_R}{n} + K_s + 2\frac{K_R K_s}{n} C_{max}\right] = 0$$

which leads to

$$C_{max} = \left(\frac{K_R K_s}{n}\right)^{-1/2} \qquad \text{Eq. 71}$$

or

$$K_s = \left(\frac{1}{d\ C_{max}^{\ 2}}\right)$$

where $d = \frac{K_R}{n}$

Therefore

$$\left(\frac{k_{exp}}{k_w}\right)_{max} = \frac{k_M}{k_w} \frac{\frac{P_S K_R}{n}}{\left(\sqrt{\frac{K_R}{n}} + \sqrt{K_s}\right)^2} \qquad \text{Eq. 72}$$

Berezin et al. (76) interpreted the ratio $\frac{k_M}{k_w}$ as the change in reactivity when the reactants are transferred from water into the micelle. The factor on the right-hand side of Eq. 72 represents the acceleration produced by the localization of the reactants in the micelle . The observed catalysis can be therefore a result of either or both factors, and these factors may vary independently.

D. Comparison between Berezin's and Present Model

To compare the two models, we will work with Eqs. 31 and 69, neglecting k_w, which simplifies the equation and doesn't affect the argument.

$$k_{exp} = k_{obs}/(S)_T$$

Berezin Model	Present Model
$\dfrac{\frac{k_M{}^B K_R{}^B K_s{}^B C}{V}}{(1 + K_R{}^B C)(1 + K_s{}^B C)}$	$\dfrac{\frac{k_M{}^C K_R{}^C P_s{}^C C}{n}}{(1 + \frac{K_R{}^C C}{n})(1 + K_s{}^C C)}$
where $K_R{}^B = P_R{}^B V$	where $K_s{}^C = \dfrac{N_A \sqrt{}_M (P_s{}^C - 1)}{n}$
$K_s{}^B = P_s{}^B V$	$V = \dfrac{N_A \sqrt{}_M}{n}$
$V = \dfrac{V_M}{V_T C}$	when $P_s{}^C \gg 1$
	$K_s{}^C = P_s{}^C V$
	exactly as in Berezin's equation.

k_M^B may be different from k_M^C because of the way the hypothetical rate equations are written. In Berezin's model, the concentration $(R)_M$ is given by $P_R^B(R)_w$. But in the present model, the concentration (MSR) is $(R)_T - (R)_w$, or

$$(MSR) = (R)_T - \frac{(R)_T}{1 + K_R^C(MS)} \qquad \text{Eq. 40}$$

Substitute Eq. 43 into Eq. 40

$$(MSR) = (R)_T - \frac{(R)_T}{1+\dfrac{K_R^C C/n}{1+ K_R^C(R)_w}}$$

But the equations assumed the condition $1 \ggg K_R(R)_w$, so we can write

$$(MSR) = (R)_T \left[\frac{\dfrac{K_R^C C}{n}}{1 + \dfrac{K_R^C C}{n}} \right]$$

Returning to our comparison of the two models :

Berezin's Model	Present Model
$k_M^B = \dfrac{V_M}{P_R^B(R)_w(S)_M}$	$k_M^C = \dfrac{V_M}{(R)_T\left(\dfrac{\frac{K_R^C C}{n}}{1+\frac{K_R^C C}{n}}\right)(S)_M}$

Since $(R)_T = (R)_w + (MSR)$ Eq. 39

$$(R)_T = (R)_w + (R)_T \left(\frac{dC}{1+dC}\right) \quad \text{Eq. 40}$$

where $d = K_R{}^C/n$

$$\therefore \quad (R)_T \left[\left(\frac{dC}{1+dC}\right)\right] = (R)_w \quad \text{Eq. 73}$$

Thus, continuing :

Berezin's Model	Present Model
	$k_M{}^C = \dfrac{V_M}{(R)_w(1+dC)\left(\frac{dC}{1+dC}\right)(S)_M}$
$k_M{}^B = \dfrac{V_M}{P_R{}^B(R)_w(S)_M}$	$k_M{}^C = \dfrac{V_M}{(R)_w \frac{K_R{}^C C}{n} (S)_M}$

Divide one by the other :

$$\frac{k_M{}^B}{k_M{}^C} = \frac{\dfrac{V_M}{P_R{}^B(R)_w(S)_M}}{\dfrac{V_M}{(R)_w \frac{K_R{}^C C}{n}(S)_M}} \quad \text{Eq. 74}$$

$$\frac{k_M^B}{k_M^C} = \frac{\frac{1}{P_R^B}}{\frac{1}{K_R^C/n}} \qquad \text{Eq. 75}$$

$$\therefore k_M^B = \left(\frac{K_R^C/n}{P_R^B}\right) k_M^C \qquad \text{Eq. 76}$$

This is how the two fundamental rate constants are related in the two models.

Let us use our relationships to see how the present model equation is related to Berezin's.

Berezin's Model	Present Model
$\frac{k_M^B K_R^B K_s C/V}{(1+K_R^B C)(1+K_s^B C)}$	$\frac{k_M^C K_R^C P_s^C C/n}{(1+\frac{K_R^C}{n})(1+K_s^C C)}$

We have seen that

$$K_s^B = K_s^C$$

$$k_M^B = \left(\frac{K_R^{C/n}}{P_R^B}\right) k_M^C$$

Berezin proposed Eq. 31 to describe the dependence of the experimental rate constant for reaction between 1-fluoro-2,4-dinitrobenzene and the methyl ester of N-benzoyl-L-histidine on surfactant concentration. If we write Eq. 31 in the form

$$k_{exp} = \frac{LC}{(1 + MC)(1 + NC)} \qquad \text{Eq. 77}$$

Then, $L = \dfrac{k_M^B K_R^B K_s}{V}$

$M = K_R^B$

$N = K_s$

We proposed a "binding-partitioning" model to describe the cetyltrimethylammonium bromide catalyzed reaction of 1-fluoro-2,4-dinitrobenzene with amines (Eq. 70). Writing Eq. 70 in the form of Eq. 77, we obtain

$L = \dfrac{k_M^C K_R^C P_s^C}{nV}$

$M = K_R^{C/n}$

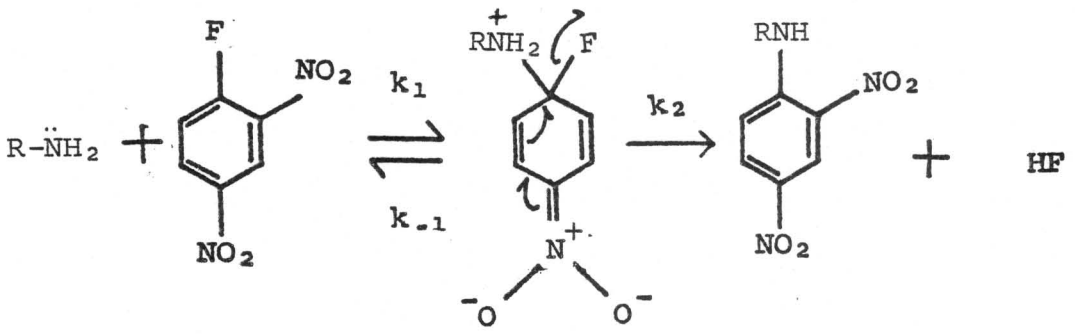
$N = K_s$

where $k_M^B = \left(\frac{K_R^{C/n}}{P_R^B}\right) k_M^C$

The equation derived from Berezin's "partitioning-partitioning" and the equation we proposed based on a "binding-partitioning" model are formally equivalent. Parameters L, M, and N determined experimentally will have different interpretations as shown above.

E. Interpretation of the Mechanism of the Cetyltrimethylammonium Bromide Catalyzed Reaction of 1-Fluoro-2,4-dinitrobenzene with Amines.

The products of the reaction of 1-fluoro-2,4-dinitrobenzene with amines in the presence of surfactant cetyltrimethylammonium bromide were identified as 2,4-dinitrophenylamine and 2,4-dinitrophenol. The reaction is first-order in amine and in 1-fluoro-2,4-dinitrobenzene. The pH rate profile suggested that the free amine form is the attacking species. This is consistent with a two-step mechanism as shown :



Applying the steady-state approximation to the intermediate gives

$$v = \frac{k_1 k_2 [DFB][Amine]}{k_{-1} + k_2}$$

where v is the rate of the reaction. This equation shows the bimolecular nature of the kinetics. The experimental second-order rate equation is therefore

$$k = \frac{k_1 k_2}{k_{-1} + k_2}$$

If $k_2 \gg k_{-1}$,

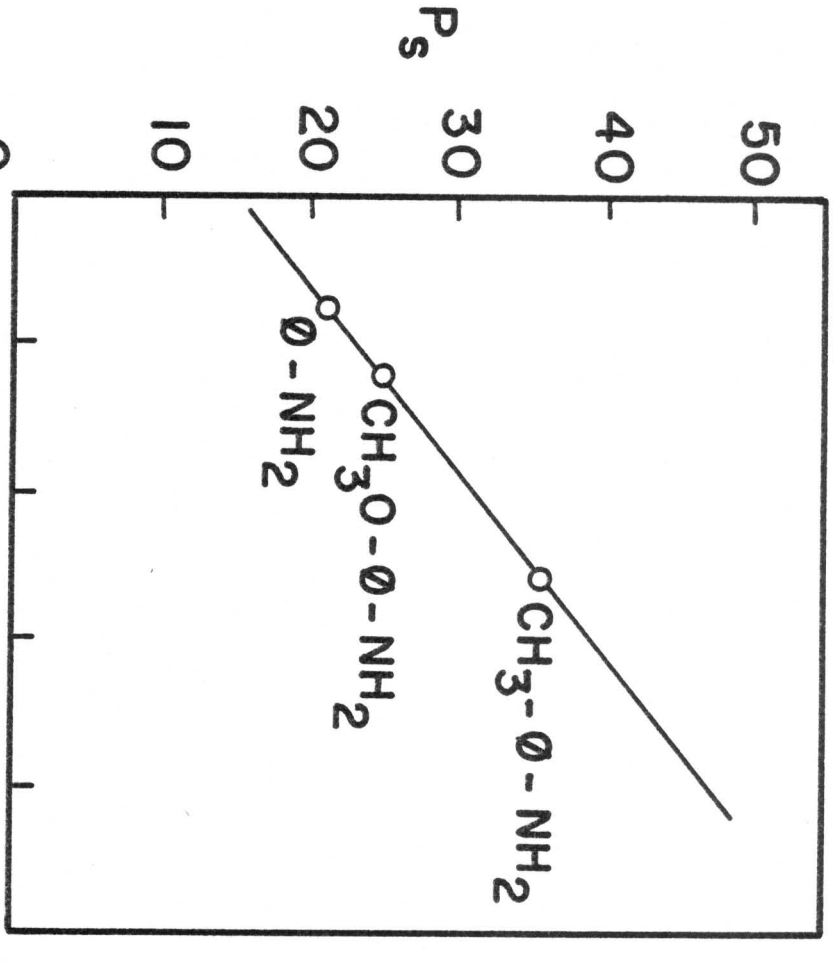
$$k \simeq k_1$$

The formation of the intermediate would be rate determining. The data in Table V for the substitution of several 1-substituted-2,4-dinitrobenzenes by piperidine exemplify this kinetic behavior (60).

The dependence of the observed rate constant on surfactant concentration shows a maximum. The physical signifigance of the presence of a maximum is that the increase in the volume of the micellar phase is accompanied both by an increase in the quantity of solutes passing into it from water and by a decrease in the concentration of solutes in the micellar phase. The first process tends to accelerate the reaction by increasing the local concentration of solutes (reactants) in the micellar phase. This process predominates at low surfactant concentrations. The second process, the dilution of the reactants in the micellar phase, slows the reaction. The second process predominates at high surfactant concentrations when the reactants are mainly present in the micellar phase. This situation makes the model qualitatively different from the usual enzyme (Michaelis-Menten) model of catalysis. Berezin et al. (76) proposed a partitioning-partitioning model to describe the acceleration of aminolysis of N-benzoyl-L-histidine methyl ester. We propose a formally equivalent binding-partitioning model for the cetyltrimethylammonium bromide catalyzed reaction of 1-fluoro-2,4-dinitrobenzene with amines. The binding-partitioning model describes

the dependence of the catalytic observed rate constant on surfactant concentration for the five amines alanine, phenylalanine, aniline, p-methylaniline and p-methoxyaniline. The parameters K_R/n, $k_M P_s$, K_s or $V(P_s-1)$ for the five amines studied were shown in Table XVII. Berezin and co-workers (77) estimated the molar volume of the surfactant cetyltrimethylammonium bromide to be 0.35 liter/mole based on the finding that the densities of a number of surface-active agents in micelles lie in the range from 0.9 to 1.1 g/ml (78). If we assume the molar volume of cetyltrimethylammonium bromide V to be 0.35 liter/mole, parameter P_s and hence k_M could be obtained (Table XVIII). The parameter P_s evaluated in this work correlated linearly with the partition coefficients between octanol and water $P_{octanol}$ reported by Hansch et al. (79) as shown in Fig. 33.

Fig. 33. Correlation between the P_s values determined in this work and the $P_{octanol}$ values reported by Hansch *et al*.


P_s
0
10
20
30
40
50
$P_{octanol}$
0
10
20
30
40
50
$\emptyset - NH_2$
$CH_3O - \emptyset - NH_2$
$CH_3 - \emptyset - NH_2$

We have shown in Eq. 72 that the rate enhancement is a result of two factors, k_M/k_w and K' where K' is given by Eq. 78.

$$K' = \frac{K_R P_s/n}{\left(\frac{K_R}{n} + K_s\right)^2} \qquad \text{Eq. 78}$$

The parameters P_s, k_M, k_w, $\frac{k_M}{k_w}$ and K' for the five amines studied, are shown in Table XIX.

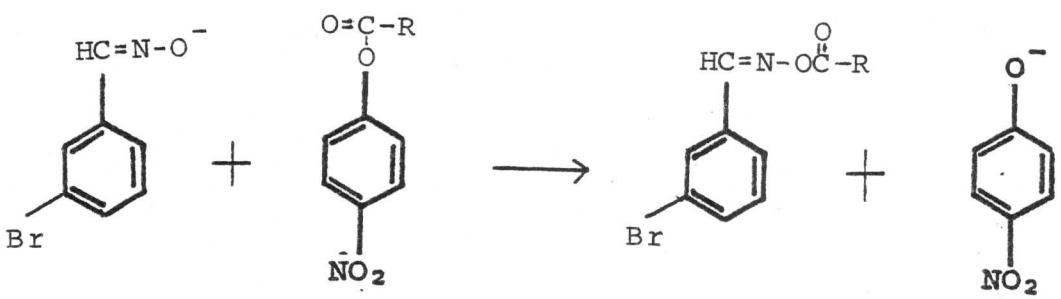
Table XIX. Parameters P_s, k_M, k_w, k_M/k_w and K' for the reactions of amines and 1-fluoro-2,4-dinitrobenzene in presence of CTAB.

Amine	P_s	k_M ($M^{-1}sec^{-1}$)	k_W ($M^{-1}sec^{-1}$)	k_M/k_w	K'[a]
alanine	20	$3.8x10^{-2}$	$2.5x10^{-2}$	1.5	12.6
phenyl-amine	63	$4.9x10^{-1}$	$8.9x10^{-2}$	5.5	26.1
aniline	21	$3.0x10^{-4}$	$4.9x10^{-4}$	0.6	12.8
p-methyl-aniline	35	0.107	0.142	0.7	17.6
p-methoxy-aniline	25	$2.26x10^{-2}$	$2.23x10^{-2}$	1.0	12.4

[a] Defined by Eq. 78.

The factor K', which represents the acceleration produced by the localization of the reactants in the micelle, is greater than 10 for all five amines studied. The rate constant of the reaction in the micellar phase is close to the value which characterizes the reaction in water for alanine, aniline, p-methylaniline and p-methoxyaniline. The factor K' is highest for phenylalanine. It is surprising that k_M/k_w for phenylalanine is also much higher. Since the mechanism of the reaction of amines with 1-fluoro-2,4-dinitrobenzene should not depend on the structure of the closely related amines, approximately the same change in reactivity, namely k_M/k_w on transfer of the reactants to the micellar phase, should be produced by the environment and by interaction with the micellar charge. The most probable reason for the difference may be orientation of the reactants in the micelle. The partition coefficients P_s for the three anilines correlate linearly with the partition coefficients between octanol and water reported by Hansch and co-workers (79). The values of K_R/n are fairly constant. We would expect K_R/n determined from the reaction of the five amines with 1-fluoro-2,4-dinitrobenzene to be slightly sensitive to amine identity since the presence

of different amines in the micelle phase might affect the binding or affinity of 1-fluoro-2,4-dinitrobenzene to the micelle. We can conclude that the main driving force of the micellar catalysis for the five reactions studied is the concentration of the reagents in the micelle. Berezin and co-workers (77) arrived at the same conclusion studying the mechanism of the acceleration of the aminolysis of 2,4-dinitrofluorobenzene with N-benzoyl-L-histidine methyl ester in the presence of surface-active agent cetyltrimethylammonium bromide. The acylation of aromatic oximes by p-nitrophenyl carboxylates in the presence of cetyltrimethylammonium bromide was also examined by Berezin et al. (80).



The experimental results were analyzed with the "partitioning-partitioning" model. They concluded that the acceleration of the reaction is a result of

concentration of reactants in the micellar phase. Thus for micellar catalysis of nucleophilic reactions by cationic micelles, numerous examples have now been shown in which the rate enhancement is largely a consequence of the local concentration effect by the micelles.

V. SUMMARY

We described the use of micellar catalysis to increase the derivative formation rate prior to spectrophotometric measurement of a product. [K. A. Connors and M. P. Wong, J. Pharm. Sci., 68, 1470 (1979)]. This thesis reports the further development of this system to the quantitative determination of many amines, amino-acids and peptides. All rate enhancements are analytically useful, and this method can be applied with the reaction carried out at room temperature. An additional rate enhancement factor of 3 was obtained by increasing the temperature to 45 °C. The concentration-response curves are linear at 25° and 45 °C, and the slopes of these curves are identical.

The observed rate constant dependence on surfactant concentration could be fitted by Berezin's model, and by an equivalent "binding-partitioning" model derived in this work. These models allow the separation of catalysis into intrinsic rate effects and concentration effects. Investigation of the mechanism of the micellar catalyzed reaction of 1-fluoro-2,4-dinitrobenzene with five amines, namely alanine, phenylalanine, aniline, p-methylaniline and p-methoxyaniline, reveals that catalysis is mainly a result of the concentration of

reactants in the micelle. The factor which represents the acceleration produced by the localization of the reactants in the micelle is highest for phenylalanine among the five amines studied. Phenylalanine possess a hydrophobic moiety and a negatively charged carboxyl end. Two factors contribute in the rate enhancement : one is the possession of a negative charge, the other is the possesion of a nonpolar moiety. When both features are present, as indicated by phenylalanine, very high rate enhancement can occur.

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