EFFECTS OF SODIUM CHOLATE ON THE SPONTANEOUS

DECARBOXYLATION OF 4-NITROBENZISOXAZOLE-3-CARBOXYLIC ACID

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ABSTRACT

Effects of Sodium Cholate on the Spontaneous

Decarboxylation of 4-Nitrobenzisoxazole-3-carboxylic Acid. (April 1979).

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The spontaneous decarboxylation of 4-nitrobenzisoxazole-3-carboxylic

acid (4-NB-3-CA) was studied kinetically in dimethyl sulfoxide (DMSO)

in the presence and absence of the naturally occurring bile salt sur-

factant, sodium cholate (NaC). The observed rate constants exhibited

a sigmoidal dependence followed by a plateau over the NaC concentration

range of 1.5×10^{-3} to 0.2 M. The observed rate constants for the

decarboxylation of 4-NB-3-CA in the presence of aggregated sodium

cholate in DMSO are enhanced by a factor of ca. 5×10^{5}-fold as compared

to that in pure DMSO solution. The sigmoidal dependence is explicable

in terms of solubilization of 4-NB-3-CA by NaC via H-bonding and

abstraction of the acidic carboxylic proton of 4-NB-3-CA by the NaC

anion. Mechanisms for the NaC catalysis are postulated and discussed,

in the light of previous work, in terms of both physical and chemical

interactions.

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DEDICATION

This paper is dedicated to my parents, Mrs. Mary D. Sonnier and Mr. Donald J. Sonnier.

ACKNOWLEDGEMENTS

INTRODUCTION

The effects of surfactants (ref. 1 and Appendix A) upon the interactions and reactions of the spontaneous decarboxylation of 4-nitrobenzisoxazole-3-carboxylate anion have been investigated in aqueous solution since 1970.[2] In the absence of surfactants in water,[2-4] and dipolar aprotic[3,4] and non-polar[3,4] solvents. The reaction proceeds via a spontaneous elimination of carbon dioxide (equation 1):
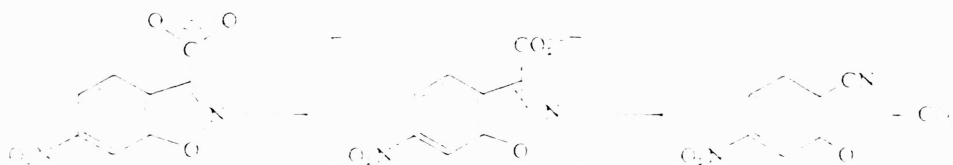
Investigation of the rate of reaction of this compound is both physico-chemically and biomedically important, and has been chosen for the following reasons: (1) decarboxylation reactions are common in vivo (e.g., β-ketoacids and thiamin pyrophosphate undergo spontaneous decarboxylations). (2) the spontaneous decarboxylation occurs as a one-step unimolecular reaction. It occurs without the interaction of an electrophile or nucleophile. Since the reaction is a unimolecular one, the complication of incorporating more than one

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reactant into the micelle (ref. 5 and Appendix A) is avoided, (3) both 4-nitrobenzisoxazole-3-carboxylic acid and its decarboxylation product can be studied spectrophotometrically. (4) Micellar catalysis of the spontaneous decarboxylations has been elucidated both in the presence and absence of synthetic surfactants and other additives. The data on these synthetic systems provides an excellent background for investigation of the naturally occurring ones, (i.e., bile salt systems; see Appendix B).

Studies of the spontaneous decarboxylation of benzisoxazole and other carboxylate anions have shown the reaction rate to be affected by the type of solvent[3,4] and surfactants[2,6-11] used. The reaction rate is also sensitive to the presence of various electrolytes in surfactant systems.[6-8]

Investigation of the solvent effects on the decomposition of 3-carboxybenzisoxazoles indicate that these reactions undergo exceedingly large rate accelerations as the solvent is changed from protic to polar aprotic ones. The most striking features of these spontaneous decarboxylations with varying solvent is the rate increases observed in dipolar aprotic solvents, relative to water (see Table 1).

The effects of different surfactants on the reaction rate of 6-nitrobenzisoxazole-3-carboxylate and other carboxylate anions have been elucidated. The studies show that the rate constants are generally enhanced by cationic, non-ionic, dicationic and zwitterionic surfactants. Anionic surfactants however do not catalyze the reaction (Table II). Polymeric surfactants, such as poly (2-ethyl-1-vinylimidazole) have been found to catalyze the rate by a factor of up to 350-

Table I. Rate Constants for Decarboxylation of 6-Nitrobenisoxazole-3-carboxylic Acid in Various Solvents at 30°C.[a,b]

Solvent	k, sec^{-1}	Log k
Water	7.4×10^{-6}	-5.13
Methanol	2.5×10^{-4}	-3.60
Formamide	7.4×10^{-4}	-3.13
Chloroform	8.0×10^{-4}	-3.09
Ethanol	1.0×10^{-3}	-3.00
Carbon tetrachloride	1.5×10^{-3}	-2.82
Benzene	8.1×10^{-3}	-2.09
N-Methylformamide	8.1×10^{-3}	-2.09
Dimethoxymethane	3.6×10^{-2}	-1.44
Dioxane	4.0×10^{-2}	-1.39
Dichloromethane	4.7×10^{-2}	-1.33
Diethyl ether	9.0×10^{-2}	-1.05
Nitromethane	5.8×10^{-1}	-0.24
Benzonitrile	2.5	0.40
Acetonitrile	2.9	0.46
Tetrahydrofuran	4.0	0.60
Diglyme	5.0	0.70
Dimethyl sulfoxide	1.0×10	1.00
Acetone	2.4×10	1.38
Dimethylformamide	3.7×10	1.56
Tetramethylenesulfone	6.4×10	1.81
Dimethylacetamide	1.6×10^{2}	2.20
N-Methylpyrrolidone	2.5×10^{2}	2.40
Hexamethylphosphoramide	$\approx 7.0 \times 10^{2}$	2.80

[a]Data taken from reference 2.

[b]6-Nitrobenzisoxazole-3-carboxylic acid] = 2.3×10^{-4} M; (tetramethylguanidine] = ca. 3×10^{-3} M.

Table II. Surfactant Effects on Spontaneous Decarboxylation of 6-Nitrobenzisoxazole-3-Carboxylic Acid in Aqueous Solution.[a]

Surfactant	Relative Rate[b]
Cationic	k_ψ/k_o
$R_{12}(CH_3)_3N^+Br^-$	65
$R_{16}(CH_3)_3N^+Br^-$	98.3
$R_{16}C_5H_5N^+Br^-$	100
Dicationic	
$R_{16}(CH_3)_2N^+(CH_2)_4N^+(CH_3)_2^-R_{16}2Br^-$	330
$R_{16}(CH_3)_2N^+(CH_2)_6N^+(CH_3)_2^-R_{16}2Br^-$	410, 523
Anionic	
$R_{12}SO_4^-Na^+$	1
α-Lecithin	1
Nonionic and Zwitterionic	
Polyoxyethylene (24) dinonyphenol (Igepal)	65
$R_{12}(CH_3)_2N^+CH_2CO_2^-$	180

[a] Data from references 2, 6, and 8.

[b] k_ψ is the observed rate constant in presence of micellar surfactant and k_o is that in its absence.

fold with respect to that in the nonmicellar (aggregated) system in water.[10]

Additionally, studies of the spontaneous decarboxylation of 6-nitrobenzisoxazole-3-carboxylic acid have shown that the addition of certain electrolytes (salts) enhance the surfactant catalyzed reaction rate. The salt order for rate enhancement of the micellar catalysis is: $SO_4^- > Br^- > Cl^- > F^- \approx CH_3CO_2$. The reaction rate of 6-nitrobenzisoxazole-3-carboxylic acid was found to be inhibited in aqueous solutions by such compounds as: NaCNS, Na pivalate, Na cholate, testosterone, urea, dioxane and phenols (See Table III).

Due to their physiological importance and their sensitivity to solvent effects (vide supra), the spontaneous decarboxylation of carboxylate anions is of considerable interest. Although the effects of synthetic surfactants have been investigated in aqueous solution no studies have been carried out in the presence of biological sur-factants (e.g., bile salts and lipids). Bile salts are physiologically essential surfactants (see Appendix B); however very little in known about their physico-chemical behavior. Therefore, a study of the effects of bile salts on the spontaneous decarboxylation of 4-nitro-benzisoxazole-3-carboxylic acid was undertaken in dimethylsulfoxide (a dipolar aprotic solvent which closely mimics the micro environment of the bile salts in vivo).

Table III. Salt Effects on the Spontaneous Decarboxylation of 6-Nitrobenzisoxazole-3-Carboxylic Acid in Aqueous Solution.[a]

Surfactant[b]	Effect of Salts	
	Catalysis	Inhibitor
$R_{16}(CH_3)_3N^+Br^-$	$SO_4^- > Br^- > Cl^-$	NaCNS > Napivalate
	F^- $CH_3CO_2^-$	Nacholate, testoster-
	$p-CH_3C_6H_4SO_3^- >$	one, urea, dioxane
	$\beta C_{10}H_7SO_3^- \approx$	and by phenols.
	$C_6H_5CO_2^-$ and by	
	phenoxide ions.	
$p-CH_3C_6H_4SO_3Na$	$p-CH_3C_6H_4SO_3Na >$	
	Na_2SO_4	

[a] Data taken from references 2, 6 and 8.

[b] See references 2, 6 and 8 for the experimental conditions (concentration, solvent, pH buffer, ionic strength, temperature, etc.) employed and for additional data.

EXPERIMENTAL

Reagents and Materials

Dimethylsulfoxide, DMSO, (Fisher, spectroanalyzed grade) was dried over freshly activated 4A molecular sieves and its water content was monitored using a Photovolt Aquatest II. The purity of DMSO, other solvents, and reagents was established prior to use via their UV-visible and nmr spectra.

6-Nitrobenzisoxazole-3-carboxylic acid was prepared according to modified literature procedures[13-17] (see Figure 1).

Methyl 2,4-dinitrophenyl acetate. Methanol (Fisher, spectro-analyzed grade; 12.3 ml, 0.30 mole) was added to a solution of pulverized 2,4-dinitrophenyl acetic acid (Eastman; 22.6 g, 0.10 mole) in 30 ml of CH_2Cl_2. Concentrated sulfuric acid (1.5 ml) was added with rapid stirring and the mixture was refluxed for ca. 15 hours. (The solution separated into a top dark yellow layer and a bottom light brown layer). The layers were separated and the bottom layer was washed successively with cold double-distilled water and with dilute sodium bicarbonate solution. White crystals which came out of solution were suction filtered and dried in vacuo over P_2O_5. After recrystallization from CH_2Cl_2, the white crystals had mp 82-83°C (lit.[13] 82-83°C).

Methyl 6-nitrobenzisoxazole-3-carboxylate. Freshly distilled iso-amylnitrite (2.19 ml, 0.024 mole) was added to 5.0 g (0.021 mole) 2,4-dinitrophenyl acetate with rapid stirring.

To this solution sodium methoxide, which was prepared from 0.41 g (0.018 mole) and 15 ml of anhydrous methanol, was added very slowly

SYNTHESIS OF BENZISOXAZOLE-3-CARBOXYLIC ACID

O_2N —⟨benzene ring⟩— CH_2COOH + CH_2Cl_2 + CH_3OH

with NO_2 substituent

↓ conc. H_2SO_4

O_2N —⟨benzene ring⟩— $CH_2\overset{O}{\overset{\|}{C}}OCH_3$

with NO_2 substituent

+

$NaOCH_3$

+

$CH_3CH_2CH_2\overset{\underset{CH_3}{|}}{C}H\,NO_2$

↓ conc. H_2SO_4

⟨benzisoxazole ring with $\overset{O}{\overset{\|}{C}}OCH_3$ and C=N, O, NO_2⟩

↓ conc. H_2SO_4
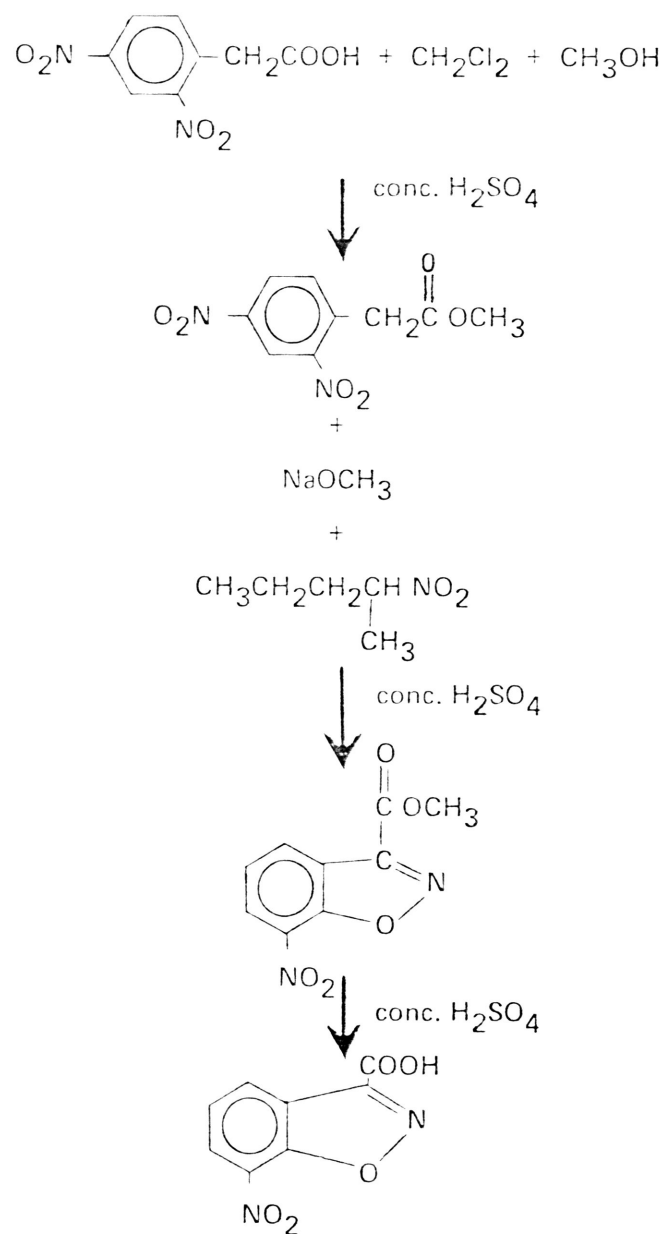
⟨benzisoxazole ring with $COOH$, N, O, NO_2⟩

Figure 1.   Synthesis scheme of 4-nitrobenzisoxazole-3-carboxylic acid.

(total time for addition ca. 45 minutes). The solution was then stirred for 6 hours followed by concentration on a steam bath. Upon cooling a high yield of shiney white crystal formed. Notably no crystals were obtained upon rotary evaporation. The crystals were filtered with suction, washed with cold methanol, recrystallized from methanol, and dried in vacuo over P_2O_5. The product melted at 131°-132° C (lit.[14] 130-313°C). A ^{1}H nmr spectrum of the product in DMSO-d_6 indicated no impurities and had resonances at 4.05, 6.83, and 7.38 ppm (Figure 3).

6-Nitrobenzisoxazole-3-carboxylic acid. Dilute sulfuric acid (conc. H_2SO_4/H_2O, 1/5, V/V) was added to methyl 6-nitrobenzisoxazole-3-carboxylate (0.50 g, 0.024 mole), heated with stirring for ca. 20 min., and poured over crushed ice. The white crystalline precipitate was filtered, washed with cold water, and dried in vacuo over P_2O_5 (mp 168-169° C (d), lit.[15] 167-169°C). The purity of the product was confirmed via its UV-visible and nmr spectra.

Kinetic Methods and Techniques

In general, only one type of serial solution was used in the kinetic studies. The series differed only in the concentration of the solute (i.e., Na cholate). Variance in the Na cholate concentrations achieved by volumetric dilution of a Na cholate stock solution with the desired amount of DMSO. Alternatively, several of the volumetric dilutions were prepared by weighing the Na cholate into the volumetric and diluting to volume with DMSO.

The water concentrations of the solute and substrate (4-NB-3-CA in DMSO) were monitored using a Photovolt Aquatest II, Model 702 coulometer,

Figure 2. ^{1}H nmr spectrum of methyl-2-nitrobenzisoxazole-3-carboxylate in DMSO-d$_6$ at 60 MHz and 35.2°C: filter bandwidth = 1 Hz, R. F. field = .16 m, G, sweep time = 250 seconds, spectrum amplitude = 40, upper spectrum sweep offset = 200 Hz (3.3 ppm).
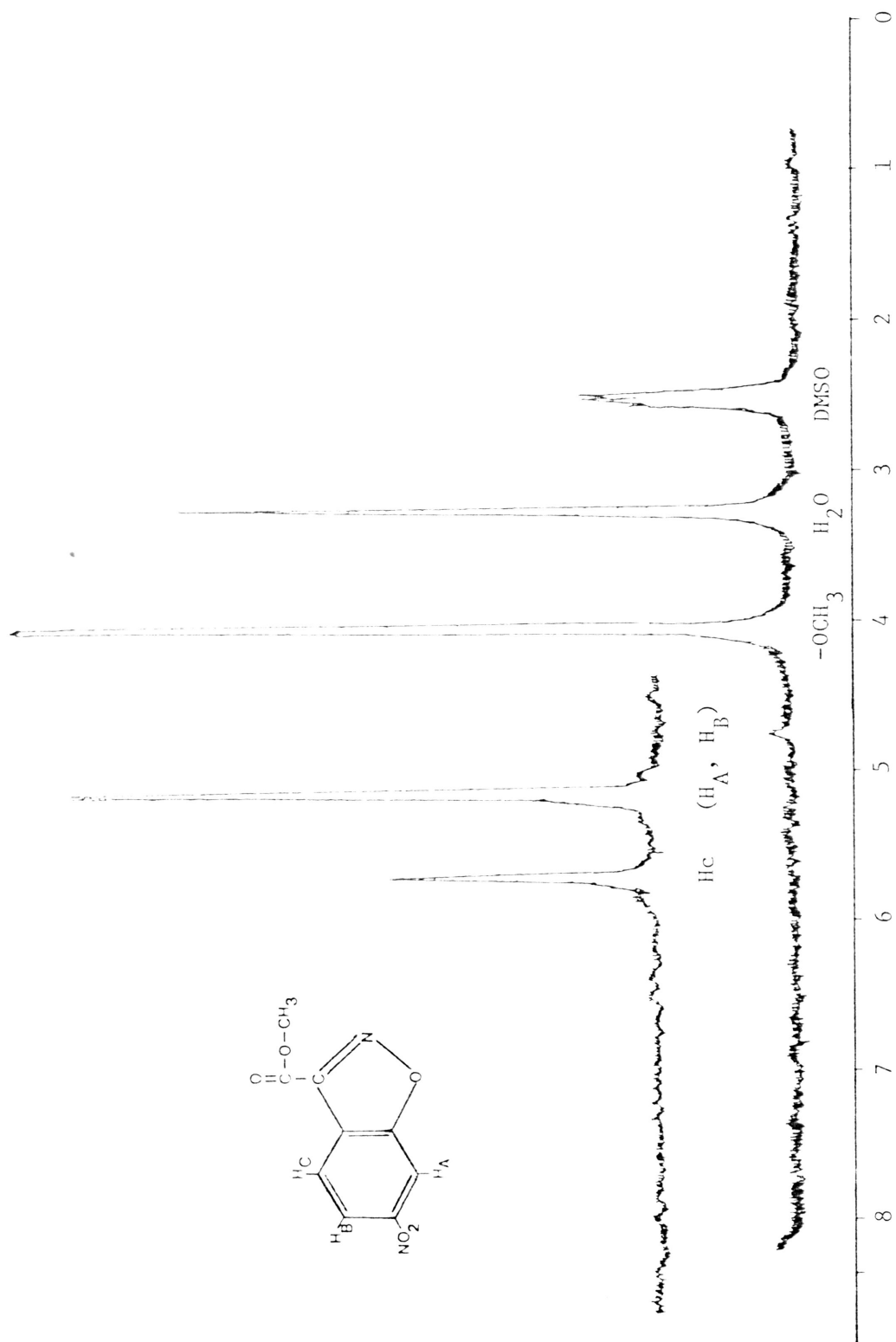
Chemical shifts, PPM(δ)

designed specifically for Karl Fisher titrations.[19] The instrument directly monitored micrograms of water, was operated only with standard (Photovolt) solutions.

Kinetic spectra were recorded using Durrum-Gibson Model D-10 T-jump stopped-flow, automated Gilford Model 252, Cary Model 118-C recording spectrophotometers. All runs were made using matched quartz cells (cuvettes), 1-cm path length, with Teflon stoppers. All spectra were recorded against the appropriate reference (blank) solution identical in composition to the sample solution except for the absorbing species of interest.

The Gilford Model 252 was used to measure the reaction rate of 4-nitrobenzisoxazole-3-carboxylic acid (4NB3CA) in DMSO (i.e., in the absence of Na cholate). A UV and visible spectrum scan (700-250 nm) of the starting material (4NB3CA in DMSO) and end product (4NB3CA, Na cholate in DMSO) was run on the Cary Model 118-C (Figures 4 and 5); the kinetic data of 4NB3CA in DMSO and Na cholate was obtained on the Durrum Gibson Model D110 stopped-flow spectrophotometer.

The spontaneous decarboxylation of 4-nitrobenzisoxazole-3-carboxylic acid in DMSO proved to be much too fast for conventional kinetics in the presence of sodium cholate. The mode of study was therefore switched from conventional kinetics to stopped-flow spectrophotometry. The stopped-flow technique is based on the rapid mixing of two reagents has a time range as short as one millisecond.

As shown in Figure 5, a stopped-flow apparatus in its simplest form consists of two "drive" syringes (1) that hold the reactants A and B; the plungers of these syringes are pushed forward simultaneously.

Figure 3. UV-visible absorption spectra of 4-NB-3-CA in DMSO at 25.0°C; [4-NB-3-CA] = 3 x 10^{-5} M.

Absorbance

λ, nanometers
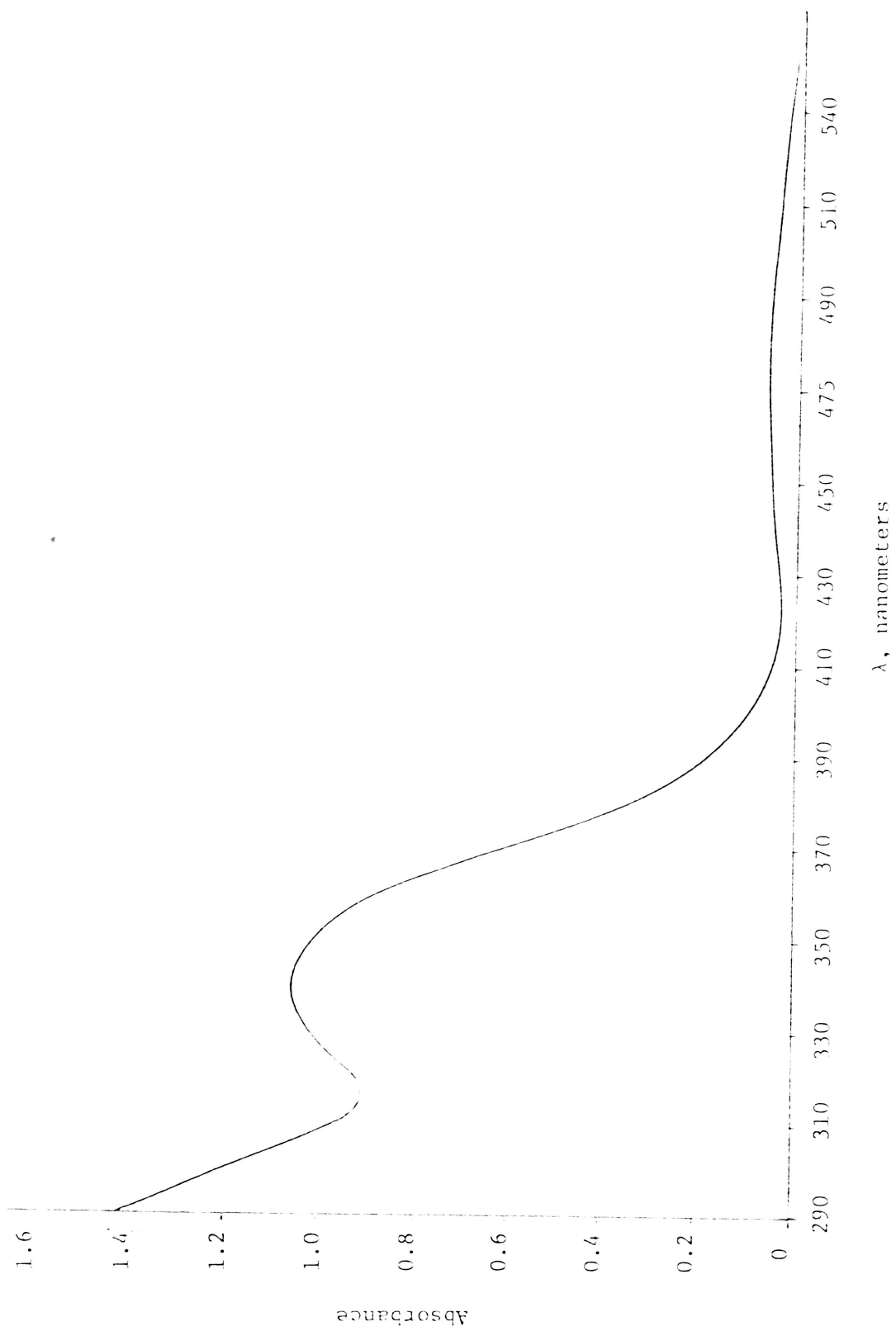
Figure 4. UV-visible spectra of decarboxylated 4-NB-3-CA in DMSO at 25.0°C; [4-NB-3-CA] = 3 x 10^{-5} M and [NaC] = .25M.

λ, nanometers

Absorbance
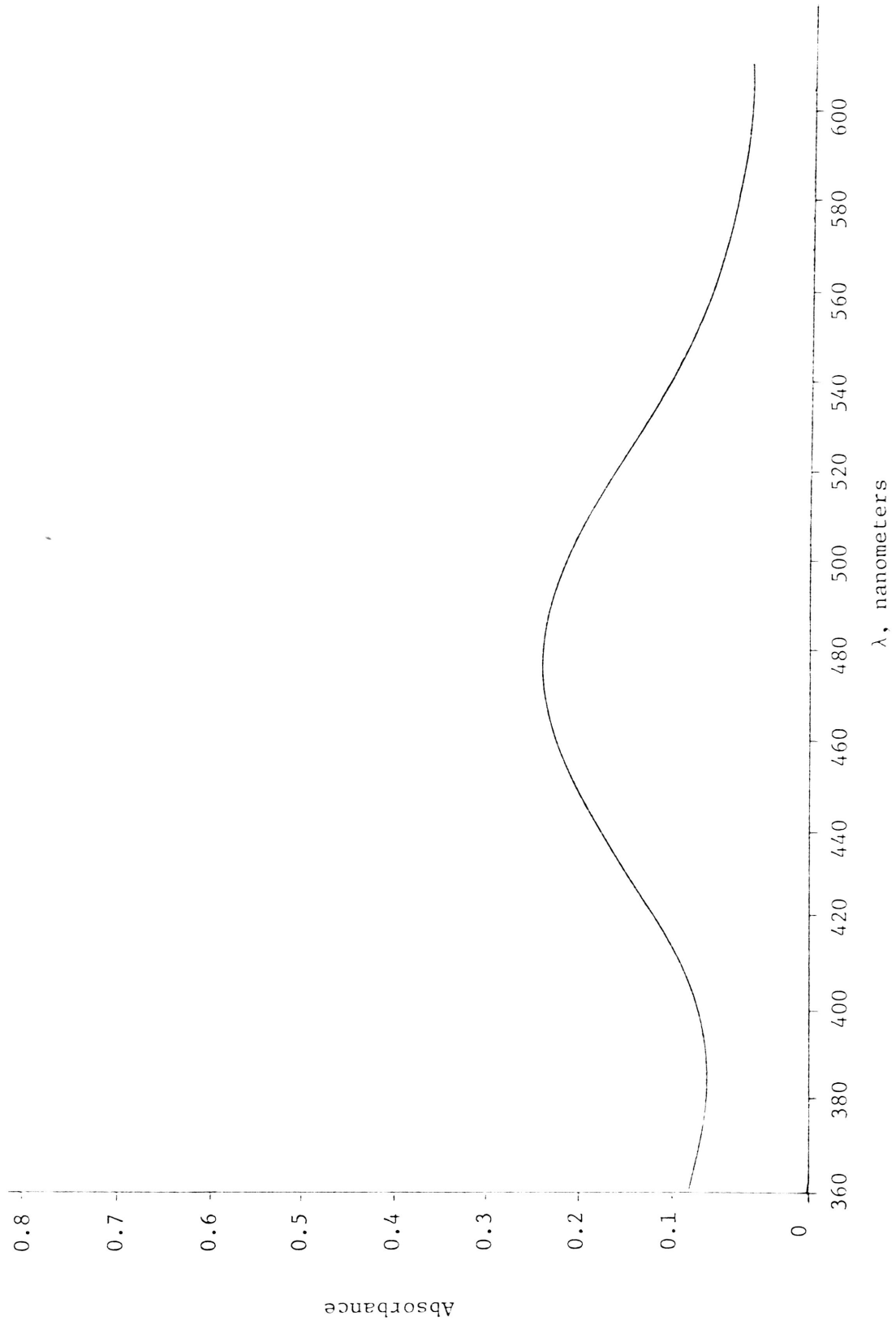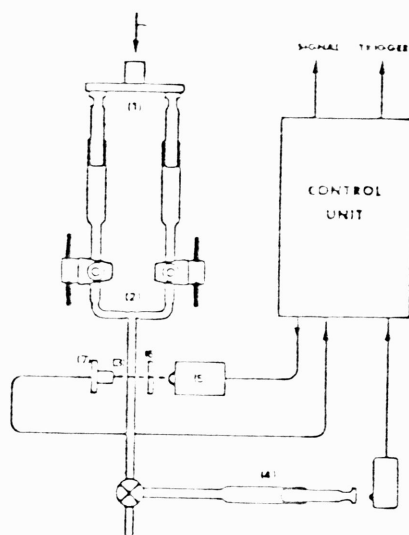
Figure 5. Diagram of stopped-flow spectrophotometer; (1) reactant syringes, (2) mixing chamber, (3) observation point, (4) collecting syringe, (5) light source, (6) light filter, (7) photocell.

The streams of the reactants meet in a mixing chamber (2) where the reaction starts. The mixture then flows down stream past the observation point (3) and into a third syringe (4), the plunger of which is pushed back by the pressure until it comes against a stop. When the flow is thus stopped suddenly the solution at the observation point is of freshly mixed reactants. The reaction now proceeds and it can be followed by the absorption of the beam of light by one of the reactants or products. The intensity of light transmitted by the sample at the observation point is displayed on an oscilloscope as a function of time following the stoppage of flow. The light source (5) emits a monitoring beam which passes through a filter (6). The intensity of light transmitted by the sample is measured by a photocell (7).[18]

The actual stopped-flow technique consisted of injecting the stock solution of 4-NB-3-CA in DMSO (7.69×10^{-4} M) into one of the

drive syringes. Next the desired concentration of Na cholate in DMSO $(3 \times 10^{-3} - .4m)$ is injected into the second drive syringe. The rate of end product formation was measured by monitoring the increase in absorbance at 475 nm. All kinetics were measured in thermostated cell compartments with the desired temperature (25°C), as measured inside the cells, maintained within $\pm$ 0.1°C as monitored by NBS thermometers. The results were stored on an oscilloscope and photographed with a Polaroid camera for future reference using established stopped-flow procedures.[20-22]

The kinetic data was plotted on a graph as log Δ absorbance vs. time (seconds) (see Figures 6-14). The observed pseudo first-order rate constant, k, was given by 2.303 times the slope of the best fit line. The half-life of the reaction was determined by dividing k into .693 (natural log of 2).

The 60 MHz ^{1}H nmr spectra were obtained using a Varian Associates A-60-A spectrophotometer at an ambient probe temperature of 32.5$\pm$0.5°C. Chemical shifts of the δ scale were measured in ppm relative to TMS (δ = 0 ppm), were taken from spectra with sweep widths of 500 Hz and were usually accurate to $\pm$ 0.3 Hz. The ^{1}H nmr spectra were determined on solutions of DMSO-d_6 (Aldrich, 99.5 atom % D).

Figure 6. Plot of the log of the change in absorbance $\underline{vs}$. time
for the spontaneous decarboxylation of 4-NB-3-CA in DMSO at
25.0°C; [4-NB-3-CA] = 3.85 x 10^{-5} M and [NaC] = 1.5 x 10^{-3} M.

Fig. 7. Plot of the log of the change in absorbance vs. time for the spontaneous decarboxylation of 4-NB-3-CA in DMSO at 25.0°C; [4-NB-3-CA] = 3.85 x 10^{-5} M and [NaC] = 2.5 x 10^{-3} M.

Time, sec.

log Δ Abs.

2.2
2.0
1.8
1.6
1.4
1.2
1.0
.8
.6
.4
.2
0

1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9

Figure 8. Plot of the log of the change in absorbance $\underline{vs}$. time for

the spontaneous decarboxylation of 4-NB-3-CA in DMSO at 25.0°C;

$[4\text{-NB-3-CA}] = 3.85 \times 10^{-5}$ M and $[\text{NaC}] = 5 \times 10^{-3}$ M.

Log Δ Abs.

Time, sec.

Figure 9. Plot of the log of the change in absorbance <u>vs</u>. time for the spontaneous decarboxylation of 4-NB-3-CA in DMSO at 25.0°C; [4-NB-3-CA] = 3.85 x 10^{-5} M and [NaC] = 1 x 10^{-2} M.

Time, sec.

log Δ Abs.

Figure 10. Plot of the log of the change in absorbance _vs_. time

for the spontaneous decarboxylation of 4-NB-3-CA in DMSO at 25.0°C;

[4-NB-3-CA] = 3.85 x 10^{-5} M and [NaC] = 1.5 x 10^{-2} M.

Time, sec.

log J Abs.

Figure 11. Plot of the log of the change in absorbance <u>vs</u>. time

for the spontaneous decarboxylation of 4-NB-3-CA in DMSO at 25.0°C;

[4-NB-3-CA] = 3.85 x 10^{-5} M and [NaC] = 3 x 10^{-2} M.

Time, sec.

Log Δ Abs.

Figure 12. Plot of the log of the change in absorbance <u>vs</u>. time
for the spontaneous decarboxylation of 4-NB-3-CA in DMSO at 25.0°C;
[4-NB-3-CA] = 3.85 x 10^{-5} M and [NaC] = 5 x 10^{-2} M.

log Δ Abs.

Time, sec.

Figure 13. Plot of the log of the change in absorbance <u>vs</u>. time for the spontaneous decarboxylation of 4-NB-3-CA in DMSO at 25.0°C; [4-NB-3-CA] = 3.85 x 10^{-5} M and [NaC] = 0.1 M.
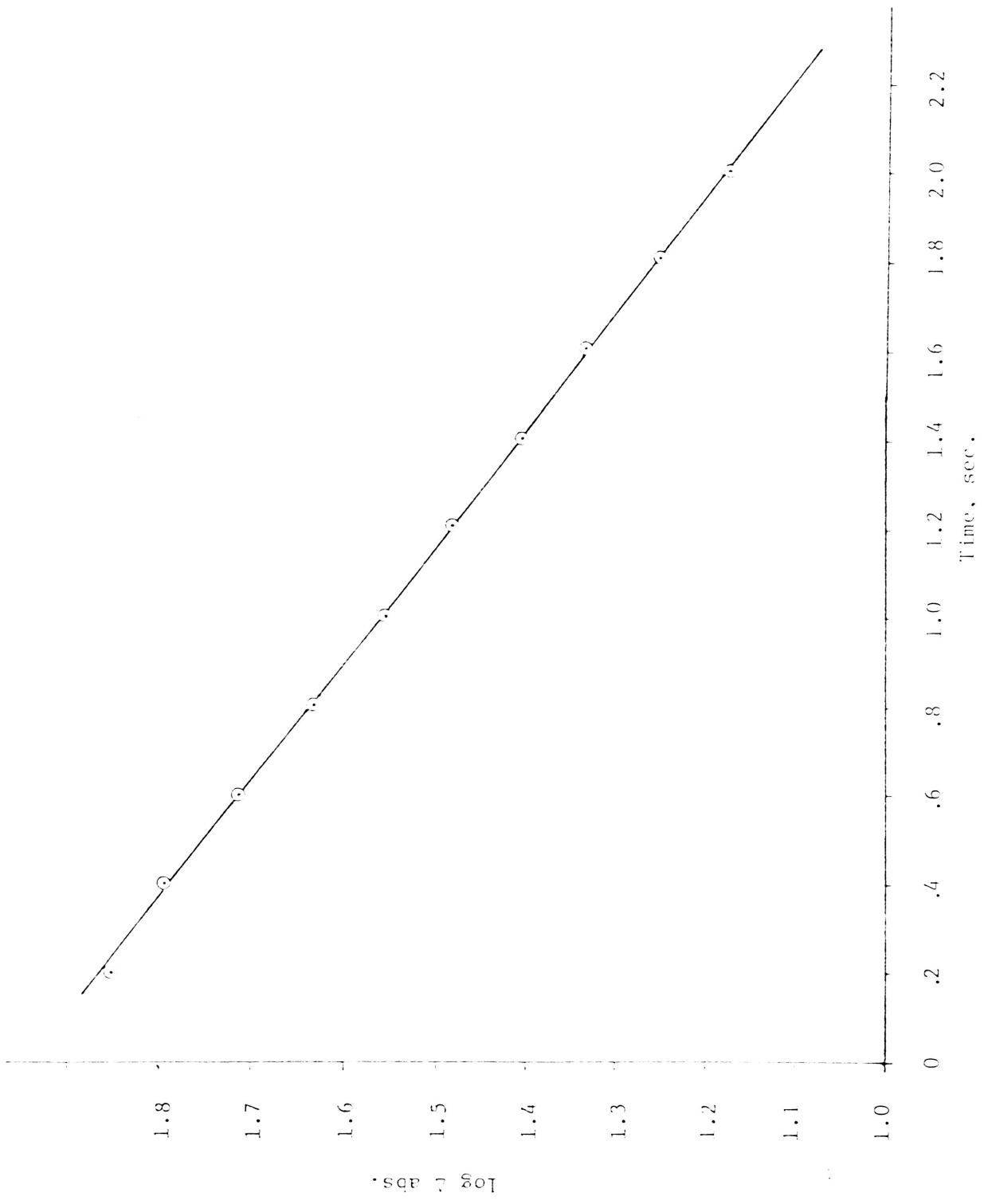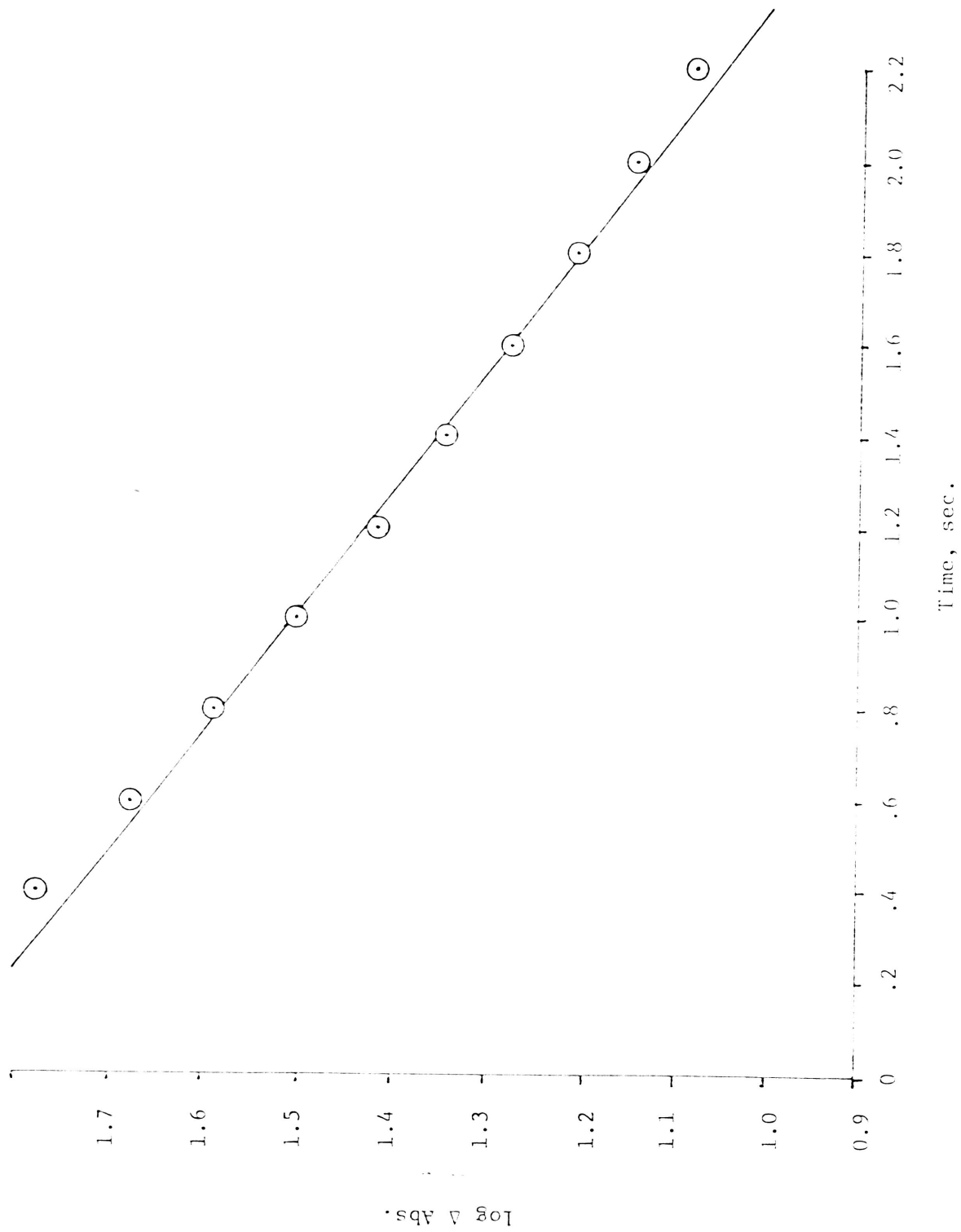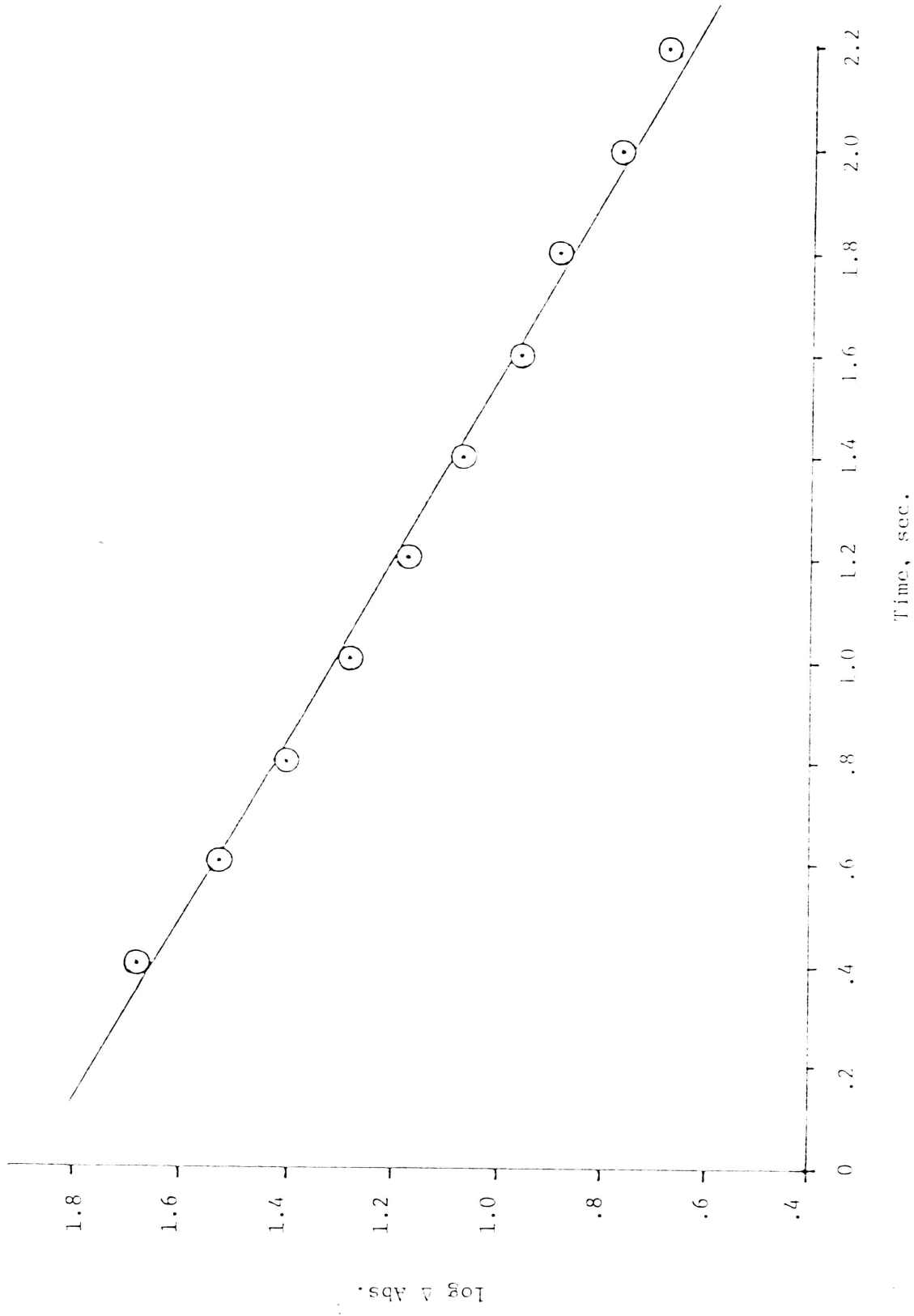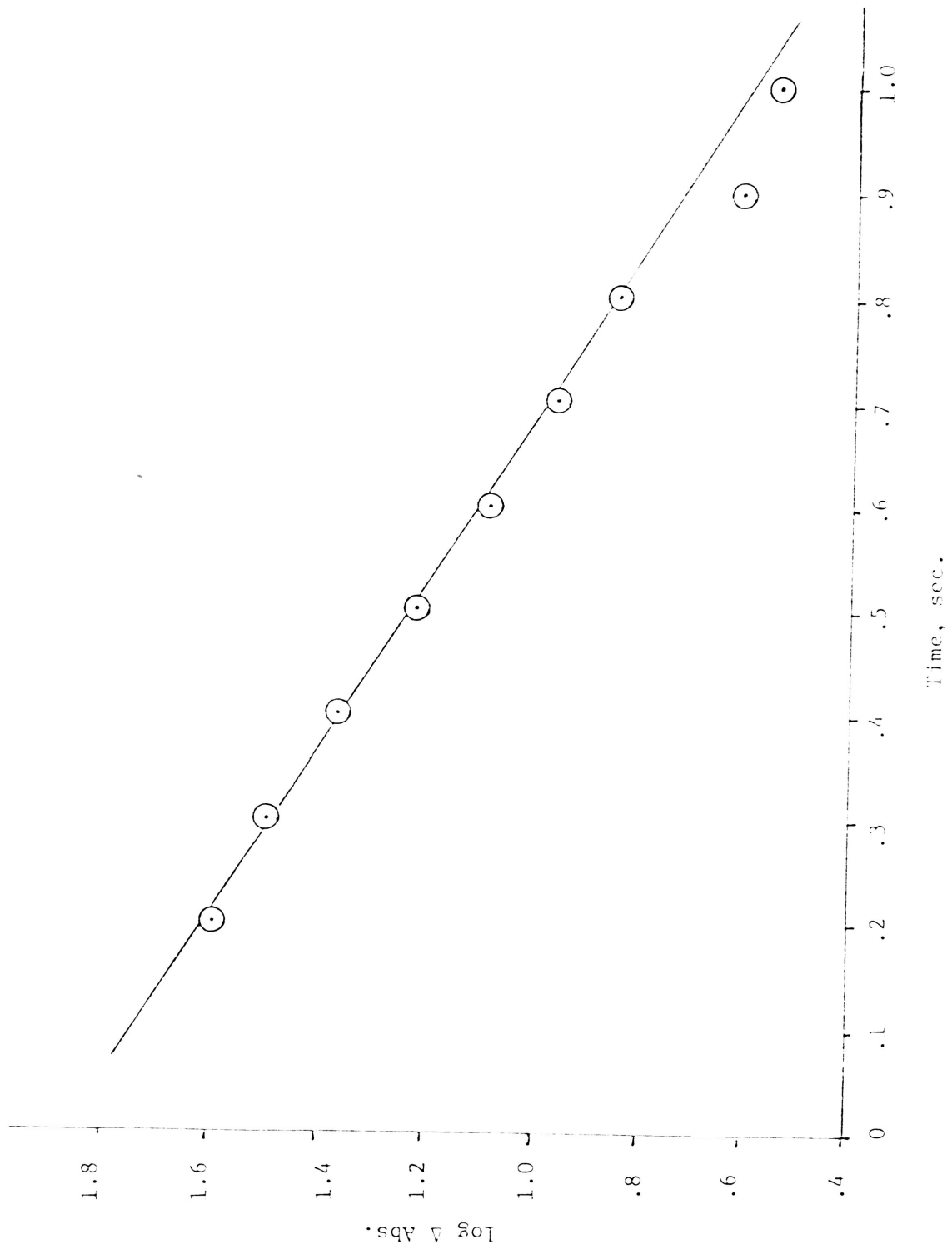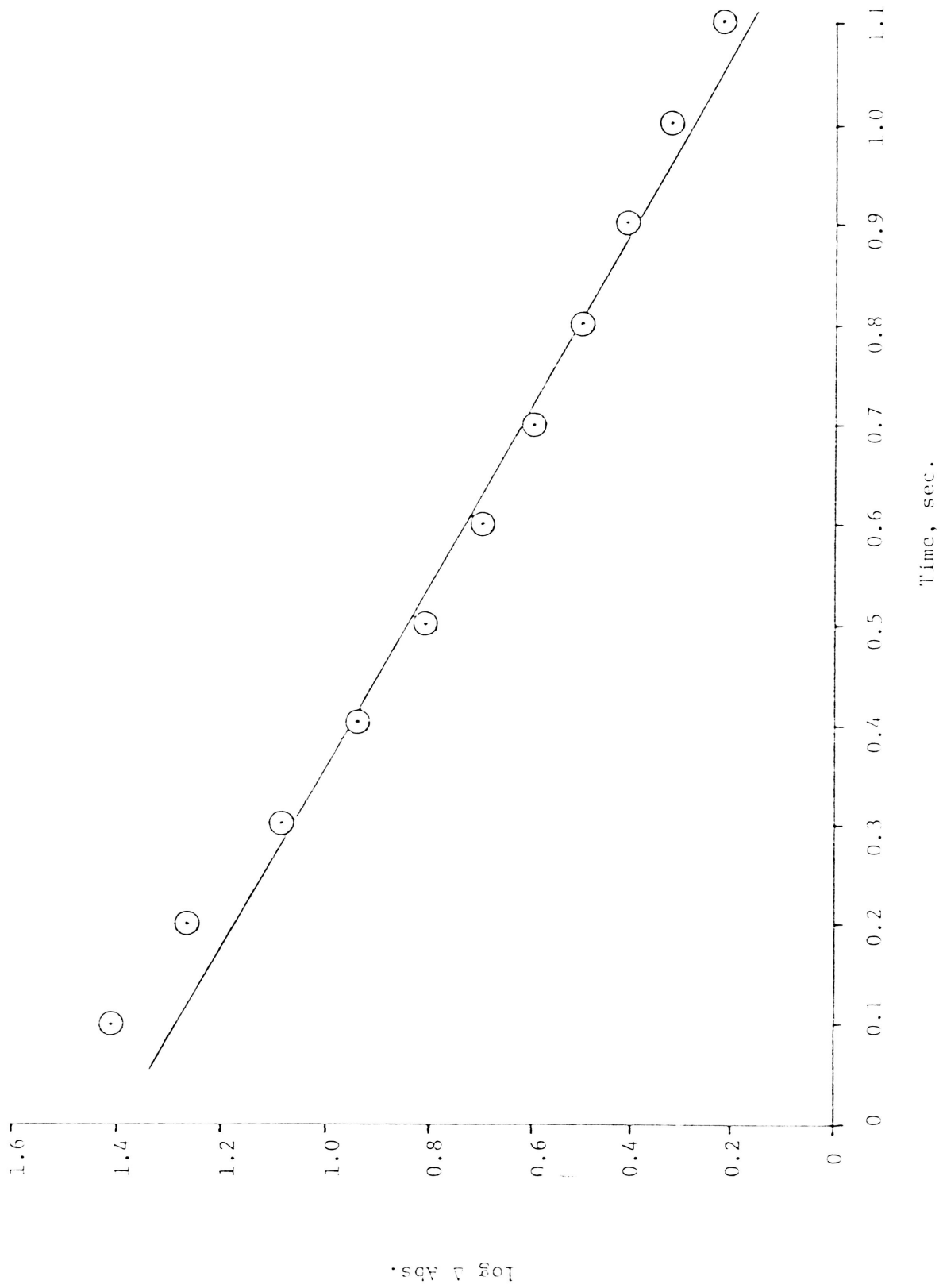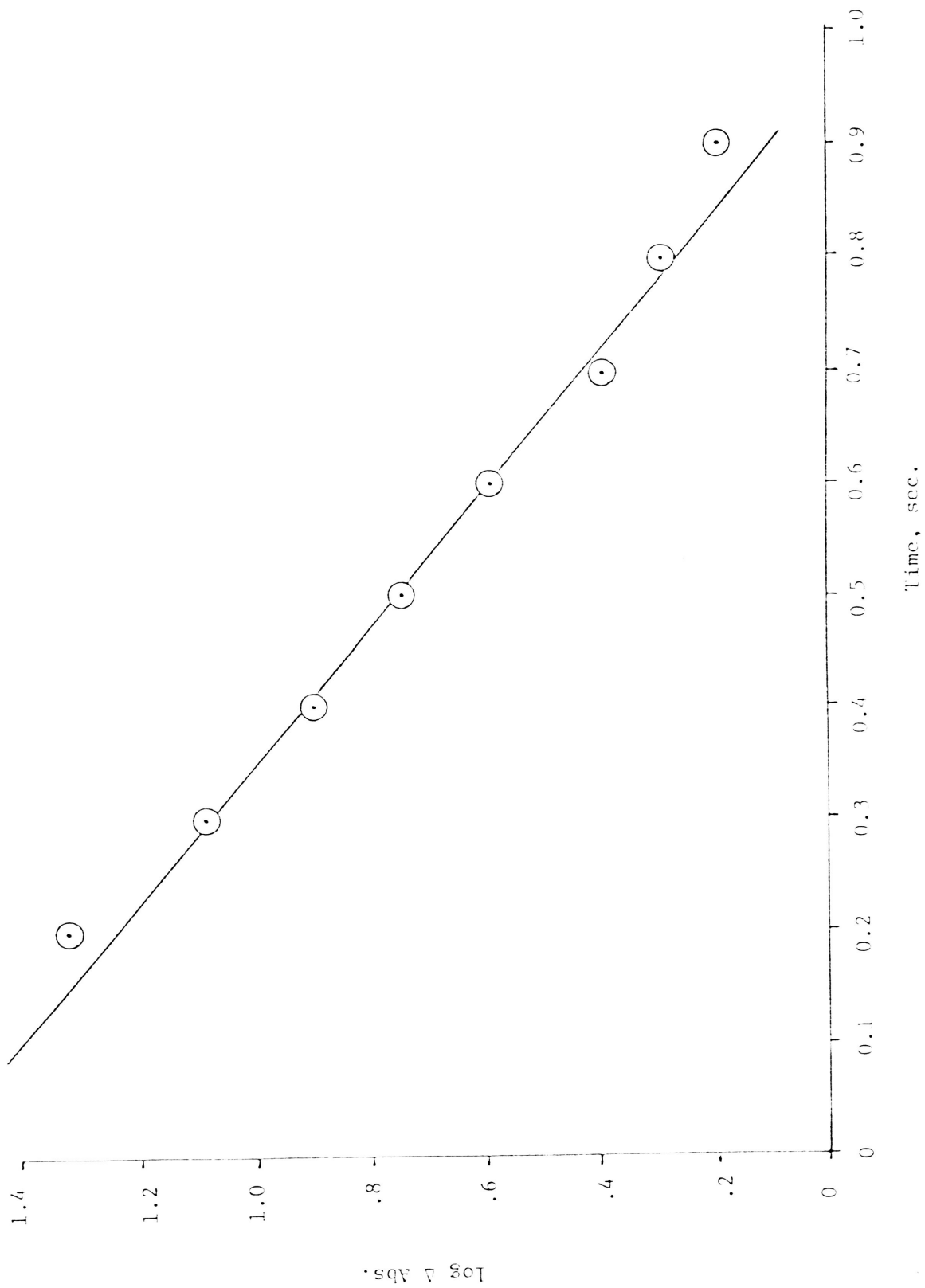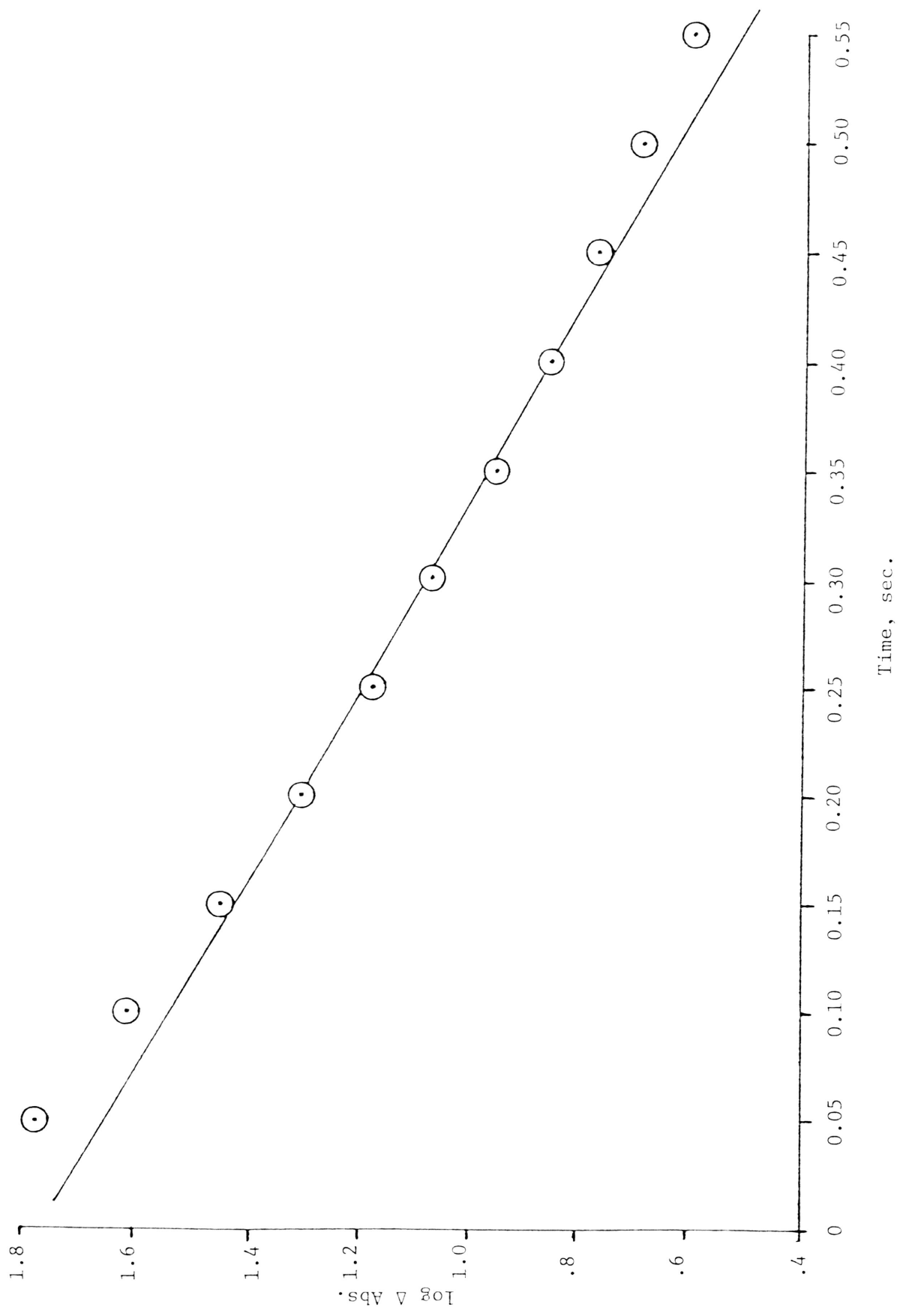
Figure 14. Plot of the log of the change in absorbance <u>vs.</u>
time for the spontaneous decarboxylation of 4-NB-3-CA in DMSO
at 25.0°C; [4-NB-3-CA] = 3.85 x 10^{-5} M and [NaC] = 0.2 M.

log Δ Abs.

Time, sec.

RESULTS AND DISCUSSION

Results and Treatment of Data

The observed rate constants for the spontaneous decarboxylation of 4-nitrobenzisoxazole-3-carboxylic acid (4-NB-3-CA) in dipolar aprotic DMSO in the presence and absence of sodium choalte (NaC) have been determined (see Figures 6-14). The observed rate constant, k_o, in the absence of sodium cholate was found to be 1.73×10^{-6} sec^{-1} with a corresponding half-life, $t_{1/2}$, of 4×10^5 sec. The observed rate constants, k_ψ, and half-lives in the presence of sodium cholate in DMSO were determined in surfactant concentrations ranging from 0.003 M to 0.40 M (Table IV). It is apparent that the observed rate constants for the decarboxylation (Figure 16 and Table IV) increase with increasing sodium cholate concentration. A sigmoidal dependence, characteristic of micelle-substrate interaction,[12,23,24] is also apparent (Figure 16). At high surfactant concentrations, the concentration dependence approaches a plateau, which is indicative of substrate saturation of the aggregated sodium cholate catalyst. The rate constant for the 4-NB-3-CA reaction in the presence of sodium cholate in the plateau region and at 1.5×10^{-3} M are 3×10^6 and 5×10^5-fold greater than that in the absence of sodium cholate, respectively.

In view of the apparent rate constant-surfactant concentration relationship (Figure 16), the data in Table IV could be treated in of a simple substrate-micelle (or aggregate) association, as given in 2:

$$M + S \underset{}{\overset{K}{\rightleftharpoons}} MS$$
$$\downarrow k_o \qquad \downarrow k_m \qquad\qquad (2)$$
$$P \qquad\quad P$$

Table IV. Decarboxylation of 4-Nitrobenisoxazole-3-Carboxylic Acid in the Presence of Sodium Cholate (NaC) in DMSO at 25.0°C.[a]

[NaC], M	k_ψ, sec^{-1}	$t_{1/2}$, sec	$\dfrac{k_\psi - k_o}{k_m - k_\psi}$	[H$_2$O], g/l
1.5×10^{-3}	0.872	0.794		5.8
2.5×10^{-3}	0.901	0.769		3.4
5.0×10^{-3}	1.38	0.50	1.38	4.8
1.0×10^{-2}	3.11	0.222	1.51	4.2
1.5×10^{-2}	3.55	0.195	2.19	5.0
3.0×10^{-2}	4.4	0.157	5.91	4.9
5.0×10^{-2}	5.11	0.136		5.6
0.20	3.97	0.175		10.6

[a] [4-NB-3-CA] = 3.85×10^{-5} M.

Figure 15. Plot of reaction half-life <u>vs</u>. [NaC] for the spontaneous decarboxylation of 4-NB-3-CA in DMSO at 25.0°C. [4-NB-3-CA] = 3.85 x 10^{-5} M.

t 1/2, sec.

[NaCl], M

0.1   0.075   0.005   0.025   0

.80 .70 .60 .50 .40 .30 .20 .10 0
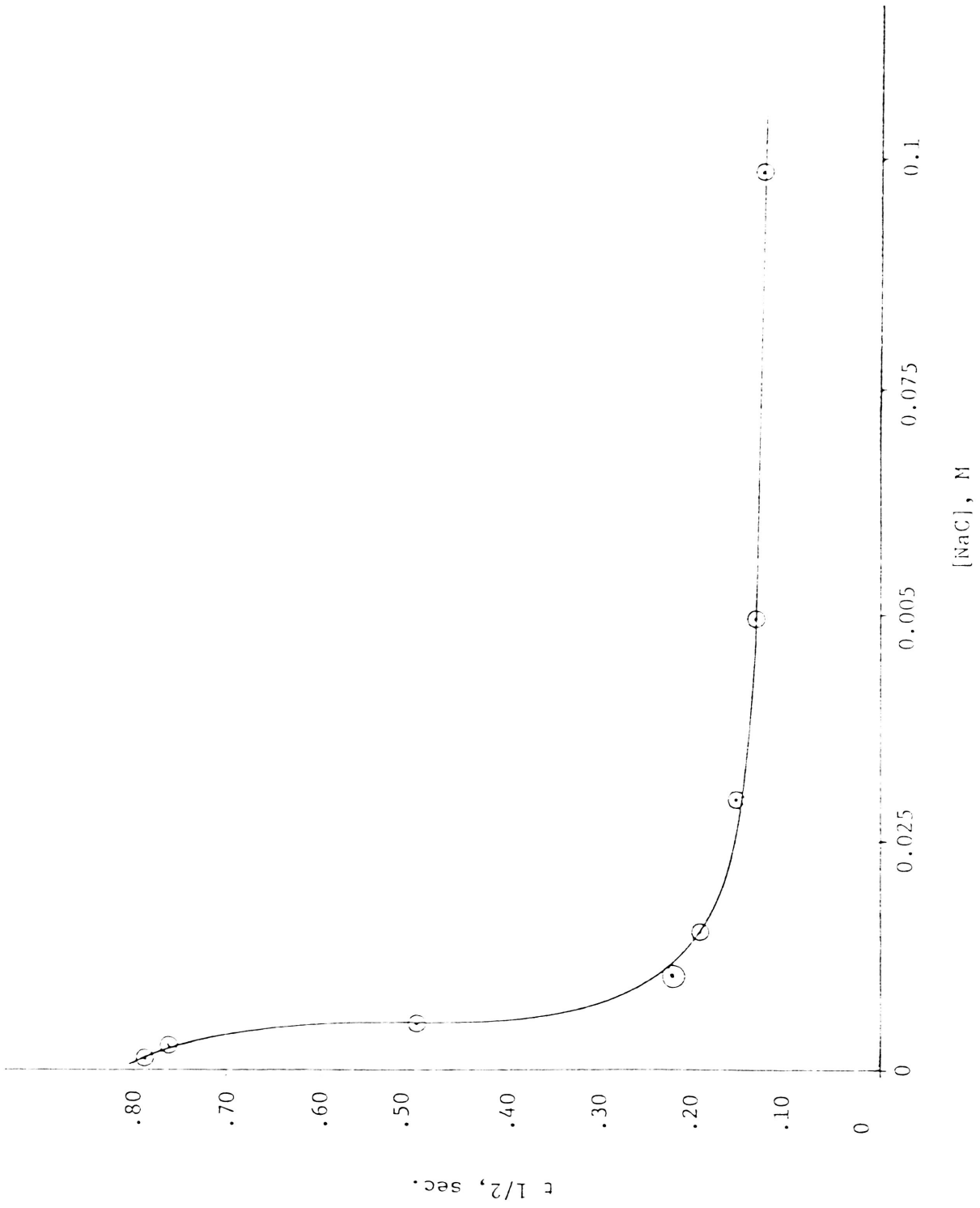
Figure 16. Plot of k_ψ vs. [Na cholate] for the spontaneous

decarboxylation of 4-NB-3-CA in DMSO at 25.0°C. [4-NB-3-CA] =

3.85 x 10^{-5} M.
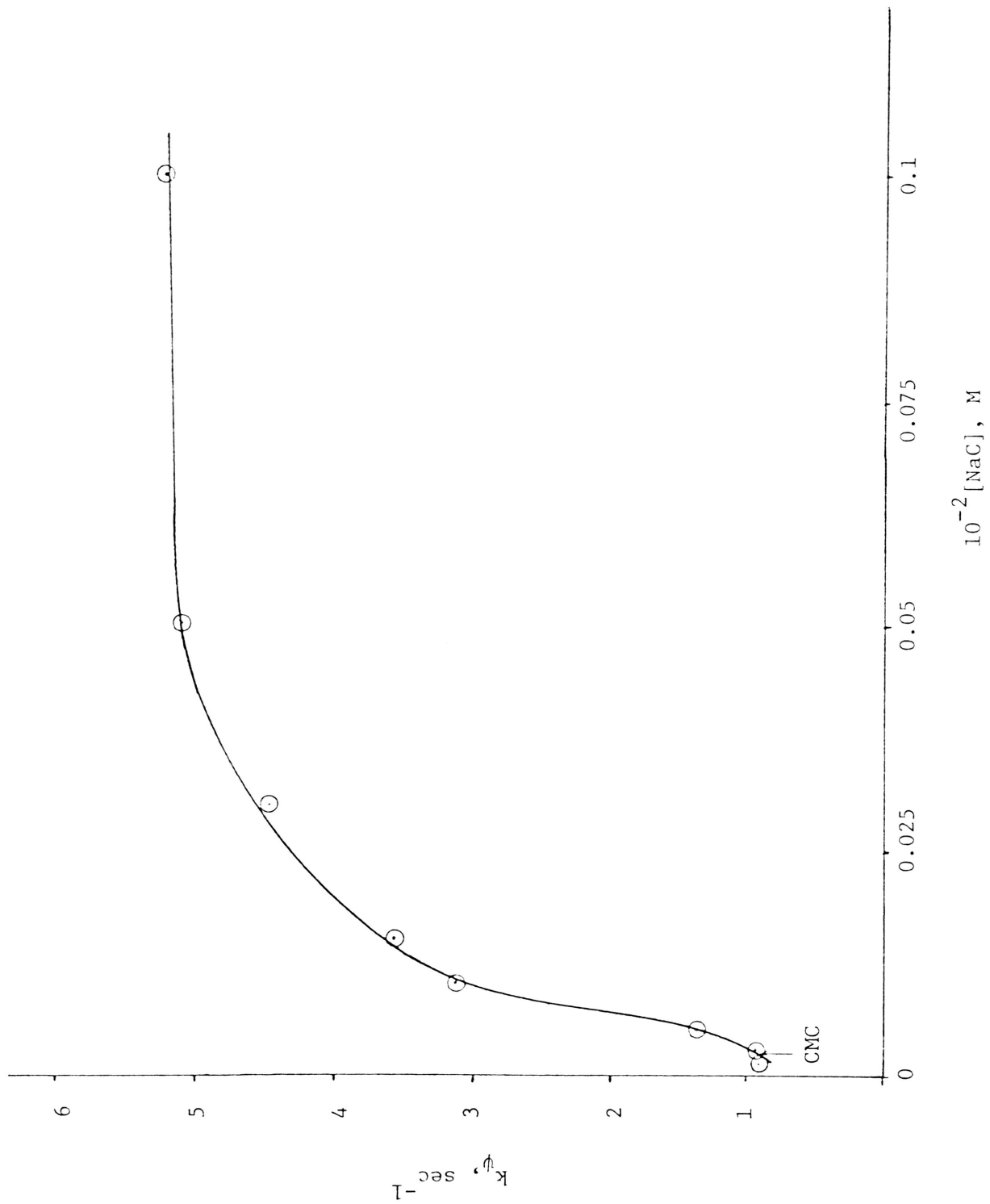
Figure 17. Plot of $\dfrac{k_\psi - k_o}{k_m - k_\psi}$ vs. [Na] for spontaneous decarboxylation of 4-NB-3-CA in DMSO at 25.0°C; [4-NB-3-CA] = 3.85 x 10^{-5} M.

$$slope = \frac{K}{N}; \ intercept = \frac{-K \cdot CMC}{N}$$

$[NaC].M$

$.03$

$.01$

$\dfrac{k_{\psi} - k_o}{k_m - k_{\psi}}$

4

2

0

-1
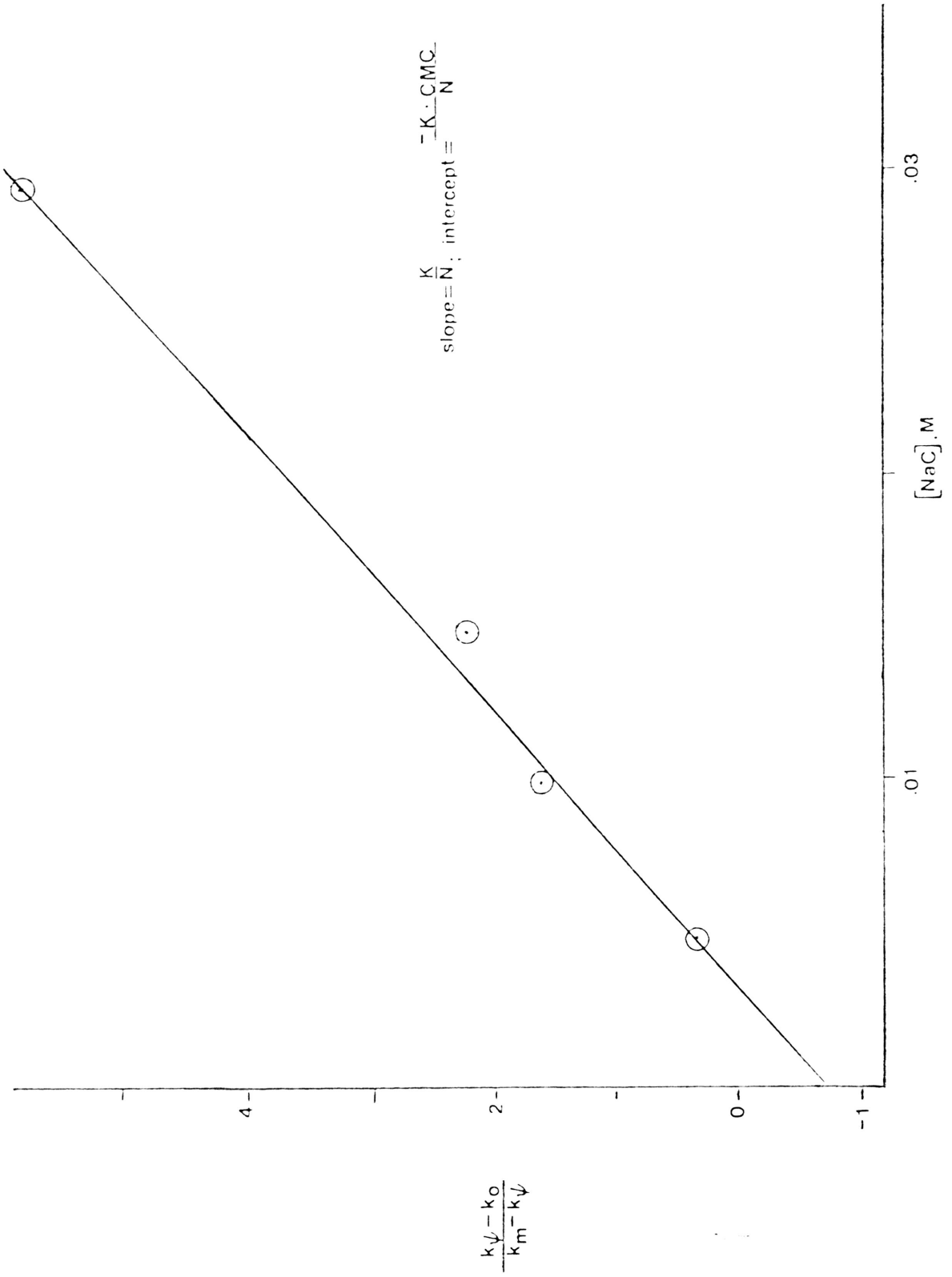
where M = bile salt (sodium cholate), S = substrate (4-NB-3-CA), MS = bile salt - substrate complex, K = binding constant (K = [MS]/[M][S]), p = product (decarboxylated 4-NB-3-CA), k_o = observed rate constant in the absence of sodium cholate), and k_m = maximum rate constant in the presence of sodium cholate.

Assuming that the micelle-substrate association is a one to one stoichiometric relationship and the substrate does not complex with the monomeric surfactant, then the observed pseudo-first order rate constant, k_ψ, could be given by equation 3.

$$k_\psi = \frac{k_o + k_m K[M]}{1 + K[M]} \tag{3}$$

Assuming that the concentration of monomers remains constant above the CMC (equation 4), equations 5 and 6 can be derived:[12]

$$[M] = \frac{C_D - CMC}{N} \tag{4}$$

Where C_D = stoichiometric concentration of sodium cholate CMC = critical micelle concentration (see Appendix A) N = aggregation number (the number of monomeric molecules per micelle).

$$\frac{1}{k_o - k_\psi} = \frac{1}{k_o - k_m} + \left(\frac{1}{k_o - k_m}\right)\left(\frac{N}{K(C_D - CMC)}\right) \tag{5}$$

$$\frac{k_\psi - k_o}{k_m - k_\psi} = \frac{KC_D}{N} - \frac{K \cdot CMC}{N} \tag{6}$$

According to equation 6, plots of $\frac{k_{\psi}-k_o}{k_m-k_{\psi}}$ vs. C_D should be linear,[12] with the slope equal to K/N and an intercept of $\frac{-K(CMC)}{N}$.

Discussion of Results

An advantage of use of equation 6 rather than equation 5 is that no knowledge of the CMC is necessary; however, it limits useful data to the region of a marked increase in k_{ψ}, i.e., there is an uncertainty in the ordinate when $k_{\psi} \simeq k_o$ or $k_{\psi} \simeq k_m$.

The treatment of kinetic data (Figure 17 and Table IV) according to equation 6 gave a straight line, from the slope and intercept which K/N and the CMC were calculated (Figure 17). The CMC (see Appendix A) was calculated to be 3.29×10^{-3} M. This value is quite similar to those found for a wide variety of ionic and non-ionic surfactants which aggregate appreciably in both aqueous and non-aqueous solvents.[2] An aggregation number, N, of 3-4 is attained over the sodium cholate concentration range employed in this study,[25,26] from which the binding constant, K, was calculated to be 636. This value lies within the range of 10^2-10^3, which is common for surfactants in both aqueous and non-aqueous solvents.[12]

The catalysis of decarboxylation of 4-NB-3-CA by sodium cholate resembles that of ionic micelles in aqueous solutions, which also feature sigmoidal dependence on surfactant concentration.[27] The rate constant is almost independent of the surfactant concentration below the CMC, while it rapidly rises with increasing concentration of surfactants above the CMC, and finally a plateau is reached at surfactant concentrations well above the critical concentrations for aggregation.

Bile salts do not undergo a monomer-n-mer type of self-association which is typical of synthetic micelles in aqueous solutions.[25,26] Instead, bile salts aggregate via a sequential definite or indefinite types[28] of association in dipolar aprotic solvents (DMSO), water, and other non-aqueous solvents.[25,26] The data accumulated on 4-NB-3-CA in the presence of sodium cholate was treated as though aggregation occurred via a monomer-n-mer type of self-association. The treatment of the data in this manner was justified in view of the similarity between data of synthetic micellar systems and the data acquired in the presence of sodium cholate.

The rate of acceleration by sodium cholate is explicable in terms of both physical (i.e., proximity) and chemical catalysis. Solubilization of 4-NB-3-CA in dipolar aprotic DMSO can occur by both hydrophobic interactions between the aromatic ring of 4-NB-3-CA and the steroid nucleus of sodium cholate. Solubilization also occurs via H-bonding of the 4-NB-3-CA with the 3-hydroxyl groups and carboxylate anion of sodium cholate. Orientation or binding in the aggregated sodium cholate can then facilitate the extraction of the carboxylic acid proton of 4-NB-3-CA (equation 7) resulting in the spontaneous decarboxylation and regeneration of the catalyst.

Abstraction of the acid proton is essential for "spontaneous" decarboxylation of 4-NB-3-CA to occur. A test of this postulated mechanism would be the effect of bases on 4-nitrobenzisoxazole-3-carboxylic acid, rather than its anion in non-aqueous solvents. Indeed, Kemp and Paul[3,4] report a rate constant of 10 sec^{-1} for the decarboxylation in the presence of tetramethylguanidine in DMSO at
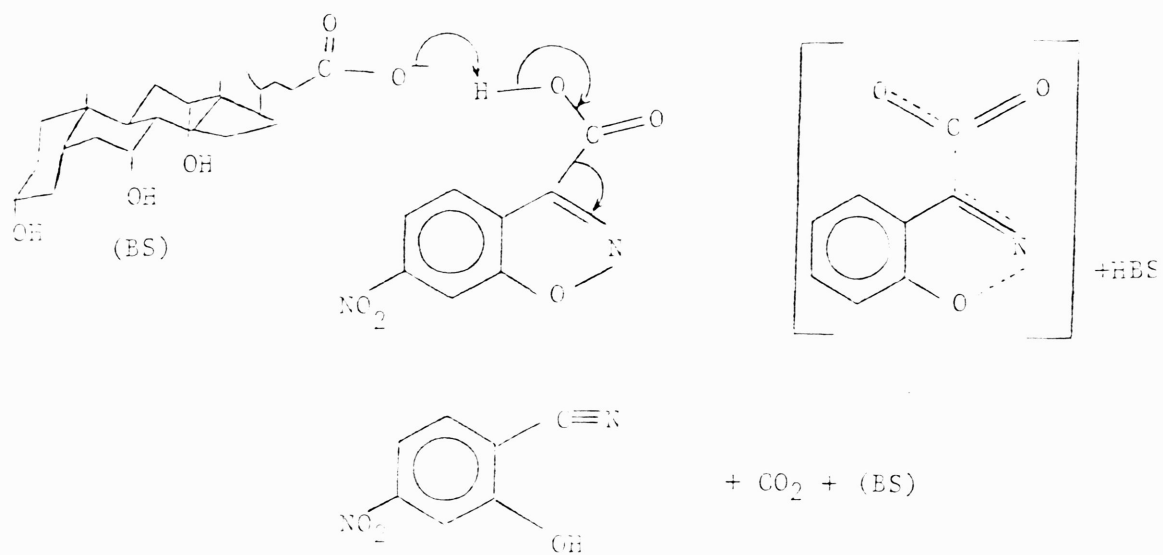
$$(7)$$

30°C and comparable rate constant in other dipolar aprotic and nonpolar

solvents (see Table I), while the rate constant obtained here was

calculated to be 1.73×10^{-6} sec^{-1} in pure dry DMSO at 25.0°C. Con-

sistent with the postulated mechanism (equation 7), the discrepancy

between these two rate constants in DMSO may be attributed to the

presence of the tertiary base in the former which logically could also

enhance the reaction via extraction of the acid proton from 4-NB-3-CA.

CONCLUSION

These results clearly demonstrate that bases and/or aggregated bile salts are involved in the "spontaneous" decarboxylation of 4-nitrobenzisoxazole-3-carboxylic acid. A mechanism consistent with all of the data for this reaction to date, has been postulated. It is clear, however, that further research is necessary to elucidate both roles of aggregated bile salts and bases on this reaction. Specifically, the effects of non-self associating bases in DMSO should be determined. Likewise, the effects of both cationic synthetic and bile salt surfactants should be elucidated, both as a function of water concentration and temperature. The binding site of the 4-nitro-benzisoxazole-3-carboxylic acid should be determined using ^{1}H and ^{13}C nmr. Such studies would serve to elucidate two primary factors involved in this reaction in non-aqueous media, namely the respective roles of bile salts and bases.

LITERATURE CITED

1. Surfactant-(surface active agents) are amphiphilic substances that lower the surface and interfacial tension of a liquid and aggregate or self associate in solution forming micelles or association colloids. Amphiphiles are substances which contain both water soluble and water insoluble groups.

2. C. A. Bunton and M. Minch, Tetrahedron Lett., 3881, (1970).

3. D. S. Kemp and K. G. Paul, J. Amer. Chem. Soc., 97, 7305 (1975).

4. D. S. Kemp, D. D. Cox and K. G. Paul, J. Amer. Chem. Soc., 97, 7312 (1975).

5. Micelle - can be defined as an aggregate of monomeric amphiphilic molecules possessing hydrophobic and hydrophilic moieties which exhibits distinct physico-chemical properties differing from those of monomers.

6. C. A. Bunton, M. Minch and L. Sepulveda, J. Phys. Chem., 76, 2707 (1971).

7. C. A. Bunton, A. Kamego and J. Minch, J. Org. Chem., 37, 1389 (1972).

8. C. A. Bunton, M. J. Minch, J. Hildago and L. Sepulveda, J. Amer. Chem. Soc., 95, 3262 (1973).

9. C. A. Bunton, A. A. Kamego and P. Ng, J. Org. Chem., 39, 3469 (1974).

10. T. Kunitake, S. Shinkai and S. Hirotsu, J. Org. Chem., 42, 306 (1977).

11. D. Spinelli, J. Org. Chem., 43, 4042 (1978).

12. J. H. Fendler and E. J. Fendler, "Catalysis in Micellar and Macromolecular Systems." Academic Press, New York, 1975; and references cited therein.

13. R. O. Clinton and S. C. Laskowski, J. Amer. Chem. Soc., 70, 3135 (1948).

14. W. Borsche, Chem. Ber., 42, 1310 (1909).

15. H. Lindemann and H. Cissee, Justus Liebigs Ann. Chem., 469, 44 (1929).

16. H. Lindemann and C. Cissee, J. Prakt. Chem., 122, 232 (1929).

17. W. Borsche, *Justus Liebigs Ann Chem.*, 390, 14 (1912).

18. P. Suppan, Amer. Lab., p. 41-51 Nov. 1977.

19. J. Mitchell, Jr. and D. M. Smith in "Chemical Analysis", B. L. Clarke and I. M. Kolhoff, Eds., Vol. V, Interscience, New York, 1948.

20. "Operation and Maintenance Manual, Durrum Stopped-Flow Spectrophotometer," Durrum Instrument Corporation, Palo Alto, 1972.

21. G. H. Czerlinski, "Chemical Relaxation," Marcell Dekker, New York, N. Y. 1966, p. 275.

22. J. E. Stewart, "Durrum Application Notes," No. 2, Durrum Instrument Corporation, Palo Alto, 1972.

23. K. L. Mittal, "Micellization, Solubilization and Microemulsions," Vol. 1, Plenum Press, New York, 1977, and references cited therein.

24. K. L. Mittal, "Micellization, Solubilization and Microemulsions" Vol. 2, Plenum Press, New York, 1977, and references cited therein.

25. E. J. Fendler and S. N. Rosenthal, in "Solution Chemistry of Surfactants," K. L. Mittal, Ed., Plenum Press, New York, in press, 1979.

26. E. J. Fendler, D. J. Koranek, M. A. McLaughlin and S. N. Rosenthal, in "Solution Chemistry of Surfactants," K. L. Mittal, Ed., Plenum Press, New York, in press, 1979.

27. C. A. Bunton, M. J. Minch, J. Hildago and L. Sepulveda, *J. Amer. Chem. Soc.*, 95, 3262 (1973).

28. F. Y. Lo, B. M. Escott, E. J. Fendler, E. T. Adams, Jr., R. D. Larsen, P. W. Smith, *J. Phys. Chem.*, 79, 2609 (1975).

29. E. H. Cordes, "Reaction Kinetics in Micelles," Plenum Press, New York, 1973.

30. C. A. Bunton, *Prog. Solid State Chem.*, 8, 239 (1973).

31. E. H. Cordes and C. Gitler, *Prog. Bioorg. Chem.*, 2, (1973).

32. T. C. Bruice, in "The Enzymes" 3rd Ed., P. D. Boyer, Ed., Vol. 2, Academic Press, New York, 1970. p. 217.

33. E. J. Fendler and J. H. Fendler, *Adv. Phys. Org. Chem.*, 8, 271 (1970).

34. E. H. Cordes and R. B. Dunlap, Accounts Chem. Res., 2, 239 (1969).

35. F. Y.-F. Lo, B. M. Escott, E. J. Fendler, E. T. Adams, Jr., R. D. Larsen, and P. W. Smith, J. Phys. Chem., 79, 2609 (1975).

36. A. S. Kertes and H. Gutman in "Surface Colloid Science" E. Matijevic, Ed., Vol. 8, New York, 1974, pp. 193-295.

37. E. J. Fendler, V. G. Constein and J. H. Fendler, J. Phys. Chem., 79, 1917 (1975).

38. M. C. Carey and D. M. Small, Arch. Intern. Med., 130, 506 (1972).

39. D. M. Small, in "Molecular Association in Biological and Related Systems," American Chemical Society, Washington, D. C., 1968, p. 31.

40. K. Fontell, Chim. Phys. Appl. Prat. Ag. Surface, C. R. Congr. Int. Deterg. 5th, 9-13 Sept., 1968 (Publ. 1969), 2(Pt. 2), 1033 (1968).

41. K. Fontell, Koll.-Z. Z. Polym., 250, 333 (1972).

42. K. Fontell, Koll.-Z. Z. Polym., 250, 825 (1972).

APPENDIX A

Micellar Chemistry

Surfactants (surface active agents or detergents) are among a variety of compounds which may aggregate or self-associate in solution, forming aggregates which are generally called micelles.[12] Surfactants and micellar solutions have been extensively studied in detail.[23,24,29-34]. Several important aspects of micellar chemistry are as follows: (1) solubilization of substrates and other additives, (2) catalysis enhancement and/or retardation) of chemical reactions, (3) effects on hydrophobic interactions and protein structure, and (4) interactions with macromolecules and related systems.[23,24]

A surfactant molecule possesses distinct hydrophilic and hydrophobic regions that impart properties of self-association. In polar protic solvents such as water, the hydrophobic, or non-polar, moieties of the surfactant monomers are oriented toward the interior of the aggregate while the hydrophilic, or polar, groups are located at or near the surface of the micelle in contact with the bulk solvent[12] (Figure 18). The narrow concentration range over which surfactant monomers first begin to aggregate is known as the critical micelle concentration, or CMC. It is important to note that micelle formation occurs over a finite concentration range rather than a precise point, i.e., it is not a thermodyanic parameter. Micelles can have spherical, ellipsoidal and cylindrical shapes and can also form bilayers (Figure 19), i.e., two parallel layers of surfactant molecules in close proximity and polar groups oriented toward the bulk solvent.[23] Formation of
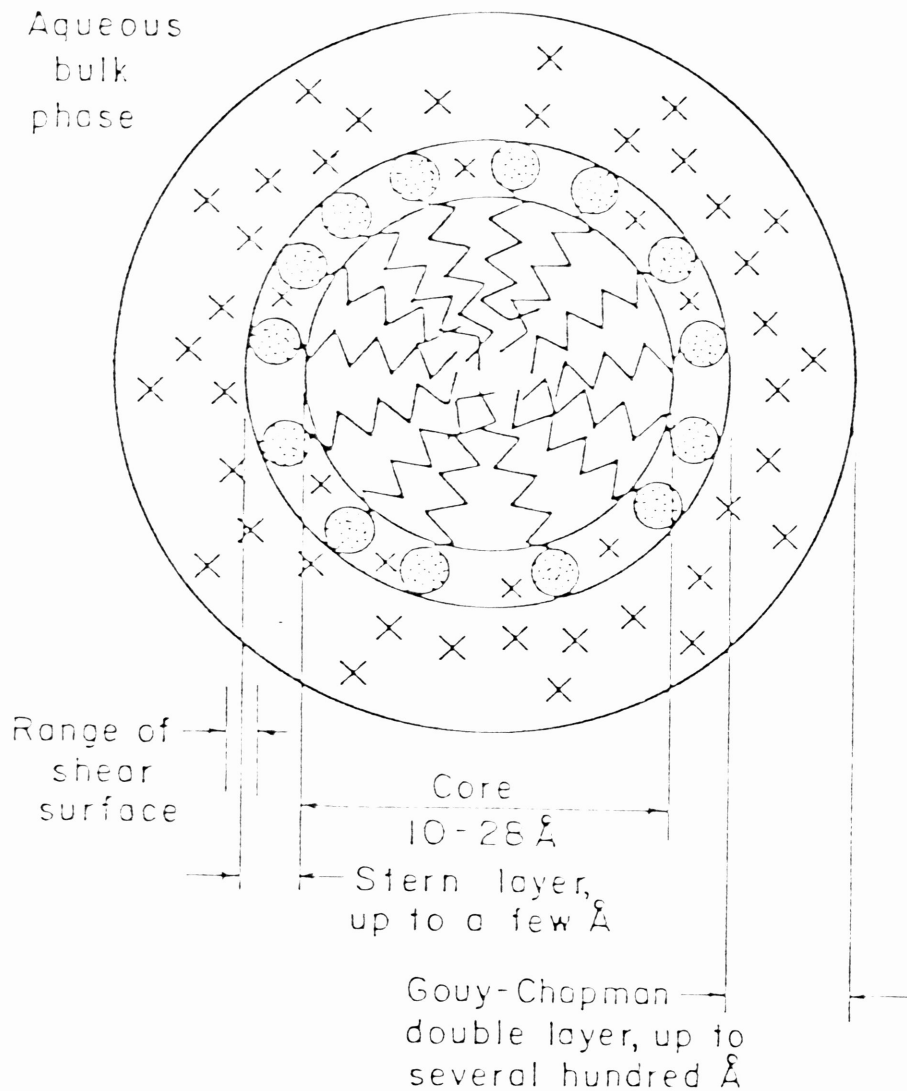
Figure 18. A two-dimensional schematic representation of the regions of
a spherical ionic micelle. The counterions (x), the head groups ( ) and
the hydrocarbon chains ( ) are schematically indicated to denote their
relative locations but not their number, distribution, or configuration.

micelles is also known to occur in non-polar and polar aprotic solvents,[12,23,24] such as carbon tetrachloride hexane, benzene, and chloroform. Since the structure of these aggregates is usually the inverse of that found in polar protic solvents, these micelles are termed reverse or inverted.[31] The polar head groups of the amphiphile forms the core of the reversed micelle with hydrophobic groups facing toward and extending into the non-polar solvent.[2] Reversed micellar systems have received considerable attention in recent years.[35-35]

APPENDIX B

The bile salts are one of the most important groups of naturally occurring surface active agents that are found in mammals. In the small intestine, these compounds, which are one of the major constituents of bile, aid in the digestion and absorption of fats and the fat soluble vitamins A, D, E and K. They are also responsible for the emulsification of the long-chain, water-insoluble triglycerides and for stimulating the hydrolytic activity of pancreatic lipase. The bile acids, which are synthesized from cholesterol in the liver, are mono-, di- or trihydroxy derivatives of cholanic acid, a 24-carbon monocarboxylic acid whose stereochemistry is determined by the cis A/B ring juncture (5-β-hydrogen) in the steroid nucleus:
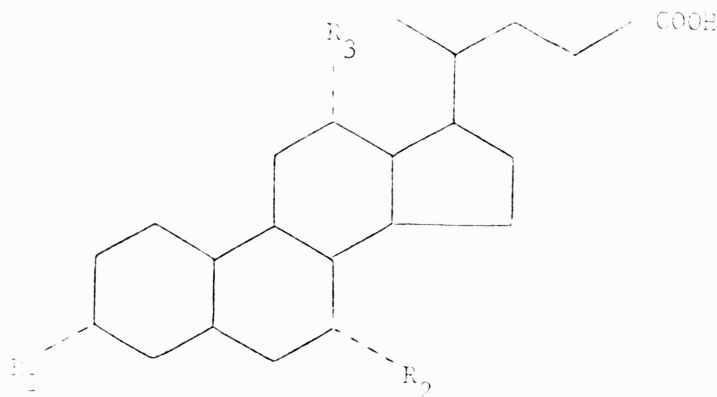
$R_1=R_2=R_3=OH$ Cholic acid (3α, 7α, 12α-trihydroxycholanoic acid)

$R_1=R_2=OH$, $R_3=H$ Chenodeoxycholic acid (3α, 7α-dihydroxycholanoic acid)

$R_1=R_3=OH$, $R_2=H$ Deoxycholic acid (3α, 12α-dihydroxycholanoic acid)

$R_1=OH$, $R_2=R_3=H$ Lithocholic acid (3α-hydroxycholanoic acid).

In man, and most higher vertebrates, there are six major bile salts – the glycine ($H_2NCH_2COO^-$) and taurine ($H_2NCH_2CH_2SO_3^-$) conjugates of cholic, chenodeoxycholic, and deoxycholic acids, and lesser amounts of the monohydroxyl compounds, salts of lithocholic acid. In vivo, conjugation with glycine and taurine are necessitated by the relatively low solubility of the unconjugated bile salts at the relatively low physiological pH (6.3-6.6) of the upper portion of the small intestine.

Quite significantly, these bile acids and salts where all the hydroxyl and acidic moieties lie on one side of the plane of the steroid nucleus and the angular methyl groups on the other, have hydrophobic and hydrophilic faces (i.e., they possess planar polarity to a striking degree). Consequently, bile salts, although structurally dissimilar to long hydrocarbon chain amphiphilic surfactants, also form small aggregates or micelles above a critical micelle concentration (CMC) when dissolved in water.[38-39] In aqueous solution, the shape of the bile salt micelle has been depicted as a cylinder, containing from four to ten or fifteen monomeric units, with the hydrophobic side in the interior and the hydroxyl and acidic substituents oriented toward the bulk solvent.[38] However, studies on ternary systems composed of bile salts, water, and n-decanol indicate that two different types of aggregates are found – one at low decanol concentrations and the other at high decanol concentrations.[40-42] In the case of the former, normal micelles or aggregates exist whose structure is essentially the same as that in aqueous solution. When the amount of n-decanol exceeds 50 mole %, the aggregate structure changes to the converse and reversed or inverted aggregates or micelles are formed, in which case water and

counter-ions are localized in the polar "cavity" or core of the aggregate with the polar groups generally surrounding this "cavity."

In dipolar aprotic solvents, such as dimethyl sulfoxide and acetonitrile, bile salts are soluble to varying extents and the catalytic efficiency appears to be related to solvent structure and solvent-bile salt interactions as well as to aggregated bile salt structure. These investigations suggest that bilamellar-type reversed aggregates are formed in both nonpolar and dipolar aprotic solvents.[25,26]

Figure 19. Perspective structural formula, Stuart-Briegleb space

filling model, and longitudinal and tranverse shorthand representa-

tions of a molecule of cholic acid.