OXIDATION KINETICS OF A NOVEL LEUKOTRIENE ANTAGONIST

BY

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by Miriam K. Franchini

(under the direction of Dr. J. T. Carstensen)

I INTRODUCTION

The chemical literature contains a fair amount of publications on the subject of oxidation. The pharmaceutical literature, on the other hand, contains only a small amount of published work in the area, for example, work by Lee and Notari,[1] Sokoloski and Higuchi,[2] Timmins *et al*,[3] and Asker *et al* [4]. It is the intent of this thesis to examine a compound of pharmaceutical interest for the purpose of proposing a means of executing preformulation studies of compounds which are prone to oxidation.

One aspect which is particularly pharmaceutical is that solutions are placed in a closed system (ampuls) at ambient temperature, and then heated to higher temperatures. In general, oxidations are studied at constant pressure, but in the pharmaceutical systems the systems are constant volume systems.

A major point of investigation is, then, the role of the oxygen, and as such it is necessary to establish the concentration of oxygen in solution. Although there are data for Henry's law constants for oxygen in water,[5,6] and for the temperature-dependence of oxygen solubility in water,[7,8] most experimental investigation of oxygen solubility in aqueous solutions of electrolytes embrace the limited temperature interval of 0 to 25°C.[9,10] There are no data on oxygen

concentration in solutions of more complicated matrices such as those commonly encountered in kinetic studies, i.e., buffers with ionic strength adjustment at temperatures above 45°C.

In order to rationally analyze oxidation reactions it is necessary to know the oxygen concentration in solution in the system under study. It is possible to monitor the oxygen concentration by external means, such as by the Winkler method or with an oxygen electrode. The Winkler method, although the standard procedure for measuring oxygen concentrations in sea water[11], is a tedious iodometric titration further complicated by the many substances which interfere with it[12]. The problem with oxygen electrodes is that none of the commercially available electrodes are functional above 50°C; preformulation studies are often carried out above this temperature. An apparatus has therefore been designed which should allow determination of oxygen concentration at any temperature, while simultaneously monitoring the degradation of the compound. Thus, the effect, if any, of the drug on oxygen concentration may also be monitored as a function of time.

The purpose of this thesis is to study, in a classical sense, a compound which undergoes oxidation in order to lay the groundwork for an advanced thesis which will utilize the aforementioned apparatus. That is, an oxidation-prone compound has been characterized by studying the kinetic salt effect, the buffer effect, the drug concentration effect, and the temperature effect.

II EXPERIMENTAL

II-A Materials

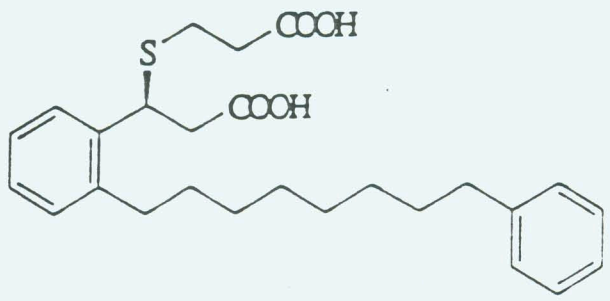
II-A-1 SKB 106203 Disodium Salt

SKB 106203 is a synthetic leukotriene antagonist proposed for the treatment of bronchial asthma (Smith-Kline Beecham, Philadelphia, PA). It has the structural formula shown in Figure II-A-1 and in part it resembles naturally-occuring leukotrienes, "highly potent bronchoconstricting substances released primarily from mast cells and basophils on antigenic challenge."[13] The studies presented herein were conducted with the disodium salt of the parent compound.

The essential molecular features are that it is a disubstituted sulfide containing two carboxylic acids. It has the following physical and chemical characteristics[14]:

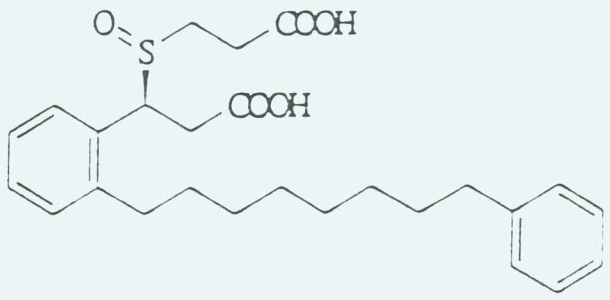
Molecular weight:	486.6 (disodium salt); 442.6 (acid)
Appearance:	white to off-white powder
pK_a Values:	5.38 and 6.70
Solubility:	poorly water-soluble (free acid)
	>350 mg/ml as the disodium salt
	5.84 mg/ml in ethanol (disodium salt)
Specific Rotation:	-12.6 (1% in water at 25°C)

Figure II-A-1 Chemical Structures of SKB106203 and Two Main Degradation Products



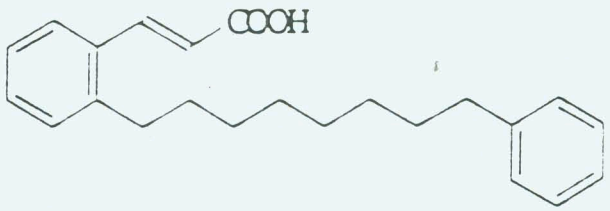
SK&F 106203

OXIDATION



SULFOXIDE ANALOGUE

ELIMINATION



CINNAMIC ACID ANALOGUE
SK&F 106736

II-A-2 Chemicals

Chemicals used were reagent grade, except acetonitrile (Baker Chemicals, Phillipsburg, NJ) which was HPLC grade. Trifluoroacetic acid was obtained from Pierce (Rockford, IL) as an ampuled solution, at a concentration of 1 g/ml of water. 0.984 N KOH solution was obtained from Aldrich (Milwaukee, WI) and concentrated H_3PO_4 from Baker. Ethylenediaminetetraacetic acid (henceforth referred to as EDTA) was obtained as the disodium dihydrate salt (MW 372.2) from Sigma (St. Louis, MO). Water was distilled, deionized (Milli^Q Water Apparatus, Millipore Corp., Milford, MA). Buffer solutions were prepared from reagent grade chemicals and filtered through a Rainin (Emoryville, CA) Nylon-66 0.45 micron membrane to remove particulates.

II-B Equipment

II-B-1 High Pressure Liquid Chromatography

The chromatographic system consisted of a Waters (Millipore Corp., Milford, MA) 484 Tunable Absorbance Detector, a Waters WISP 712 Autosampler, a Waters 501 HPLC solvent delivery system, and FIAtron (Oconomowac, WI) CH-30 column heater with TC-50 controller. Data acquisition and processing were accomplished through the Rainin Dynamax Method Manager Software on a Macintosh (Apple Computer Inc. Cupertino, CA) SE Computer.

Generally, the system was allowed to equilibrate for at least 20 minutes prior to beginning a run, and initially at least 3 standard injections preceeded the run as a check on baseline stability and system reproducibility. Standards were injected at regular intervals throughout the run and at the end of the run. Blanks consisting of pure water or similarly-stressed buffer (containing no drug) solutions were injected to confirm the absence of interferences in the sample matrix.

II-B-2 pH Meter

The pH meter used was an Orion (Cambridge, MA) Research Digital Ionalyzer/501 pH meter calibrated at two points with appropriate buffers.

II-B-3 Sample Dilutor

Samples and standards were diluted in the same proportions using an ICN Biomedicals, Inc. (Horsham, PA) Digiflex Dilutor and Pipetman and Eppendorf manual micropipets (Fisher Inc., Philadelphia, PA). Using the dilutor allowed rapid and precise dilution of samples with minimum waste of sample, glassware, and diluent. The dilutor accuracy was checked by filling volumetric ware with multiple dispensions from the same setting, e.g. by filling a 10-ml volumetric flask with water using 200 mcl increments.

Precision was monitored by making multiple dilutions of the same standard, and injecting each over the course of the HPLC run. This served as a check on the entire analytical procedure, including sampling, dilution to the analytical concentration, and sample injection by the autosampler onto the chromatographic column. Generally, the standards were not found to vary by more than 2% within a run.

Once the linearity of the procedure was confirmed by preparing several standard curves in the initial HPLC runs, subsequent runs were assayed against a single-point standard, in order to conserve solvent. At least one standard at the 50% or lower level was also prepared to check for linearity. Other levels were occasionally monitored, and were not found to deviate by more than 2% from theoretical. In all cases, at least two standards bracketed the sample concentrations.

II-B-4 Ovens and Waterbaths

Ovens used for 75 and 93°C studies were manufactured by Blue M Electric Co., Chicago, IL (Stable Therm Gravity Oven, and Model 0V12) and Hotpack (Philadelphia, PA). For 60 and 75°C studies, a thermostatically-controlled circulating water bath from Lauda (Lauda-Konigshofen, Germany) and a Haake (Berlin, Germany) immersion circulator Model E-52 were used.

II-B-5 Ampuls

For the degradation studies, samples were placed in 5-ml Wheaton (Millville, NJ) Type I Flint Glass ampuls which were then flame sealed with a Veriflow Corp. (Richmond, CA) Type 3A Blowpipe Flamesealer (oxygen-gas mixture).

II-C Software

Data analysis was aided by the use of the following software for Macintosh: CricketGraph (v. 1.3), Statworks (v. 1.2), and Student MATLAB (v. S3.5).

II-D Methods

II-D-1 Decomposition of the Disulfide

SKB 106203 reacts with oxygen to give an intermediate sulfoxide, which then breaks down to a substituted cinnamic acid, as shown in Figure II-A-1. In order to elucidate the kinetics of SKB 106203, studies were conducted to observe the effects of buffer concentration, drug concentration, ionic strength, and temperature on the drug substance.

Specifically, samples at 0.2 mg/ml, or 4.11×10^{-4} M, were prepared in pH 7 phosphate buffers (potassium salts) ranging from 0.01 to 1.0 M whose ionic strength was not adjusted in order to

observe the kinetic salt effect. These samples were stressed at 60, 75, and 93°C, and compared with samples of the same initial drug concentration prepared in buffers whose ionic strength was adjusted to 2.2 with KCl. This ionic strength was chosen because it represents the calculated ionic strength of the highest molarity buffer used in the studies (i.e., 1.0 M pH 7). By maintaining a constant ionic strength, the buffer effect could be observed.

The effects of drug concentration, ionic strength, and temperature were observed by preparing samples at four drug concentrations ranging from 0.05 to 0.5 mg/ml in both low and high ionic strength buffers, and stressing these at 75 and 93°C. Such high temperatures were chosen due to the very slow nature of the reaction at ambient temperature.

Because it was suspected, based on the chemical structure and the physical behavior of the drug substance (foaming), that the compound may form micelles[15], the critical micelle concentration was determined (by SKB at our request) by surface tension measurements. Drug concentration studies bracketing the cmc were conducted to observe, if any, a micellar effect on the rate of reaction.

Finally, in order to determine if the high levels of KCl used for ionic strength adjustment may have contained sufficient quantities of trace metals to cause degradation catalysis, studies were performed at high ionic strength at both 75 and 93°C in the presence and absence of the metal-chelating agent, EDTA (disodium salt). Low ionic strength samples containing EDTA were run as controls. Since EDTA is acidic, the initial pH of samples containing EDTA was

adjusted with KOH to bring it into the same range as the other samples on study in the same experiment. In addition to the samples, ampuls of blank buffer or test medium were stored with each set and at 4°C in order to serve as controls.

Samples for study were stored in Type I flint glass ampuls (approximately 3 ml in a 5-ml ampul), flame-sealed without precaution (i.e. the head space is air, i.e. 22% oxygen), and protected from light. Samples were drawn from time to time, and refrigerated until ready for analysis by HPLC. Unstressed samples were also kept at 4°C to serve as control.

The studies were carried out in ovens (60°C, 75°C, 93°C) and thermostatically-controlled water baths (60°C, 75°C).

II-D-2 Assay

For assay, samples at room temperature were filtered through 0.22 micron Rainin Nylon 66 filters housed in Gelman 13 mm plastic filter holders, in order to remove the insoluble cinnamic acid analog formed as a degradation product[16]. Sample solutions were assayed by HPLC[17] using a Waters (Milford, MA) Reverse Phase μBondapack™ Phenyl 125 Å 10μm column at 40°C and a flow rate of 2 mL/min. Detection was by UV at a wavelength of 215 nm. The mobile phase was 50:50 CH_3CN:H_2O with 0.1% Trifluoroacetic Acid. A sample chromatogram is shown in Figure II-D-2-a.

Prior to injection, the filtered samples were diluted appropriately with water. Failure to dilute sufficiently, especially

the high molarity and high ionic strength solutions, resulted in split peaks in the chromatography.

The samples were assayed against standards diluted with water to the same analytical concentration as the samples, generally, to a concentration of 0.00556 mg/ml. The pH of each sample was measured at room temperature.

Figure II-D-2-a

SKB106203 Kinetics: 25 days at 60°C
in 0.2 M pH 7 Phosphate Buffer μ =2.2
Initial Drug Concentration = 0.2 mg/ml

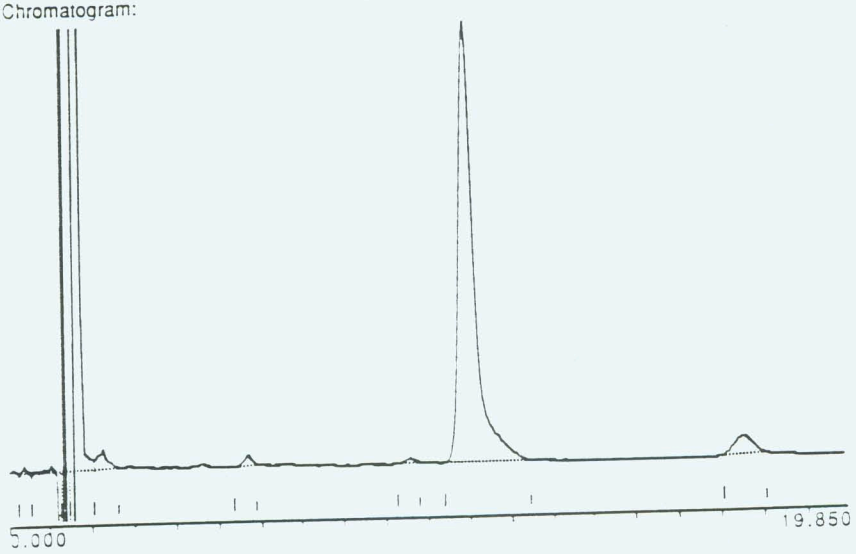
Retention Times:

Sulfoxide analog	5.6 min
SKB106203	10.9 min
Cinnamic Acid analog	17.5 min

Data: 13feb91 skb-017

Processing File:
Method: mkf skb method
Inject Vol: 100
Internal Std: 0
Sampling Int: 0.3 Seconds

Chromatogram:



Analysis: Channel A

Peak No.	Time	Type	Height(uV)	Area(uV-sec)	Area%
	0.340	N1	203	1194	0.121
1	1.005	N2	172	96871	9.896
2	1.330	N3	60522	489625	50.020
3	1.650	N4	45967	95328	9.738
	2.225	N5	478	7869	0.803
	5.650	N	244	3240	0.331
	9.545	N	117	1913	0.195
4	10.930	N	10476	268963	27.477
5	17.490	N	450	13848	1.414
Total Area				978851	99.995

II-E Summary

The present work mainly addresses the kinetics of SKB 106203 at pH 7. This pH was chosen because it was one of the pH's studied by SKB; in those studies, an increase in the buffer concentration, at constant ionic strength, was accompanied by a decrease in the observed rate constant, i.e. a buffer protective effect. The buffer concentrations studied were 0.01 and 0.05M adjusted to an ionic strength of 0.15 with NaCl; the initial drug concentration was 0.2 mg/ml. Since the SKB data contained a tremendous amount of scatter, the present study was initially undertaken in order to explore this unusual behavior suggestive of complex formation[18,19]. It was not considered initially that the compound might form micelles, however it was suspected the compound may be prone to oxidation.

It should be noted that no sodium was added to the systems in the present study other than ionization from the drug which is a disodium salt and EDTA present as the disodium salt; KOH and H_3PO_4 were used to adjust the pH of the potassium salt buffers. This is in contrast to the SKB studies which were done in sodium salt systems.

At high ionic strengths, some kinetic effects tend to be specific to the electrolyte concerned[20]. At the time these studies were commenced, it was not felt the counterion would make a significant difference, however, in the light of the results presented in this thesis, this point may require further study. Published literature results suggest oxygen solubility is a function of electrolyte identity

as well as molarity, and may be enhanced in surfactant solutions at concentrations above the cmc. [21,22,23,24] These effects may be pronounced at elevated temperatures. [25]

Table II-E summarizes the studies performed for this thesis using SKB106203; some experiments were repeated in two studies to serve as a check.

TABLE II-E Summary of Studies Performed with SKB106203

Study	Temp., °C	[Drug], mg/ml	[Buffer], M	μ
1 (Pilot)	93	0.2	0.01, 0.05, 0.1, 0.2, 0.4, 0.6, 1.0	unadjusted
2	93	0.2	0.4, 0.6, 0.8	unadjusted
			0.4, 0.6, 0.8 0.01	2.2 w/KCl
			none	drug in 2.2 M KCl
3	75, 93	0.05, 0.1, 0.2, 0.5	0.05	unadjusted
			0.6	2.2 w/KCl
4	60, 75	0.2	0.2, 0.4, 0.6, 0.8, 1.0	unadjusted
			0.2, 0.8	2.2 w/KCl
5	93	1, 2, 5, 10	none	drug in H_2O
6	93	1, 1.5, 2, 10	none; initial pH adj. w/KOH	drug in H20, +/-0.1% EDTA +/-2.2 M KCl
7	75	0.2	0.2, 0.8	unadjusted
			0.2, 0.8	2.2 w/KCl
			0.2,0.8	2.2 w/KCl, +0.1% EDTA

III RESULTS

III-A Decomposition of the Disulfide at 93°C as a Function of Ionic Strength (Kinetic Salt Effect)

The results of the three-point pilot study are listed in Table III-A; the observed rate constant, $k_{(obs)}$, is obtained as the negative of the slope of the least squares fit of the semilogarithmic plot.

III-B Decomposition of the Disulfide at 93°C as a Function of Buffer Concentration and Ionic Strength

The results are presented in Table III-B-1; $k_{(obs)}$ is obtained as the negative of the slope of the least squares fit of the semilogarithmic plot. The pH remained at 7.0 for all samples throughout the study except for the unbuffered 2.2 M KCl samples, the pH of which is shown in Table III-B-2.

III-C Degradation of the Disulfide as a Function of Drug Concentration and Ionic Strength

III-C-1 93°C, Drug Concentration Effect in Low (μ=0.11) and High (μ=2.2) Ionic Strength

These results are given in Tables III-C-1-a and III-C-1-b; $k_{(obs)}$ is obtained as the negative of the slope of the least squares fit of the semilogarithmic plot.

III-C-2 75°C, Drug Concentration Effect in Low (μ=0.11) and High (μ=2.2) Ionic Strength

These results are given in Tables III-C-2-a and III-C-2-b; where reasonable linearity was observed, $k_{(obs)}$ is reported as the negative of the slope of the least squares fit of the semilogarithmic plot. It is noted however, that there is a fair amount of scatter in these data, and that in some cases, the data are better fit to a higher order polynomial.

III-D Degradation of the Disulfide as a function of Buffer Concentration and Ionic Strength

III-D-1 75°C

These results are given in Table III-D-1; where linearity was observed, the $k_{(obs)}$ is reported as the negative of the slope of the least squares fit of the semilogarithmic plot.

III-D-2 60°C

These results are given in Table III-D-2; since these plots are not linear, first-order rate constants were not obtainable. The rate constants listed were obtained by Equation IV-8 as described in Section IV-C.

III-E Degradation of the Disulfide at 93°C as a Function of High Drug Concentration in H_2O

Degradation as a function of time is presented in Tables III-E-1; $k_{(obs)}$ is reported as the negative of the slope of the least squares fit of the semilogarithmic plot. The corresponding pH data are given in Table III-E-2.

III-F Degradation of the Disulfide at 93°C as a Function of High Drug Concentration and High Salt Concentration, in the Presence and Absence of a Metal Chelating Agent, and in the Absence of Phosphate

These results are presented in Tables III-F-1; $k_{(obs)}$ is reported as the negative of the slope of the least squares fit, where the semilogarithmic plots were observed to be reasonably linear. It is noted that in some cases, the $k_{(obs)}$ has been reported with the omission of the last time-point. In these instances, the data are

better fit to a higher order-polynomial, however the first-order rate constant is reported for the purpose of making rough comparisons.

The corresponding pH data are reported in Table III-F-2.

III-G Degradation of the Disulfide at 75°C at pH 7 in the Presence and Absence of a Metal Chelating Agent

These results are presented in Table III-G. The data contain a fair amount of scatter, and in some instances an initial lag time and curvature appear to be present as in other studies done at 75°C. The $k_{(obs)}$ obtained from first-order plots are shown for comparison only.

Table III-A SKB106203 Kinetics, Study 1 (Pilot), 93°C:

Kinetic Salt Effect in pH 7 Buffers with no Ionic Strength Adjustment

Initial Drug Concentration = 0.2 mg/ml (4.11×10^{-4} M)

			% Remaining				
M	μ*	pH	7 Days	15 Days	$k_{(obs)}$**	y-int.	R^2
0.01	0.02	7.1	55.4	26.8	0.088	4.6137	1
0.05	0.11	6.9	48.9	23.5	0.096	4.5911	0.999
0.1	0.22	6.8	48.1	20.2	0.107	4.6103	1
0.2	0.45	7.0	42.4	18.7	0.112	4.5783	0.998
0.4	0.89	7.2	30.0	10.0	0.153	4.5592	0.998
0.6	1.3	7.0	40.2	2.7	0.243	4.8801	0.971
1.0	2.2	7.0	14.3	n.d.	0.278	4.6052	1

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n.d. not detected

* calculated

** Zero days is taken as 100% Remaining

Table III-B-1 SKB106203 Kinetics, Study 2, 93°C:
Kinetic Salt Effect in pH 7 Buffers Unadjusted for Ionic Strength, or Adjusted to the Ionic Strength of a 1.0 M pH 7 Phosphate Buffer
Initial Drug Concentration = 0.2 mg/ml

	% Remaining							
days	0.4M	0.4M	0.6M	0.6M	0.8M	0.8M	2.2M*	0.01M
0	100	100	100	100	100	100	100	100
1	88.2	89.6	89.0	86.0	86.9	88.9	87.6	80.2
3	51.2	46.8	22.0	50.5	39.2	44.2	47.4	43.1
6	29.1	30.5	28.0	32.5	17.1	27.1	7.2	21.3
9	21.7	17.2	14.5	20.5	6.5	16.6	n.d.	n.d.
13	10.3	n.d.	6.0	5.0	3.0	7.0	n.d.	n.d.
μ	0.9	2.2	1.35	2.2	1.80	2.2	2.2	2.2
$k_{(obs)}$/day	0.174	0.198	0.220	0.178	0.307	0.205	n.l.	0.285
R^2	0.987	0.984	0.998	0.998	0.990	0.978	n.l.	0.996

n.d. not detected, or below the limit of detection
n.l. not linear
*Control, drug in 2.2 M KCl with no Buffer

Table III-B-2 SKB106203 Kinetics, Study 2:
pH of Drug Solution in 2.2 M KCl

days	pH
0	7.9
1	7.5
3	7.4
6	7.0
9	6.9
15	6.6

Table III-C-1-a SKB106203 Kinetics, Study 3, 93°C:
Drug Concentration Study In 0.05 M pH 7 Buffer
with no Ionic Strength Adjustment ($\mu_{calculated}$ = 0.11)
Initial Drug Concentration as Indicated

	% Remaining					
days	0.05 mg/ml	0.1 mg/ml	0.2 mg/ml	0.5 mg/ml	mean	S D
0	100	100	100	100	100	0
1	94.8	94.0	85.7	88.0	90.6	3.9
2	76.9	77.6	75.6	79.7	77.4	1.5
4	82.6	69.7	62.7	65.3	70.1	7.6
7	58.4	55.4	56.8	61.7	58.1	19.9
9	53.7	47.3	47.6	54.7	50.8	3.4
11	43.2	39.6	44.7	45.6	43.3	2.3
16	n.a.	27.8	30.2	32.2	30.1	1.8
18	27.4	25.9	26.8	29.5	27.4	1.3
$k_{(obs)}$/day	0.072	0.076	0.068	0.065		
R^2	0.980	0.992	0.985	0.986		

n.a. not analyzed

Table III-C-1-b SKB106203 Kinetics, Study 3, 93°C;
Drug Concentration Study In 0.6 M pH 7 Buffer
with Ionic Strength Adjusted to 2.2 with KCl
Initial Drug Concentration as Indicated

	% Remaining					
day	0.05 mg/ml	0.1 mg/ml	0.2 mg/ml	0.5 mg/ml	mean	S D
0	100	100	100	100	100	0
1	88.1	90.8	89.4	74.5	85.7	7.5
2	n.a.	(29.3)	57.6	68.0	67.3	9.3
4	15.4	31.1	42.5	53.8	35.5	3.6
7	7.3	13.8	27.1	15.4	15.9	8.2
9	6.2	4.2	18.6	8.3	9.3	6.4
11	n.a.	n.a.	12.8	11.7	12.2	0.6
$k_{(obs)}$/day	0.338	0.344	0.198	0.231		
R^2	0.940	0.977	0.990	0.921		

n.a. not analyzed

Table III-C-2-a SKB106203 Kinetics, Study 3, 75°C:
Drug Concentration Study in 0.05 M pH 7 Buffer with
No Ionic Strength Adjustment ($\mu_{calculated}$ = 0.11)
Initial Drug Concentration as Indicated

	% Remaining					
days	0.05 mg/ml	0.1 mg/ml	0.2 mg/ml	0.5 mg/ml	mean	S D
0	100	100	100	100	100	0
9	97.6	91.8	89.4	91.5	92.6	3.0
19	92.9	80.9	84.9	87.3	86.5	4.3
30	74.8	80.3	80.9	83.7	79.9	3.2
45	73.4	76.7	74.6	81.8	76.6	3.2
69	67.2	34.2	72.4	75.0	62.2	16.4
128	57.6	5.8	59.9	61.5	46.2	23.4
$k_{(obs)}$/day	0.0044	n.l.	n.l.	0.0036		
R^2	0.879	n.l.	n.l.	0.936		

n.l. not linear

Table III-C-2-b SKB106203 Kinetics, Study 3, 75°C:
Drug Concentration Study in 0.6 M pH 7 Buffer
with Ionic Strength Adjusted to 2.2 with KCl
Initial Drug Concentration as Indicated

	% Remaining					
days	0.05 mg/ml	0.1 mg/ml	0.2 mg/ml	0.5 mg/ml	mean	SD
0	100	100	100	100	100	0
9	91.8	95.3	91.8	90.4	92.3	1.8
19	84.5	42.6	82.3	81.7	72.8	17.4
30	79.3	38.6	75.2	66.0	64.8	15.9
45	72.6	34.3	42.7	58.7	52.1	14.7
69	10.9	13.8	33.5	49.1	26.8	15.5
$k_{(obs)}$/day	n.l.	n.l.	0.017	0.011		
R^2	n.l	n.l.	0.943	0.974		

n.l. not linear

Table III-D-1 SKB106203 Kinetics, Study 4, 75°C:
Kinetic Salt Effect in pH 7 Buffers of Indicated Molarity with No Ionic Strength Adjustment, Except 0.2 and 0.8 M Buffers Whose Ionic Strength was Adjusted to 2.2 with KCl
Initial Drug Concentration = 0.2 mg/ml

	% Remaining						
days	0.2M	0.2M *	0.4M	0.6M	0.8M	0.8 M*	1.0M
0	100	100	100	100	100	100	100
2	n.a.	98.5	97.7	98.9	97.5	96.1	96.9
4	98.6	92.6	97.4	96.4	96.0	93.4	94.1
19	76.4	74.8	65.8	58.4	48.9	82.0	39.9
25	76.2	50.2	63.1	33.6	44.2	54.9	32.6
30	71.0	27.2	60.3	50.6	42.0	24.9	(6.1)
45	64.1	n.d.	52.4	41.0	25.6	s.m.	15.3
69	55.7	n.a.	44.6	30.6	14.4	31.6	3.5
μ	0.45	2.2	0.89	1.3	1.8	2.2	2.2
$k_{(obs)}$	0.0087	n.l.	0.012	0.018	0.029	n.l.	0.048
R^2	0.947	n.l.	0.917	0.958	0.986	n.l.	0.992

n.l. not linear
n.a. not analyzed
n.d. not detected
s.m. sample mishandled

*When the data from Studies 4 and 7 obtained in 0.2 and 0.8 M buffer (μ=2.2) are treated by Equation IV-8 (Section IV-C), the k(obs) = 0.140/day, R^2 = 0.913

Table III-D-2 SKB106203 Kinetics, Study 4, 60°C: Kinetic Salt Effect in pH 7 Buffers of Indicated Molarity with No Ionic Strength Adjustment, Except 0.2 and 0.8 M Buffers Whose Ionic Strength was Adjusted to 2.2 with KCl
Initial Drug Concentration = 0.2 mg/ml

% Remaining

days	0.2 M	0.2M*	0.4M	0.6M	0.8M	0.8M*	1.0M
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
2	n.a.	100.0	n.a.	n.a.	n.a	n.a.	n.a.
4	n.a.	98.6	97.2	100.1	99.1	99.5	98.5
6	100.3	97.4	n.a.	n.a.	n.a.	98.9	n.a.
25	82.7	83.8	95.0	95.6	90.4	92.1	88.1
45	81.1	75.2	92.4	91.5	83.1	60.5	76.0
69	76.2	47.6	68.2	57.7	46.6	57.2	35.5
91	63.7	28.6	52.1	42.6	25.8	37.5	11.8
110	54.1	i.s.	39.0	31.1	15.5	i.s.	4.8
147	37.9	i.s.	24.3	16.7	3.8	i.s.	0.4
μ	0.45	2.2	0.89	1.3	1.8	2.2	2.2
$k_{(obs)}$[a]	n.l.	0.012	n.l.	n.l.	n.l.	0.012	n.l.
R^2[a]	--	0.930	--	--	--	0.930	--
$k_{(obs)}$[b]	0.018	0.062	0.036	0.040	0.053	0.062	0.065
R^2[b]	0.973	0.896	0.962	0.929	0.963	0.896	0.994

[a] first-order
[b] by Equation IV-8
n.l. not linear
n.a. not analyzed
i.s. insufficient sample

*Although a $k_{(obs)}$ obtained by Equation IV-8 is reported, the data treated in this manner show an initial sharp decrease followed by a linear portion; the semi ln-transformed data are better fit to a second degree polynomial.

Table III-E-1 SKB106203 Kinetics, Study 5, 93°C

Drug Concentration Effect, Drug in H_2O

	% Remaining					
	mg/ml					
days	1	2	5	10	mean	SD
0	100	100	100	100	100	0
1	89.6	94.1	94.9	95.6	93.6	2.3
2	84.9	90.8	90.3	93.3	89.8	3.1
3	82.4	88.0	89.2	91.8	87.8	3.4
6	n.a.	81.5	83.1	85.8	83.5	1.8
7.25	69.1	80.6	80.0	84.0	78.4	5.6
8.1	67.3	i.s.	80.1	82.1	76.5	6.6
$k_{(obs)}$	0.045	0.028	0.026	0.023		
R^2	0.969	0.957	0.963	0.988		

n.a. not analyzed
i.s. insufficient sample

Table III-E-2 SKB106203 Kinetics, Study 5, 93°C:
Drug in H_2O, pH Data

Days	1 mg/ml	2 mg/ml	5 mg/ml	10 mg/ml
0	7.7	7.7	7.5	7.8
1	7.6	7.6	7.7	8.4
2	7.4	7.6	8.6	8.4
3	7.6	8.7	8.3	9.0
6	---	7.9	8.1	9.1
7.25	7.6	8.4	9.1	8.8
8.1	8.6	---	9.1	8.3

Table III-F-1 SKB106203 Kinetics, Study 6, 93°C: Drug Concentration Effect, Effect of Trace Metals, Kinetic Salt Effect; in the Absence of Phosphate

	% Remaining			
	A*	B	C	D
1.0 mg/ml				
Initial	100	100	100	100
1 d	100.9	91.1	97.1	94.5
4 d	95.1	88.4	95.6	94.5
7.1 d	90.7	79.8	92.5	93.3
10 d	88.6	78.7	85.2	94.0
16 d	84.3	77.5	80.0	91.8
32 d	80.0	59.1	51.2	82.2
86 d	54.0	(10.1)	(49.8)	55.6
$k_{(obs)}$/day	0.0066	0.026	0.020	0.0065
R^2	0.987	0.970	0.965	0.984
1.5 mg/ml				
Initial	100	100	100	100
1 d	95.1	95.6	100.8	94.9
4 d	91.9	87.5	92.5	91.9
7.1 d	88.4	81.1	84.7	91.2
10 d	89.1	75.7	n.a.	89.8
16 d	87.3	71.4	71.8	88.9
32 d	79.8	58.0	62.1	81.1
86 d	54.9	17.4	n.d.	54.6
$k_{(obs)}$/day	0.0067	0.020	0.016	0.0066
R^2	0.999	0.996	0.951	0.985

A = Drug in H_2O (*only last three points used for regression due to initial nonlinearity associated with initial sharp increase in pH)

B = Drug in 2.2M KCl C = Drug in 2.2 M KCl + 0.1% EDTA

D = Drug in 0.1% EDTA (control)

n.a. not analyzed n.d. not detected

Data in parenthesis not used in the regression due to curvature

Table III-F-1 (continued)

SKB106203 Kinetics, Study 6, 93°C:
Drug Concentration Effect, Effect of Trace Metals,
Kinetic Salt Effect; in the Absence of Phosphate

		% Remaining			
		A*	B	C	D
2 mg/ml	Initial	100	100	100	100
	1 d	90.8	99.7	102.0	98.5
	4 d	83.5	89.8	86.3	97.1
	7.1 d	80.2	81.9	79.2	94.4
	10 d	80.1	80.0	78.0	94.4
	16 d	77.3	73.3	67.0	90.7
	32 d	71.6	57.9	31.9	83.7
	86 d	48.1	n.d.	n.d.	(49.9)
$k_{(obs)}$/day		0.0069	0.017	0.035	0.0054
R^2		0.995	0.970	0.972	0.989
10 mg/ml	Initial	100	100	100	100
	1	96.9	102.2	n.a.	99.6
	4	94.5	92.5	97.9	97.0
	7.1	94.0	86.5	93.5	95.8
	10 d	93.7	79.1	91.8	95.6
	16 d	s.m.	66.5	87.8	92.4
	32 d	76.9	36.5	76.4	88.1
	86 d	51.7	n.d.	(31.9)	(58.6)
$k_{(obs)}$/day		0.0074	0.032	0.0085	0.0039
R^2		1.0	0.989	0.995	0.971

A = Drug in H_2O (*only last two (10.0 mg/ml) or three (2.0 mg/ml) points used for regression due to initial nonlinearity associated with initial sharp increase in pH

B = Drug in 2.2M KCl C = Drug in 2.2 M KCl + 0.1% EDTA

D = Drug in 0.1% EDTA (control)

n.a. not analyzed n.d. not detected s.m. sample mishandled

Data in parenthesis not used in the regression due to curvature

Table III-F-2 SKB106203 Kinetics, Study 6, 93°C: pH Data

		A	B	C	D
1 mg/ml					
	Initial	7.7	7.2	7.3	7.1
	1 d	8.7	8.2	7.6	7.5
	4 d	8.4	8.3	7.6	7.9
	7.1 d	7.9	--	7.7	7.8
	10 d	8.5	8.2	7.7	8.3
	16 d	8.5	7.8	7.5	8.0
	32 d	8.8	8.1	7.7	8.3
1.5 mg/ml					
	Initial	7.7	7.4	7.4	7.1
	1 d	8.6	8.0	7.7	7.5
	4 d	8.5	8.2	7.7	7.8
	7.1 d	8.2	7.8	7.6	7.6
	10 d	8.5	8.1	7.7	8.2
	16 d	8.4	7.9	7.6	8.0
	32 d	8.9	8.2	7.7	8.3
2 mg/ml					
	Initial	7.6	7.9	6.8	7.0
	1 d	8.6	8.1	7.0	7.5
	4 d	8.7	8.1	7.0	7.7
	7.1 d	7.9	7.9	7.0	7.8
	10 d	8.7	7.9	7.1	8.1
	16 d	8.5	8.0	7.0	7.8
	32 d	8.9	8.3	7.1	8.4
10 mg/ml					
	Initial	8.0	8.3	7.6	7.5
	1 d	8.1	8.1	7.6	7.5
	4 d	8.8	8.0	7.6	7.6
	7.1 d	8.6	7.8	7.6	7.5
	10 d	8.8	7.9	7.6	7.6
	16 d	--	8.0	7.6	7.6
	32 d	8.9	8.2	7.8	7.8

A = Drug in H20 C = Drug in 2.2 M KCl + 0.1% EDTA
D = Drug in 0.1% EDTA (control) B = Drug in 2.2 M KCl

Table III-G SKB106203 Kinetics, Study 7, 75°C:
Trace Metal Catalysis by Phosphate, and Kinetic Salt Effect In 0.2 M and 0.8 M pH 7 Phosphate Buffers

0.2 M

	A	B	C	D
Initial	100	100	100	100
5 d	77.0	87.7	90.9	77.5
11 d	90.2	82.0	89.2	84.2
25 d	65.5	45.4	56.9	57.7
81 d	n.d.	21.3	34.7	33.2
$k_{(obs)}$/day	0.014	0.019	0.013	0.013
R^2	0.678	0.954	0.941	0.945

0.8 M

Initial	100	100	100	100
5 d	94.1	93.6	92.4	94.1
11 d	90.8	92.1	93.2	95.2
25 d	48.9	53.2	61.1	60.2
81 d	6.3	34.1	43.6	29.2
$k_{(obs)}$/day	0.035	0.014	0.010	0.016
R^2	0.992	0.906	0.905	0.973

A = drug in pH 7 buffer, no ionic strength adjustment

B = drug in pH 7 buffer, μ = 2.2 with KCl

C = drug in pH 7 buffer, μ = 2.2 with KCl, + 0.1% EDTA (di Na salt)

D = drug in pH 7 buffer, + 0.1 % EDTA

IV DISCUSSION

IV-A Buffer/Kinetic Salt Effect

In order to explore the buffer and kinetic salt effects, samples were prepared in various molarities of pH 7 phosphate buffers ranging from 0.1 to 1.0 M, with and without ionic strength adjustment with KCl. Degradation of the drug substance was monitored in these buffers at 60, 75, and 93°C.

At all three temperatures, there was no buffer catalysis by phosphate, however, there was a kinetic salt effect. That is, as buffer molarity, hence ionic strength, was increased, an increase in the $k_{(obs)}$ was noticed, as may be seen in Figures IV-A-1, -2, and -3. This is also shown in Figure IV-A-4 where the data at 75 and 93°C are seen to obey the equation[26]

$$\log k_{(obs)} = \log k^{\circ} + \log(b_a + b_b - b_t)\mu \qquad \text{(IV-A-1)}$$

where a, b_a, b_b, and b_t are constants, μ is ionic strength, and $k_{(obs)}$ is obtained from the negative of the slope of the semilogarithmic plots of concentration vs time.

The 60°C data are not first-order, but may be linearized by Equation IV-8 (Section IV-B). Thus, a $k_{(obs)}$ may be obtained from the negative of the slope of the semilogarithmic plot of the ratio of % remaining to % degraded vs time; rate constants so obtained are linear in μ, as may be seen in Figure IV-A-5.

The absence of buffer catalysis by phosphate at 60, 75, and 90°C is illustrated in Figures IV-A-6, -7, and -8, which show the kinetic curves in buffers of different molarities in which ionic strength has been adjusted to 2.2 with KCl[27] to be superimposable at a given temperature.

That the same conclusion may be drawn regarding buffer catalysis and kinetic salt effect at three different temperatures is not remarkable in itself, but what is striking is the profile of the semilogarithmic plots. At 93°C, the semilogarithmic plots are linear (first-order) through several halflives, whether or not the ionic strength has been adjusted. When the studies were repeated at 60°C, all the semilogarithmic plots show curvature, indicative of oxidation. However, at 75°C, those reactions run in buffers with no ionic strength adjustment exhibited linear first-order plots, while those run in buffers also containing KCl show downward curvature in a semilogarithmic mode.[28] These findings are summarized in Table IV-A-1.

The fact that the plots are linear at 75°C but not at 60°C in the absence of KCl may simply be a consequence of the increased solubility of oxygen at a lower temperature.

However, the fact that curvature is noticed in the presence of KCl at both 60°C and 75°C, but not at 75°C in the absence of KCl, suggests that KCl may be involved in the reaction directly or indirectly, and that perhaps some critical oxygen concentration or set of conditions is required to change the degradation reaction from a simple first-order mechanism to an autocatalytic oxidation.

One way KCl may increase the reaction rate is by suppling trace metals as shown in Section IV-E, although it is not clear that this alone would result in curvature of the semilogarithmic plots since phosphate is also shown to contribute trace metals.

Adding electrolyte to an aqueous solution is known to have an effect on oxygen concentration as predicted by the Setchenow equation (Appendix F), and to lower the cmc of a surfactant.[29] In turn, solutions of surfactant above the cmc have been shown to increase the oxygen concentration in solution by an order of magnitude (Appendix G). These effects combined with trace metal catalysis may be the reason for the observed curvature at 75°C in the presence of KCl.

If one assumes KCl to behave similarly to NaCl, any concentration of KCl greater than 0.1 M could reduce the cmc of SKB 106203 to less than 0.5 mg/ml, as may be seen in Appendix G. In these studies, the initial drug concentration was 0.2 mg/ml, so that it is possible some of the solutions are just above the cmc.

In micellar form, the drug may be able to solubilize additional oxygen which would most likely be associated with the interior of the micelle and unavailable for reaction until the compound degrades to below the cmc. The bulk oxygen not associated with the interior of the micelle would obey Henry's Law or the Setchenow equation or some variation of them depending on the nature of the bulk phase. As degradation proceeds to below the cmc, a change in mechanism may be observed as curvature in the first-order plot if

the micelle is involved in the reaction. Micellar catalysis has been explained as a type of Michaelis-Menten kinetics.[30]

Even without micellization, the oxygen concentration may be affected by KCl. Although the general trend is for oxygen to be salted-out by salts between 0 and 25°C, what literature exists suggests that some salts, such as LiCl, may salt-in oxygen between 60 and 90°C.[31]

Table IV-A-1 SKB106203 Kinetics: Nature of First-Order Plots in pH 7 Phosphate Buffers With and Without Ionic Strength Adjustment

Temperature	With KCl	Without KCl
60°C	Curvature	Curvature
75°C	Curvature	Linear
93°C	Linear	Linear

Figure IV-A-1 SKB106203 Kinetics: 93°C
Kinetic Salt Effect in pH 7 Buffers with No Ionic Strength Adjustment
Initial Drug Concentration = 0.2 mg/ml
Data from Tables III-A and III-B-1

0.4M: y = 4.54 - 0.162x R^2 = 0.982
0.6M: y = 4.56 - 0.219x R^2 = 0.905
0.8M: y = 4.59 = 0.282x R^2 = 0.990

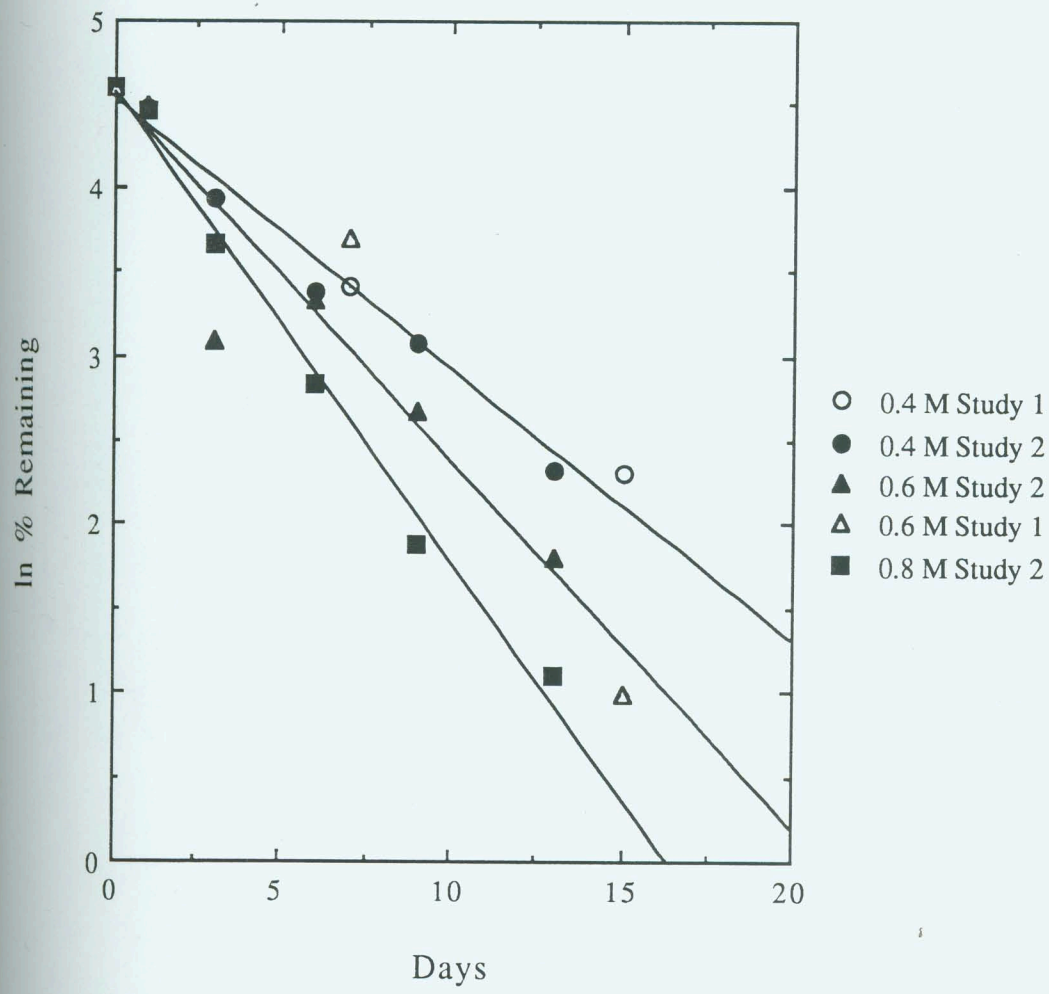


Figure IV-A-2 SKB106203 Kinetics: Studies 4 and 7, 75°C
Kinetic Salt Effect in pH 7 Buffers with No Ionic Strength Adjustment
Initial Drug Concentration = 0.2 mg/ml
Data from Tables III-D-1 and III-G

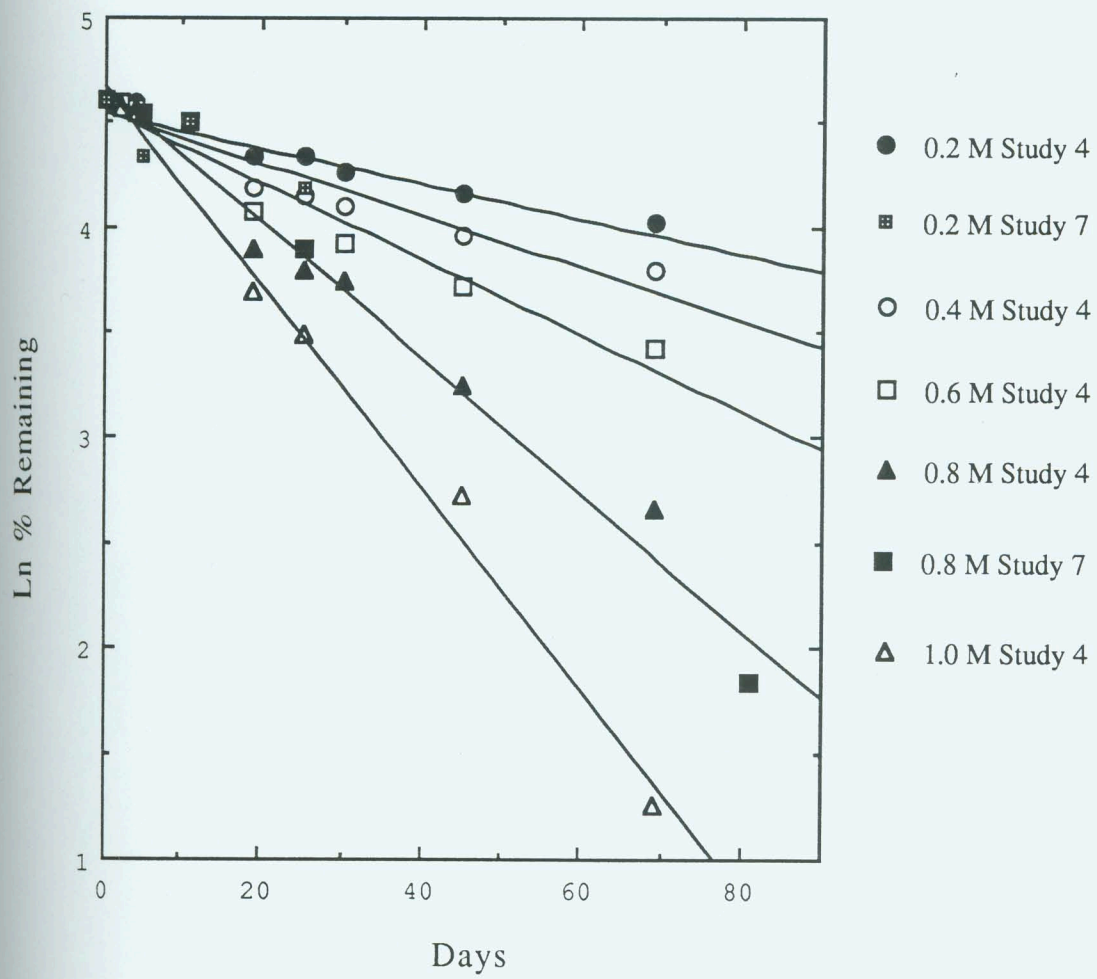


Figure IV-A-3 SKB106203 Kinetics: Study 4, 60°C
Kinetic Salt Effect in pH 7 Buffers with No Ionic Strength Adjustment
Initial Drug Concentration = 0.2 mg/ml
Data from Table III-D-2

0.2M: y = 4.58 - 2.52e-3x - 2.62e-5x^2 R^2 = 0.983
0.4M: y = 4.62 - 1.94e-3x - 5.52 e-5x^2 R^2 = 0.988
0.6M: y = 4.65 - 3.52e-3x - 6.33e-5x^2 R^2 = 0.987
0.8M: y = 4.62 - 7.01e-4x - 1.49e-4x^2 R^2 = 0.997
1.0M: y = 4.61 + 3.57e-3x - 2.82e-4x^2 R^2 = 0.998

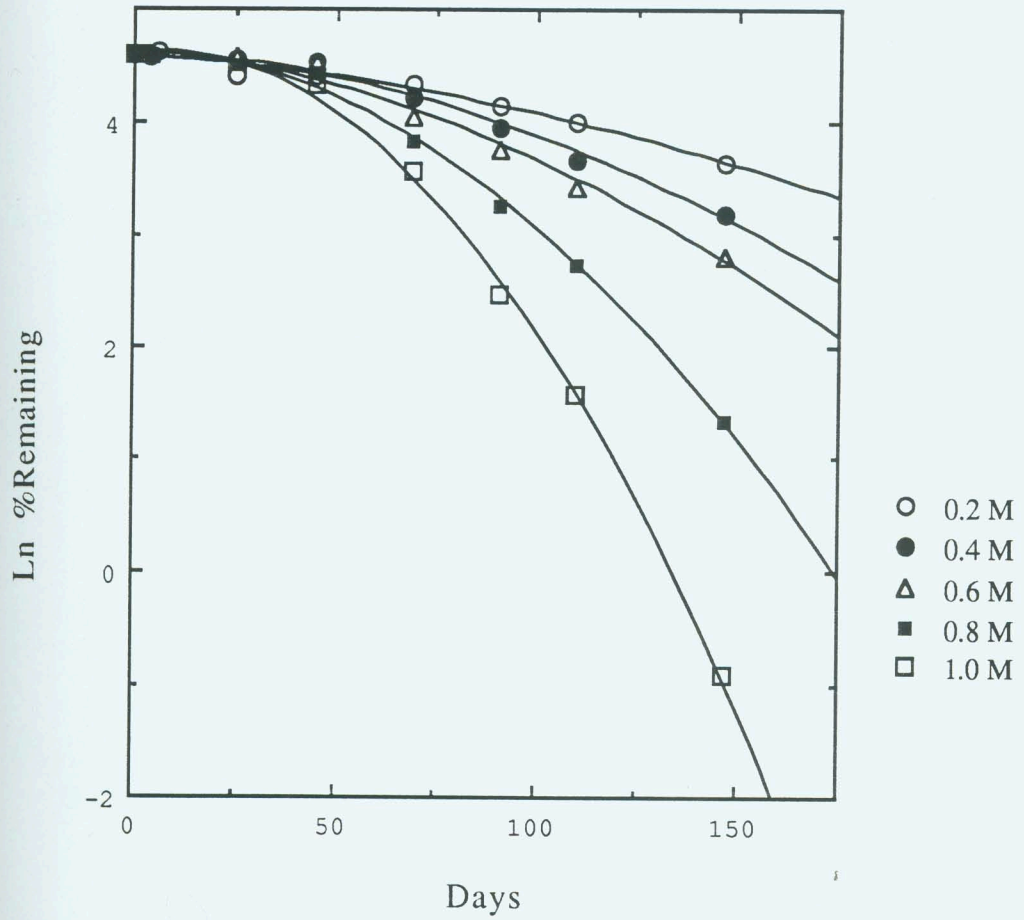


Figure IV-A-4 SKB106203 Kinetics: Kinetic Salt Effect
In pH 7 Phosphate Buffers with No Ionic Strength Adjustment (No KCl)
k(obs) from First-Order Plots
Data from Tables III-A, III-B-1, III-D-1, III-G

93°C: y = -1.04 + 0.249x R^2 = 0.964
75°C: y = -2.37 + 0.478x R^2 = 0.974

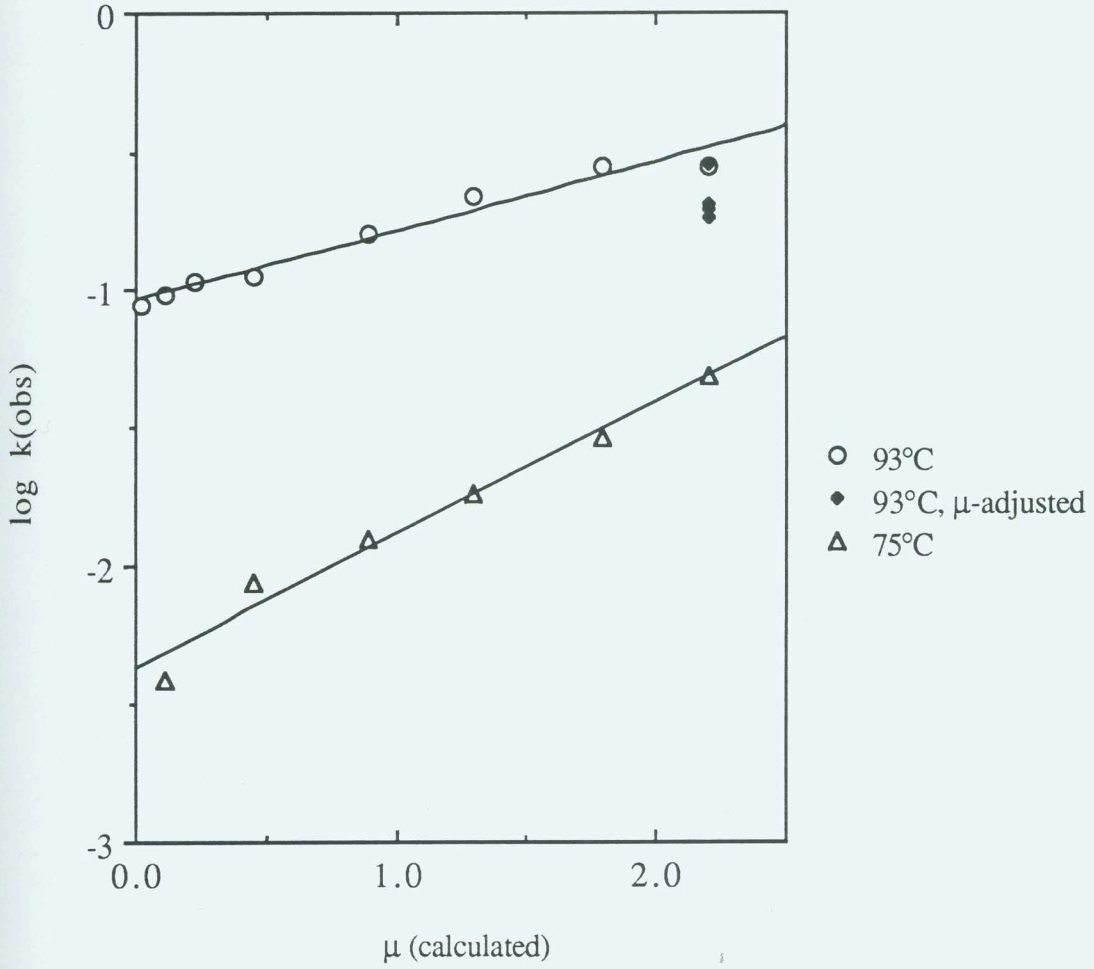


Figure IV-A-5 SKB106203 Kinetics: Study 4, 60°C
Kinetic Salt Effect
Rate Constants Obtained by Equation IV-8
Data from Table III-D-2

y = 8.68e-3 + 2.51e-2x R^2 = 0.975

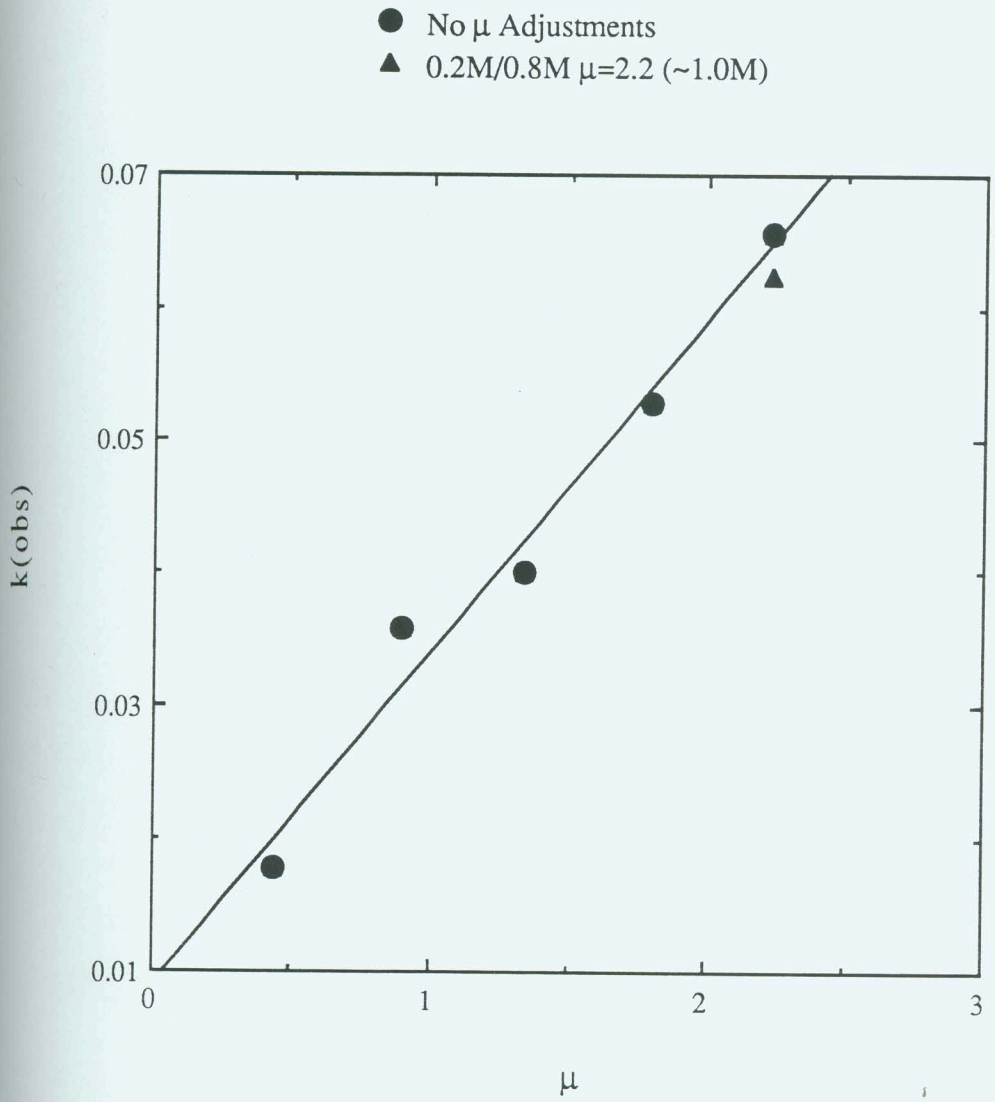


Figure IV-A-6 SKB106203 Kinetics: 93°C
Buffer Effect (Phosphate) at pH 7
Ionic Strength Adjusted to 2.2 with KCl
Initial Drug Concentration = 0.2 mg/ml
(0.01 M Data Not Used For Regression)
Data from Tables III-B-1 and III-C-1-a;
Appendix B-1

$y = 4.59 - 0.195x$ $R^2 = 0.972$

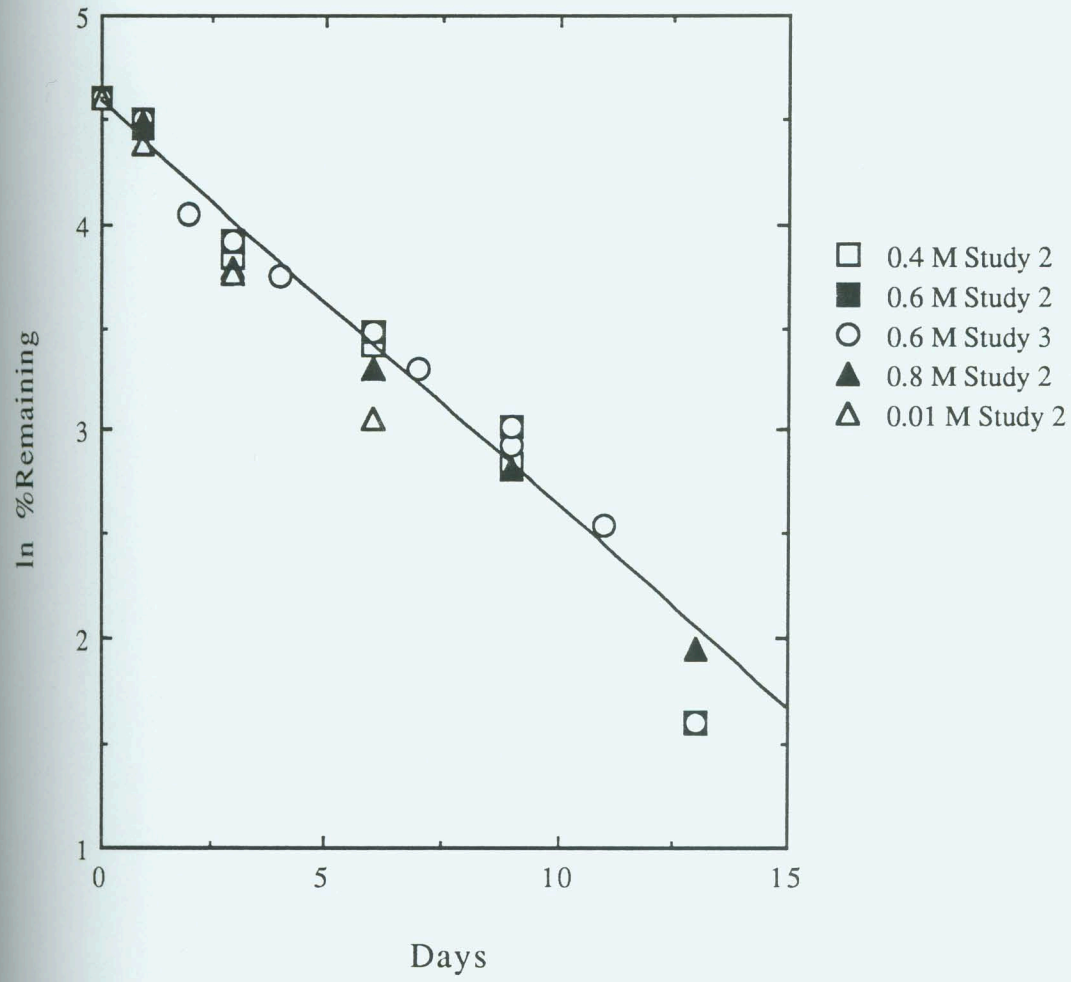


Figure IV-A-7 SKB106203 Kinetics: 75°C, Studies 4 and 7
Buffer Effect (Phosphate) at pH 7
Ionic Strength Adjusted to 2.2 with KCl
Initial Drug Concentration = 0.2 mg/ml
Data from Tables III-D-1 and III-G

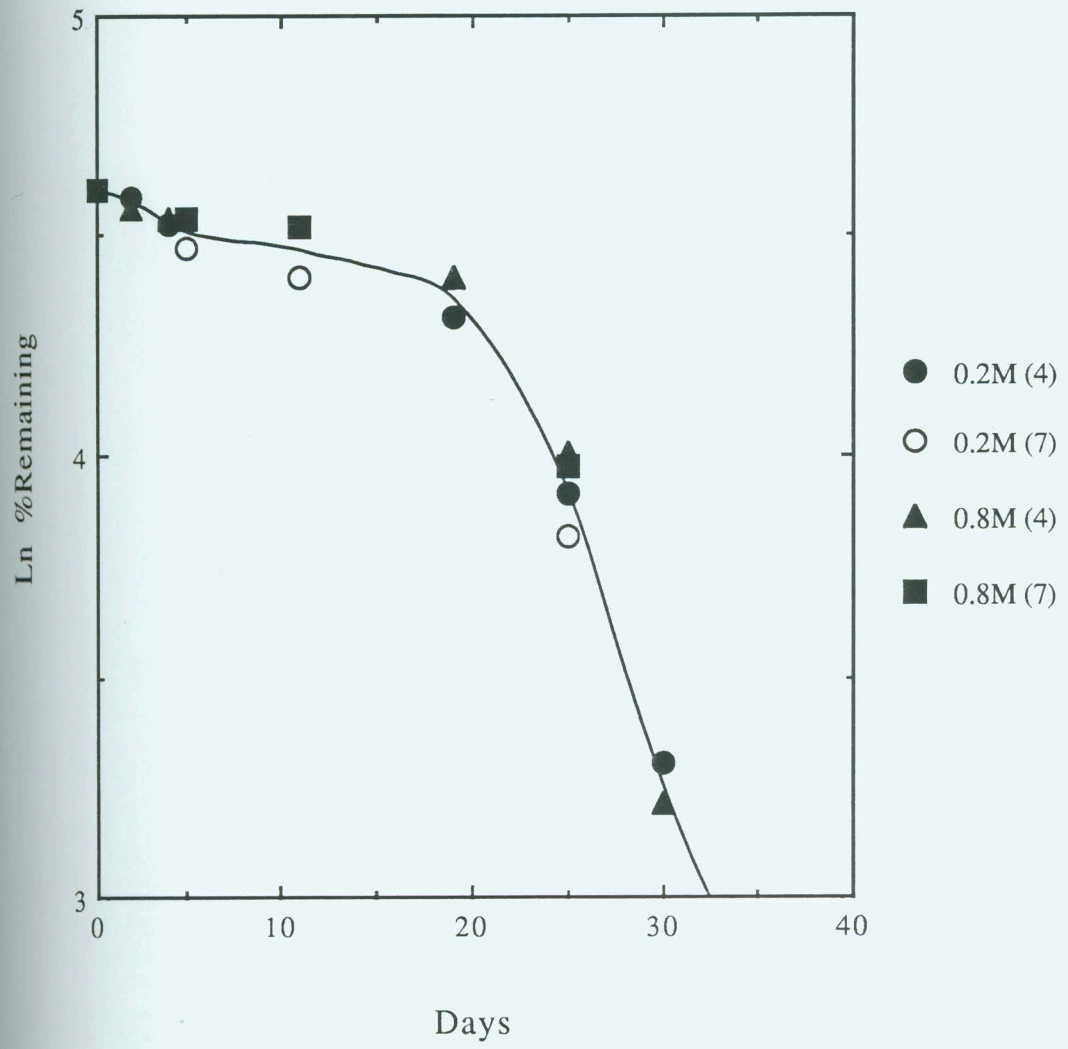
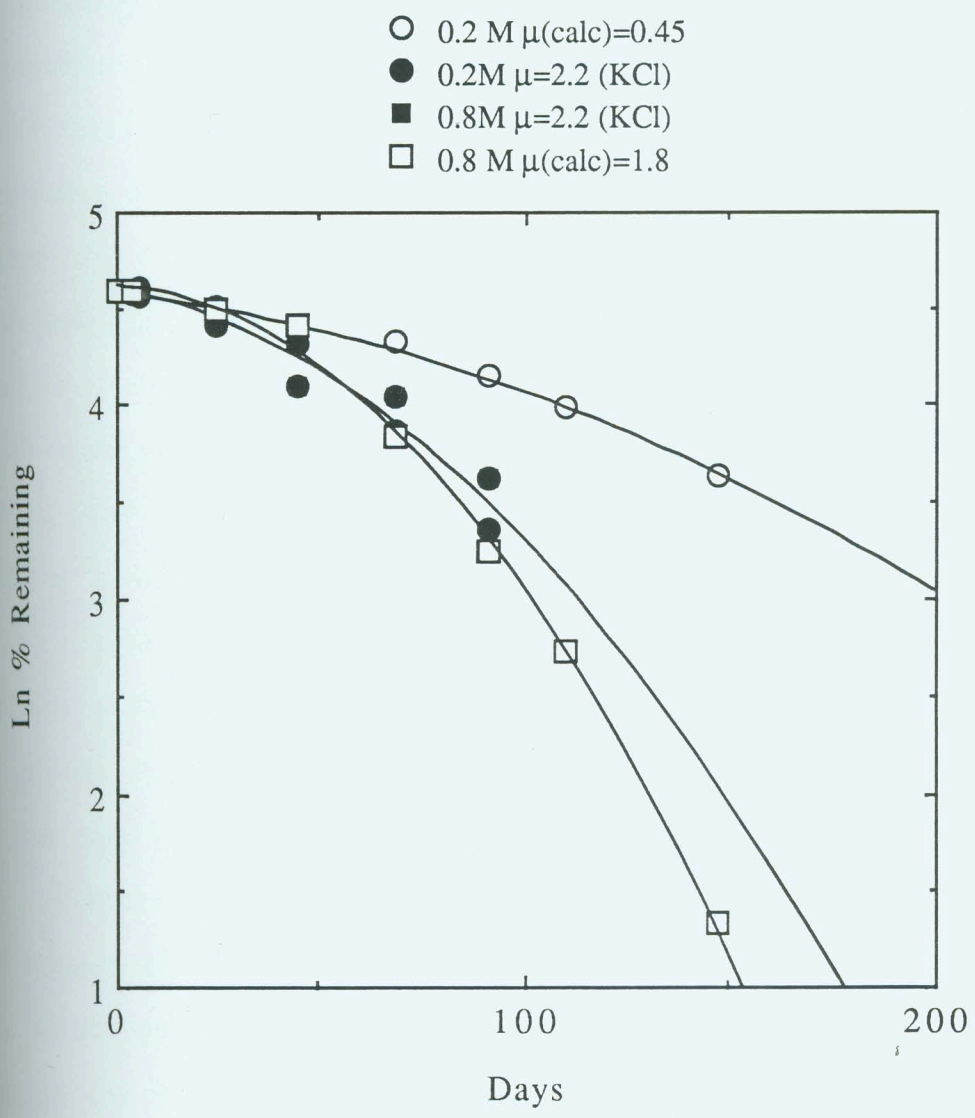


Figure IV-A-8 SKB106203 Kinetics: Study 4, 60°C
Buffer/Kinetic Salt Effect in pH 7 Phosphate Buffers
With and Without Ionic Strength Adjustment with KCl
Initial Drug Concentration = 0.2 mg/ml
Data from Table III-D-2

0.2M (μ=0.45):	y = 4.58 - 2.53e-3x - 2.62e-5x^2	R^2 = 0.983
0.2M/0.8M (μ=2.2):	y = 4.61 - 3.64e-3x -9.35e-5x^2	R^2 = 0.963
0.8M (μ=1.8):	y = 4.62 - 7.01e-4x - 1.49e-4x^2	R^2 = 0.997



IV-B Protective Effect by KCl

It is noted that at both 60 and 75°C, there is no difference in rates when the reaction is run in 0.2 or 0.8 M buffers in which the ionic strength has been adjusted to 2.2 (e.g. Figure IV-A-7). But these data are not superimposable on those generated in 1.0 M buffer having the same intrinsic ionic strength (i.e. 2.2) but not containing KCl (Figure IV-B-1). The 1.0 M samples degrade at a faster rate. Although all three buffers have the same ionic strength, somehow the presence of added electrolyte in the 0.2 and 0.8 M buffers slowed the reaction somewhat relative to the 1.0 M buffer. This observation is consistent with observations in Appendix I which show the reaction rate of a micellar-catalyzed reaction in general to be decreased by the presence of electrolyte.

A possible explanation for this behavior is that KCl may provide a shielding layer of positive charge to surround the negatively-charged micelle; this layer would make the oxidizable sulfide moiety less accessible to oxygen through steric hindrance.

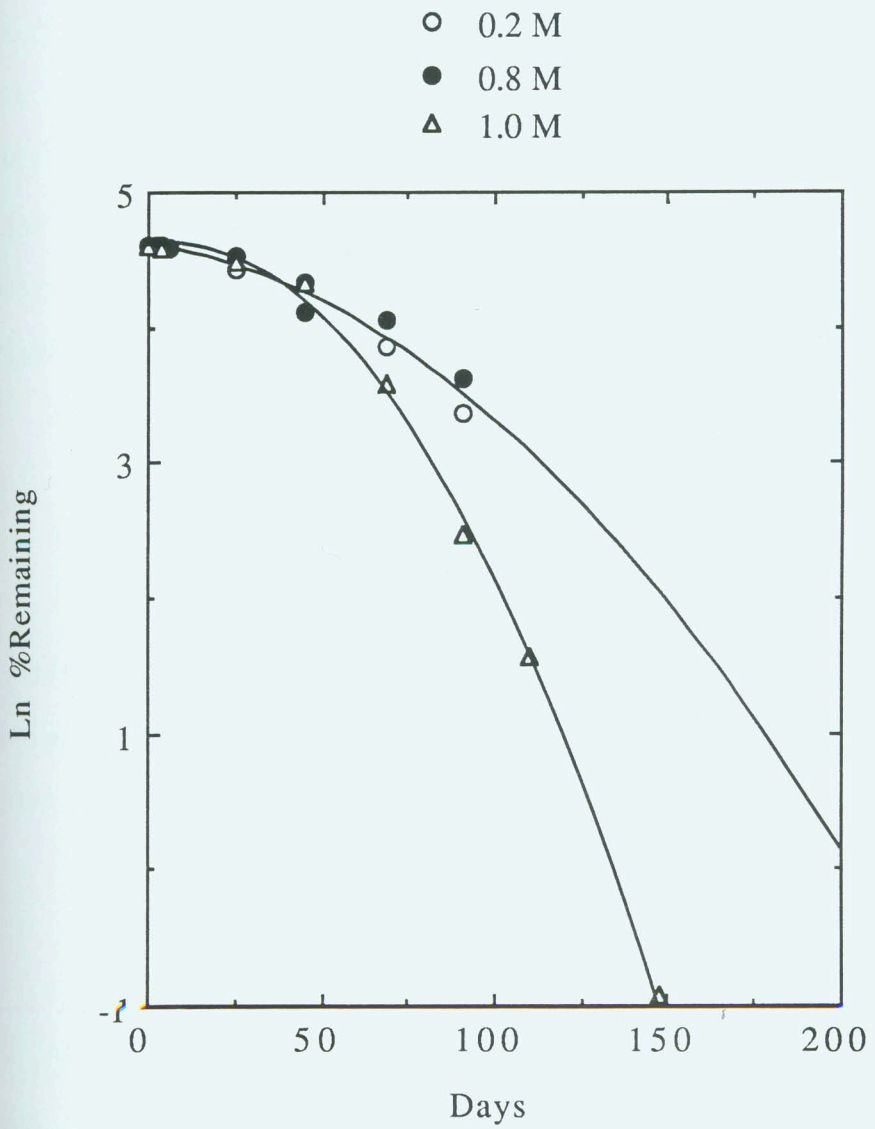
Another reason for the observed behavior may be related to the oxygen salting-out ability of KCl as compared to phosphate or the effect on the cmc by KCl; alternatively, the phosphate used to prepare the buffer may contain more trace metals on a molar basis than the KCl (see Section IV-E).

Increasing the electrolyte concentration is known to affect the cmc by decreasing it. Thus, the drug in 0.2 and 0.8 M samples

containing KCl should be in micellized form according to data in Appendix G. If the micellized drug is more stable than non-micellized drug, and one assumes that phosphate has little or no effect on cmc, then it is conceivable that degradation would be slower in 0.2 and 0.8 M buffers containing KCl than in the 1.0 M buffer of comparable ionic strength which does not contain any KCl. It remains as future work to substantiate these theories.

Figure IV-B-1 SKB106203 Kinetics: Study 4, 60°C
Buffer Effect/KCl Protective Effect in 0.2 and 0.8 M
pH 7 Phosphate Buffers μ = 2.2 with KCl vs 1.0 M
Buffer with No Added KCl (Intrinsic μ = 2.2)
Initial Drug Concentration = 0.2 mg/ml
Data from Table III-D-2

0.2/0.8M: $y = 4.61 - 3.64e\text{-}3x - 9.35e\text{-}5x^2$ $R^2 = 0.963$
1.0M: $y = 4.61 + 3.57e\text{-}3x - 2.82e\text{-}4x^2$ $R^2 = 0.998$



IV-C Oxidation Kinetics

Oxidation reactions may be broadly classed as either simple, first-order reactions, or as autocatalytic reactions. At 93°C, the drug substance under study appears to be a simple oxidation, that is, the semilogarithmic plots of percent remaining vs time are linear over several halflives. At 60 and 75°C, some of the semilogarithmic plots show a downward curvature suggestive of an autocatalytic oxidation. Some investigators believe the curvature is a result of an initial lag time during which the concentration of the oxidizing moieties builds up. The following mechanism has also been proposed to explain the observed behavior.[32]

For a simple oxidation

$$A + O_2 \text{--->} B \qquad \text{(IV-1)}$$

the observed rate constant, $k_{(obs)}$, is assumed to vary only with the concentration of oxygen, which under most circumstances is in excess and may be treated as a constant:

$$k_{(obs)}=k[O_2]=k^*$$

For an oxidation autocatalytic reaction in the presence of excess oxygen, the observed rate constant is assumed to vary with the concentrations of the products:

$$k_{(obs)} = f([B],[O_2]) = k[O_2]B = k^*B \qquad (IV\text{-}2)$$

If the results are expressed as fractions,

$$B=1-A \qquad (IV\text{-}3)$$

and

$$k_{(obs)}=k^*(1-A) \qquad (IV\text{-}4)$$

$$dA/dt = -k_{(obs)}[A] \qquad (IV\text{-}5)$$

$$=-k^*[1-A][A] \qquad (IV\text{-}6)$$

or

$$dA/[1-A][A]=-k^*dt \qquad (IV\text{-}7)$$

Integration using partial fractions gives

$$\ln[A]/[1-A] =-k^*t + \text{constant} \qquad (IV\text{-}8)$$

Therefore a plot of ln([A]/[1-A]) vs t should result in a straight line with the observed rate constant as negative of the slope, $k^*=k[O_2]$.

Figure IV-C-1 plots the 0.1 M data from Figure IV-A-3 according to Equation IV-8. Although for other buffer concentrations the fit is not as good as for the 1.0 M data, a linear relationship is suggested. Because the formula uses an input function, namely $k_{(obs)}= k^*P$, the lack of perfect linearity may indicate that the input function needs to be further refined. Nevertheless, when the remaining buffer concentrations in Figure IV-A-3 are treated according to Equation IV-8, the rate constants

obtained from the slope were shown in Figure IV-A-5 to be a linear function of ionic strength, thus supporting the use of Equation IV-8.

From a phenomenological point of view, however, it is easier to fit the data to an equation of the type

$$\ln A = - bt - ct^2 + \text{constant} \qquad \text{(IV-9)}$$

as is also shown in Figures IV-A-3 and IV-C-1. Such a fit would result from using an input function which is time-dependent in a linear fashion,

$$k_{(obs)} = b + ct \qquad \text{(IV-10)}$$

where the coefficients b and c are functions of buffer concentration.[33] Then,

$$dA/dt = -kA=-(b + ct)A \qquad \text{(IV-11)}$$

Integrating, one obtains equation IV-9.
A linear input function such as Equation IV-10 is the simplest form of input function, therefore a reasonable choice.

The data support the choice of a time-dependent linear input function. Appendix A describes a global equation of the form of Equation IV-9 which is useful in predicting degradation as a function of buffer molarity at 60°C in the absence of KCl. When the

predicted values are compared with the observed values in Figure IV-A-3, the fit is found to be quite good (Figure IV-C-2).

Although this input function works quite well for the data at hand, others may exist which fit the data equally well. For example, the data may be plotted as Weibull functions, i.e., ln(-ln fraction remaining) vs ln time, as shown in Figure IV-C-3 . The data plotted in this manner are also reasonably linear, however do not offer an explanation for the observed behavior of the compound. Furthermore, the Weibull plot which takes twice the logarithm of the fraction retained as well as the logarithm of time, smoothes the data too much so that real differences are masked. It is only presented here as an example of the fact that other input functions may linearize the data, and that the fit depends a bit on the input function.

One might ask what would cause the rate constant to vary in such a manner. Generally, such curvature is typical of oxidation reactions. It has been theorized that there is an initial lag time associated with the build-up of the oxidizing moiety; for the degradation of epinephrine, Sokoloski and Higuchi reported that their data consisted of an initial first-order phase followed by a zero-order phase.

The observed curvature may also be due to the oxygen concentration changing with time. Since this drug is capable of forming micelles, it is possible that as the compound degrades, its concentration drops below that of the cmc, which may affect the amount of oxygen available for reaction, which would in turn result

in a change in rate constant with time. That the compound is micellar is supported by the fact that samples degraded beyond a certain point no longer foam upon shaking. Although the cmc was determined in water at ambient temperature, it was not determined in any of the other diluents. It is known that temperature and electrolytes will affect the cmc[34], and in turn, the rate of degradation may be either enhanced or slowed by the formation of micelles.[35,36,37,38] Thus, the reaction rate would be dependent upon the buffer molarity as well since this affects the ionic strength.

Figure IV-C-1 SKB106203 Kinetics: Study 4, 60°C
In 1.0 M pH 7 Buffer, No Ionic Strength Adjustment;
Initial Drug Concentration = 0.2 mg/ml
Data Fit to Second Degree Polynomial vs Equation IV-8
Data from Table III-D-2

First-Order: $y = 5.26 - 3.54e\text{-}2x$ $R^2 = 0.893$
Polynomial Fit: $y = 4.61 + 3.57e\text{-}3x - 2.82e\text{-}4x^2$ $R^2 = 0.998$
Equation IV-8: $y = 4.05 - 6.55e\text{-}2x$ $R^2 = 0.994$

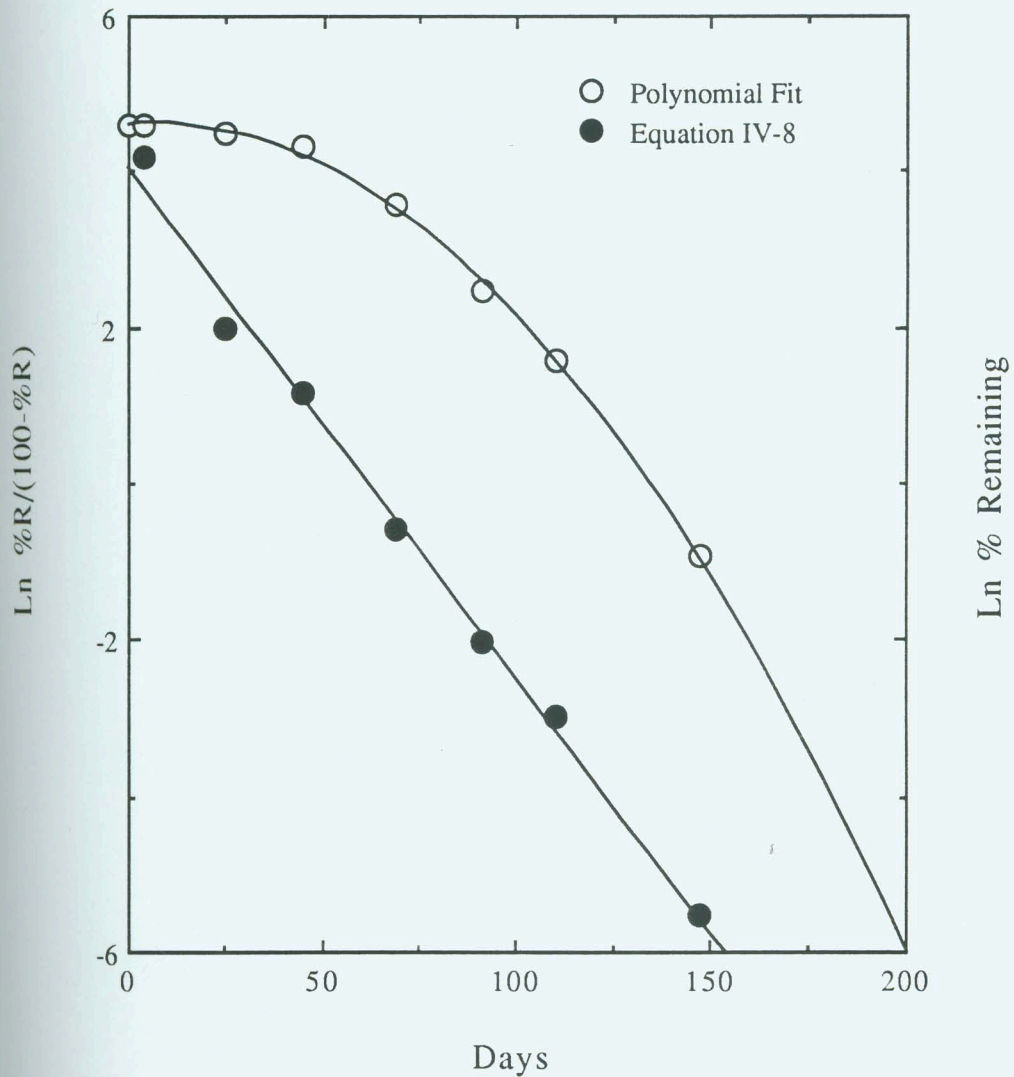


Figure IV-C-2 SKB106203 Kinetics: Study 4, 60°C
In pH 7 Buffers of Different Molarities With No Ionic Strength Adjustment
Comparison of Results Predicted by Equation A-H-1 with Observed Results in Figure IV-A-3
Data from Table A-1

y = 0.13 + 1.01x R^2 = 0.975

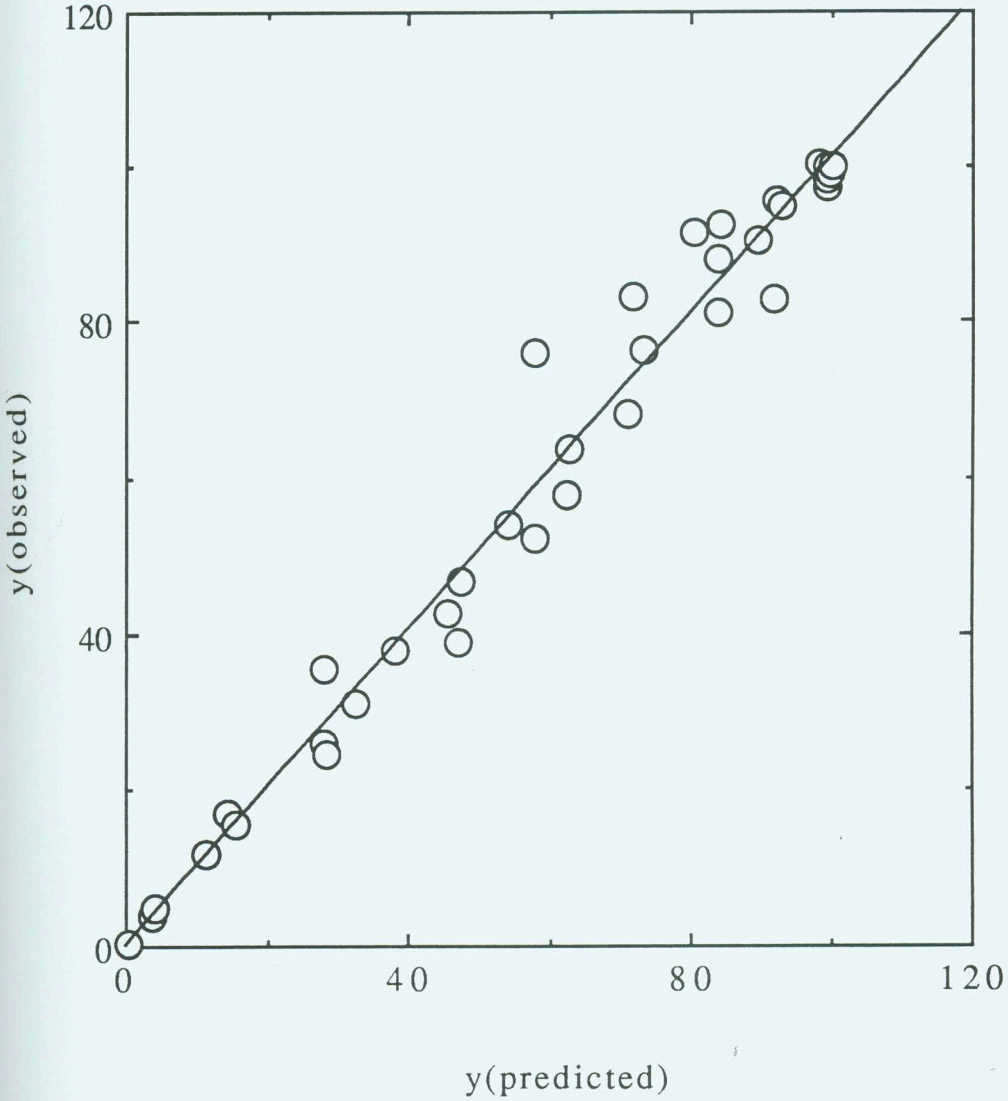
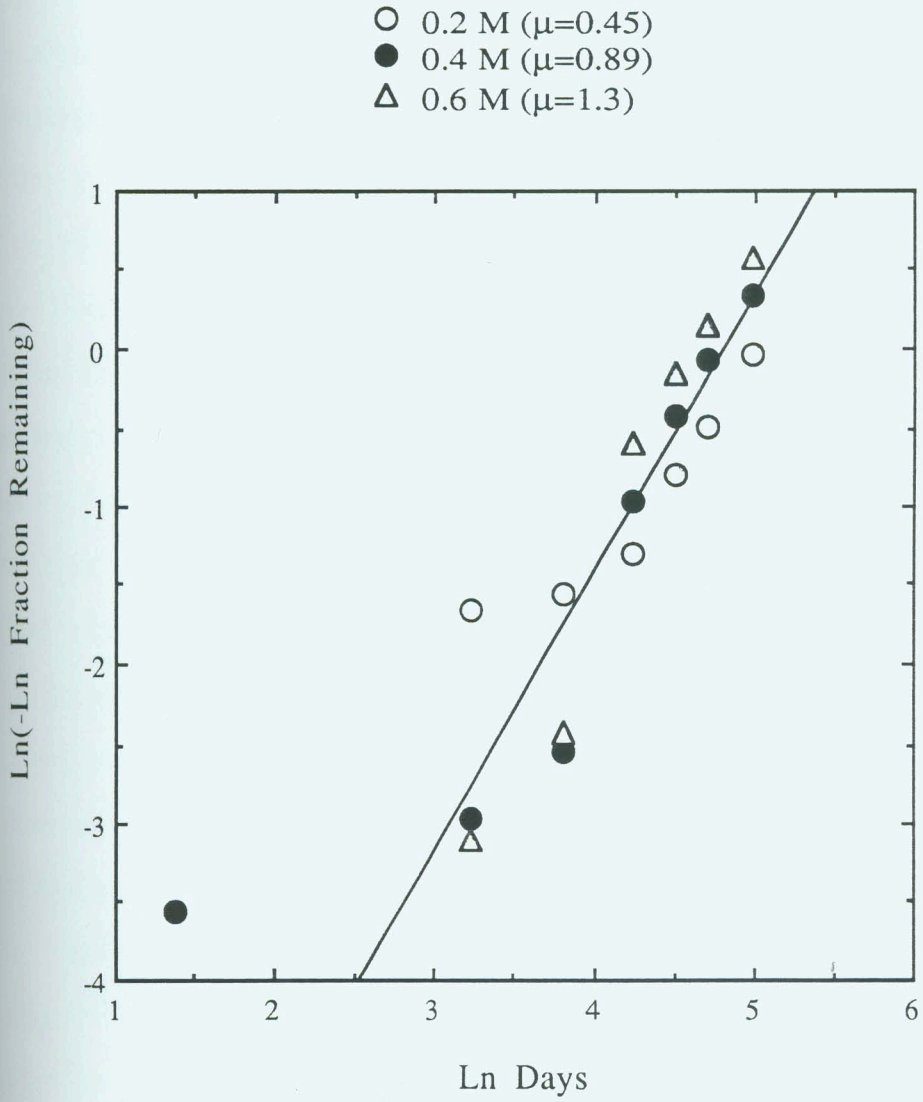


Figure IV-C-3 SKB106203 Kinetics: Study 4, 60°C
In In pH 7 Buffers of Different Molarities With No Ionic Strength Adjustment (from Figure IV-A-3)
Data Treated as a Weibull Function
(0.4 M, 4-day Data Point Omitted from Regression)
Data from Table III-D-2

$y = -8.43 + 1.75x$ $R^2 = 0.850$



IV-D Drug Concentration Effect

To study the effect of drug concentration, the reaction was monitored in two concentration ranges, between 0.05 and 0.5 mg/ml at 75°C and 93°C, and between 1.0 and 10.0 mg/ml at 93°C, with four concentrations per range. The reaction was studied in low and high ionic strength environments, and in the presence and absence of a metal chelating agent, since metals are known to catalyze oxidation reactions. In some cases, only initial rates were obtained, since at 93°C the reaction was previously shown to obey pseudo-first order kinetics.

Because the cmc for this compound was determined to be 2.4 mg/ml in H_20, it was expected that there would be a difference observed in the degradation rate above and below this value, as was shown for sodium decyl sulfate, Figure IV-D-1.[39] However, for the most part, this was not the case, and the conclusion is drawn that there was no drug concentration effect observed between 0.05 and 0.5 mg/ml and 1.0 and 10.0 mg/ml in the various matrices studied to the extent that the reactions were monitored. This does not imply a non-existence of a cmc, but only that under the conditions studied, micellization was not involved in the reaction.

Between 0.05 and 0.5 mg/ml (or 1.03×10^{-4} and 10.3×10^{-4} M) at 93°C, the data do not suggest a drug concentration effect in either low or high ionic strength environments (Figure IV-D-2). The ionic strength effect however is evident as a fourfold difference in rate constants between the kinetics observed in 0.05 M buffer

($\mu_{calculated}$ = 0.11) vs 0.6 M buffer (μ=2.2 with KCl). The data generated in the 0.6M buffer show more scatter but do not show a trend in the observed rate constants when compared with drug concentration. Statistical analyses for these studies are given in Appendix B-2 and 3.

In general, samples in high salt and or phosphate buffer tended to show more scatter than other samples; the scatter may indicate some complex phenomena involving micellization. However, at this point, it is assumed the anomalies reflect some influence on the chromatographic method, even though the samples were highly diluted prior to analysis. It is recalled that samples in high buffer and or salt concentrations required considerable dilution prior to chromatographic analysis in order to avoid peak splitting. There were other aberrant data, which are explored in detail below.

What is curious about many of the studies is that there does not appear to be a drug concentration effect if one ignores one of the intermediate concentrations. In some cases, this discrepancy is traced to pH, but in the others, this is not the case. The discrepancies are treated here as unsourced systematic error (in sample preparation, for example), but it is not ruled out that the effect is mechanistic.

For example, the data of Set C of Study 6 are not significantly different on the 95% confidence level, if one ignores the 2.0 mg/ml data.[40] In this case, the pH of the 2.0 mg/ml samples remained lower than the others over the course of the study, hence an

explanation for the higher observed degradation rate of the 2.0 mg/ml samples.

Set D, corresponding to drug in 0.1% EDTA, also shows no drug concentration effect between 1.0 and 10.0 mg/ml at 93°C as is seen in Figure IV-D-3. [41] This is one of the few sets of data where this conclusion is unequivocal, and may be due to the slight buffering capacity of the EDTA.

Studies 5 and 6 are duplicate experiments measuring the rate of degradation of the drug substance in H_2O at 93°C between 1.0 and 10.0 mg/ml. The rates in Study 5 were determined as initial rates, whereas in Study 6 the drug substance was allowed to degrade to a considerable extent. Unfortunately, the $k_{(obs)}$ of Studies 5 and 6 (Set A) , are an order of magnitude different from each other, except in the case of the 2.0 mg/ml samples. This is the difficulty associated with kinetic measurements in unbuffered systems.

Even though the quantitative results do not agree, the qualitative observation which may be made from both Studies 5 and 6 is that there is no drug concentration effect between 1.0 and 10.0 mg/ml, however something unusual occurs between 1 and 5 mg/ml. In Study 5 (Figure IV-D-4), the discrepancy in rate constants between 1 and 5 mg/ml is due to the much lower pH of the 1.0 mg/ml samples; however in Study 6, no such discrepancy exists to explain the deviations of the 2.0 mg/ml data.

Set B of Study 6, corresponding to drug in 2.2 M KCl, does not show a drug concentration effect between 1.0 and 2.0 mg/ml, however at 10.0 mg/ml the degradation rate appears to be higher as

may be seen in Figure IV-D-5.[42,43] Without additional time-points for the 10.0 mg/ml range it is difficult to conclude for certain that the difference is real. With regards to the pH trend of this set of data compared with the other three drug concentrations, the 10.0 mg/ml data are not outstanding .

At 75°C (Study 3), the data become more complicated, and it is no longer possible to draw clear-cut conclusions regarding drug concentration effects. It appears as though there is no difference in $k_{(obs)}$ between the 0.05, 0.2, and 0.5 mg/ml samples in 0.05 M pH 7 buffer, as can be seen by examining Figure IV-D-6. However, the 0.1 mg/ml data deviate significantly at the later time points and do not adhere to first-order kinetics. The 0.1 mg/ml data can be adequately fit to a second-degree polynomial, which is shown on the plot, however, the reason for the deviation is not immediately apparent. Here, the pH is not an issue, since these samples are buffered.

The 0.1 mg/ml data also deviates from the trend when the kinetics were monitored in 0.6 M buffer containing KCl (Figure IV-D-7). Again, pH is not an issue here. Curvature is present at all the drug concentrations.

Figure IV-D-1 Sodium Decyl Sulfate Kinetics, 90°C
Effect of Drug Concentration on $k_{(H)}$
Ionic Strength = 0.51, $[HClO_4]$ = 0.02 M
Reconstructed from Kurz, J., J. Phys. Chem., Nov. 1962, pp. 2239-46

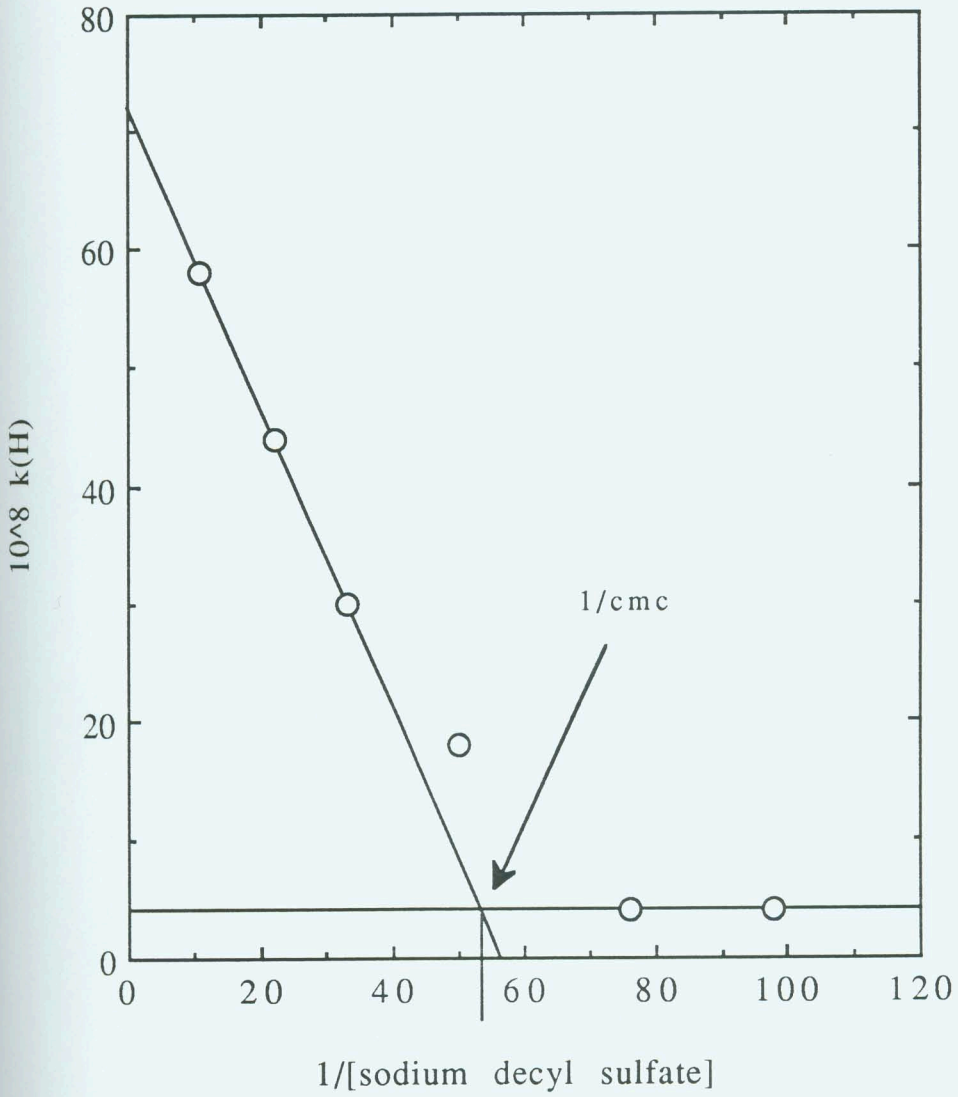


Figure IV-D-2 SKB106203 Kinetics: Study 3, 93°C
In 0.05 M pH 7 Buffer ($\mu_{calculated}$ = 0.11)
vs 0.6M (μ = 2.2 with KCl)
Data from Tables III-C-1-a and III-C-1-b;
Appendices B-2 and B-3

Mean of Four Drug Concentrations: 0.05, 0.1, 0.2, 0.5 mg/ml

0.05 M:	y = 4.55 - 7.07e-2x	R^2 = 0.995
0.6 M:	y = 4.70 - 0.30 x	R^2 = 0.995

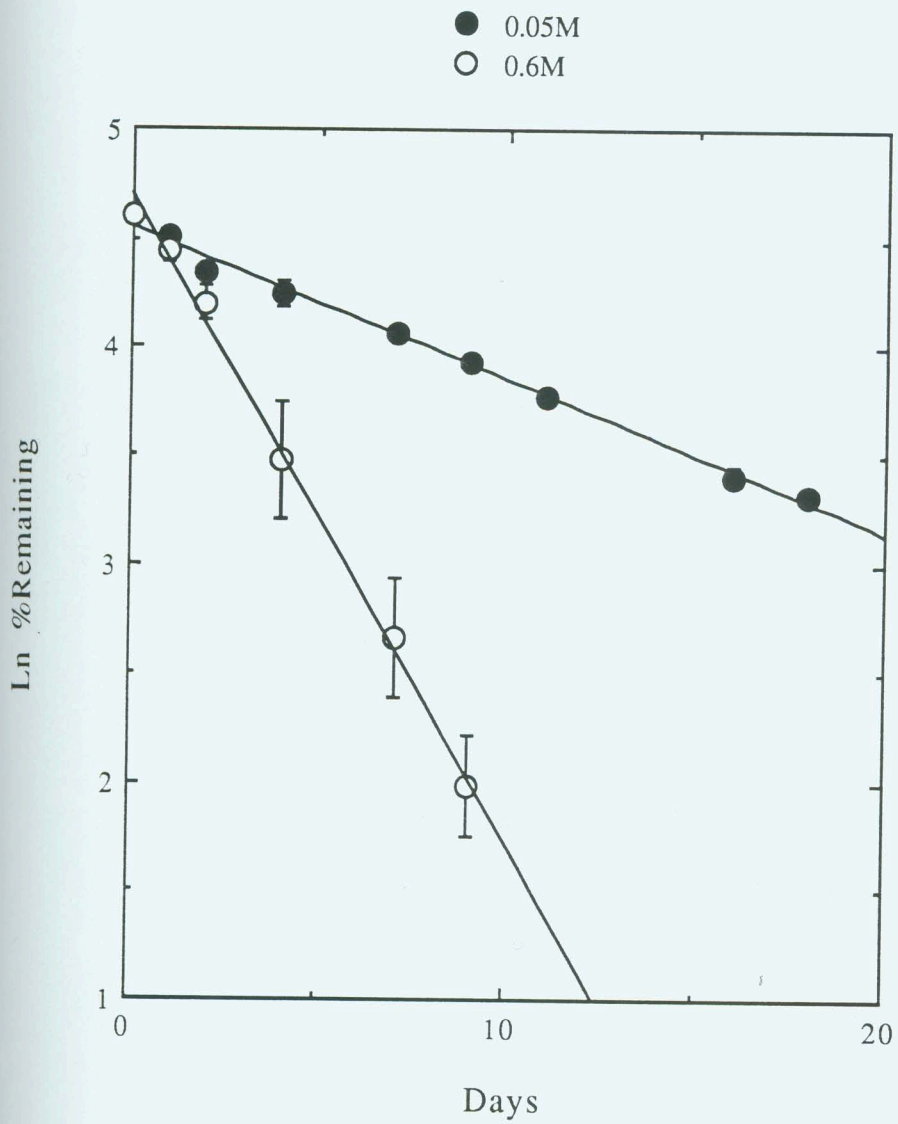


Figure IV-D-3 SKB106203 Kinetics: Study 6, 93°C
Drug Concentration Effect
Set D: Drug in 0.1% EDTA
Data from Table III-F-1; Appendix B-7

y = 4.60 - 6.78e-3x R^2 = 0.964

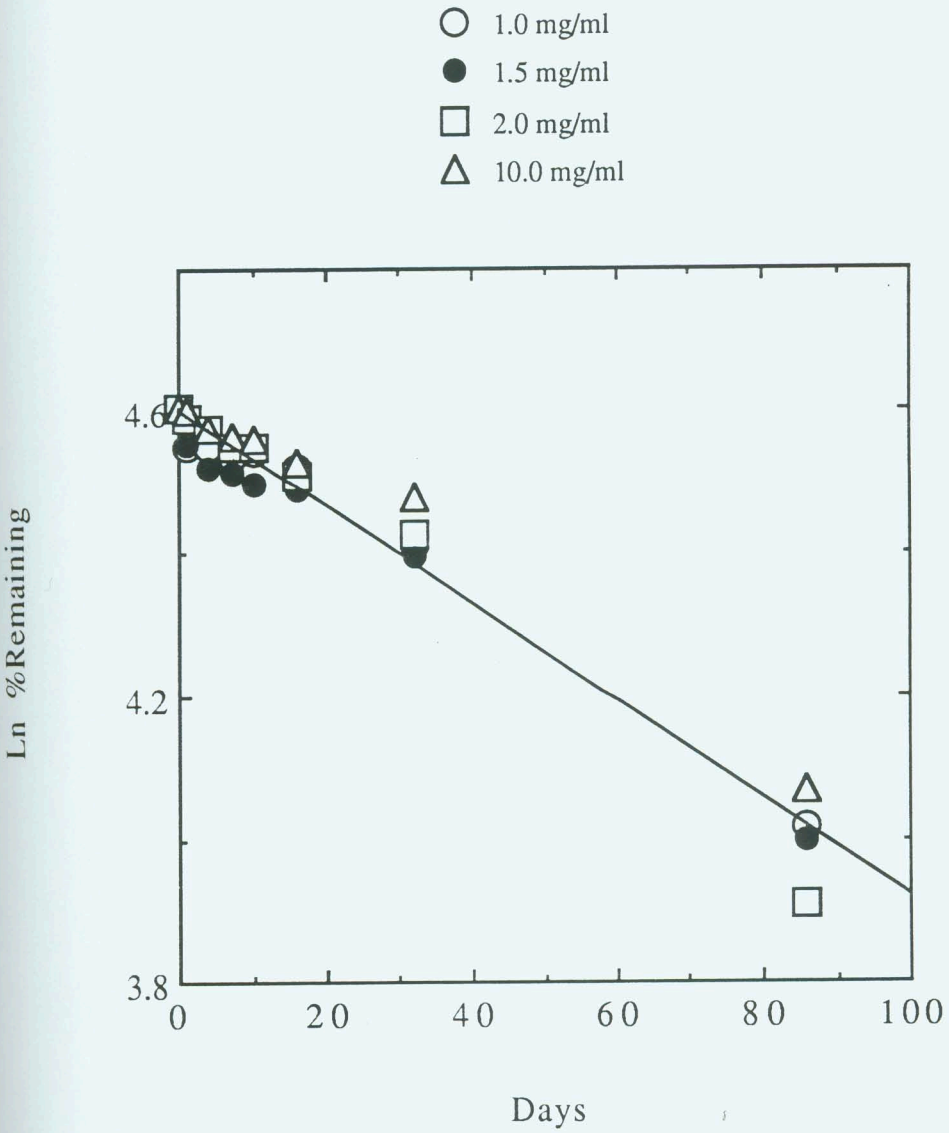


Figure IV-D-4 SKB106203 Kinetics: Study 5, 93°C
Drug Concentration Effect, Drug in H_2O
Data from Table III-E-1; Appendix B-8

2.0, 5.0, 10.0 mg/ml: y = 4.58 - 2.52e-2x R^2 = 0.927
1.0 mg/ml: y = 4.56 - 4.48e-2x R^2 = 0.969

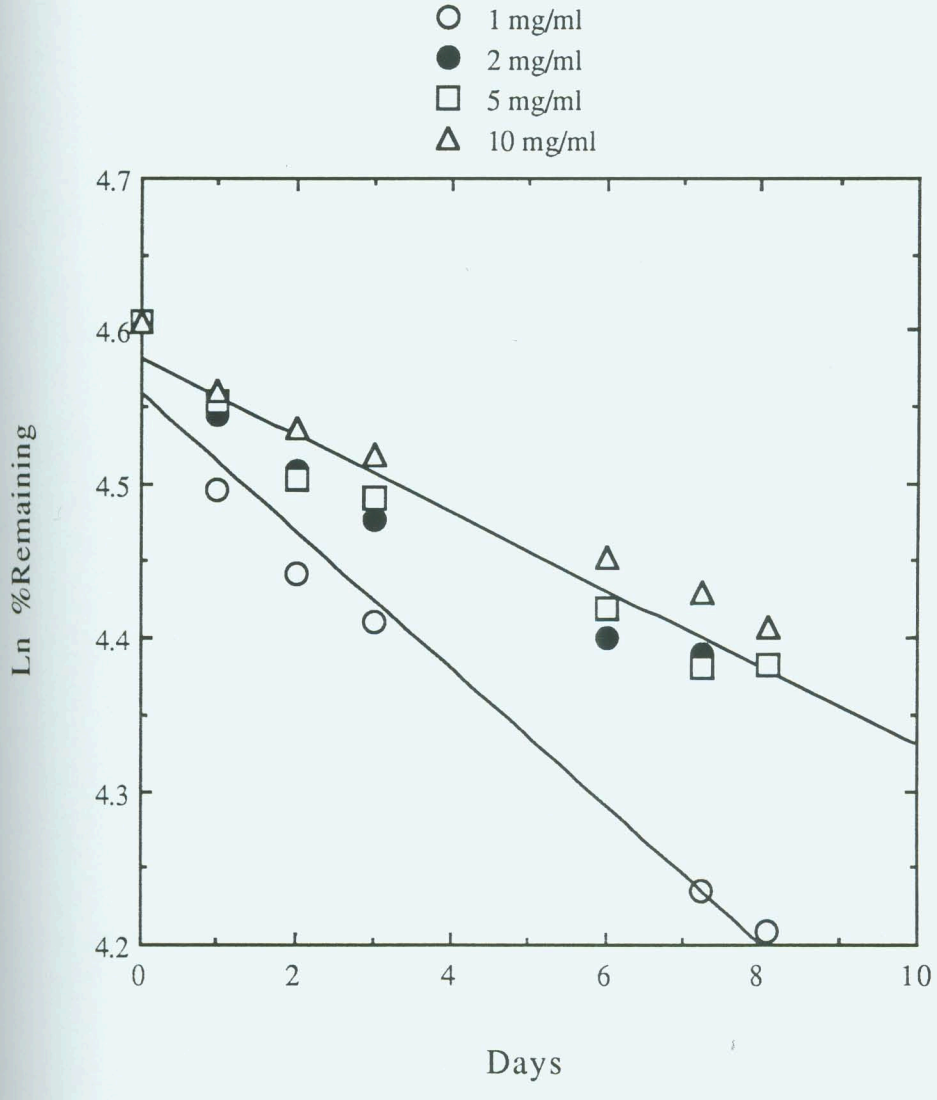


Figure IV-D-5 SKB106203 Kinetics: Study 6, 93°C
Drug Concentration Effect
Set B: Drug in 2.2 M KCl (No Buffer)
Data from Table III-F-1; Appendix B-5

y = 4.61 - 2.32e-2x R^2 - 0.996

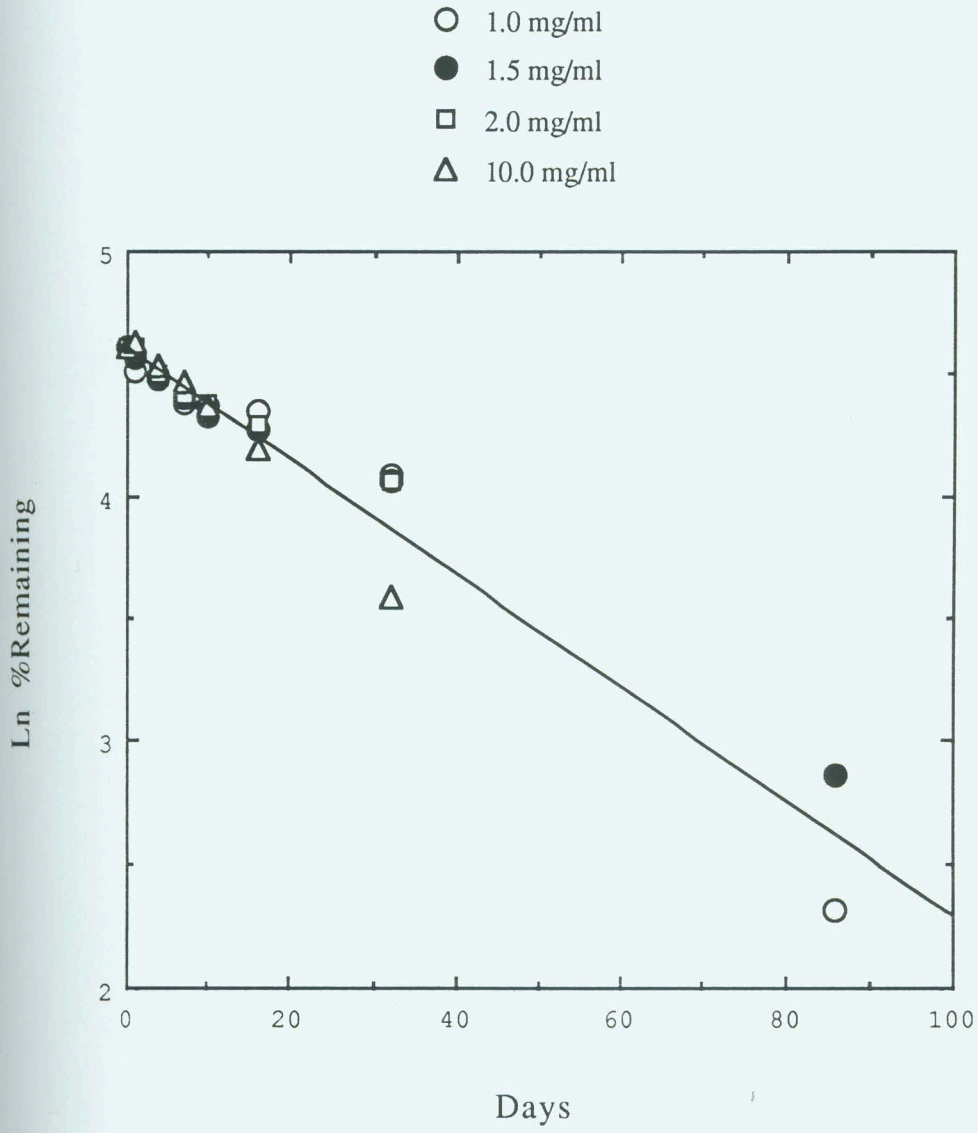


Figure IV-D-6 SKB106203 Kinetics: Study 3, 75°C
Drug Concentration Effect in 0.05 M pH 7 Buffer
No Ionic Strength Adjustment ($\mu_{calculated}$ = 0.11)
Data from Table III-C-2-a

0.05, 0.20, 0.50 mg/ml: y = 4.54 - 3.87e-3x R^2 = 0.890
0.10 mg/ml: y = 4.58 - 2.73e-3x - 1.52e-4x^2 R^2 = 0.990

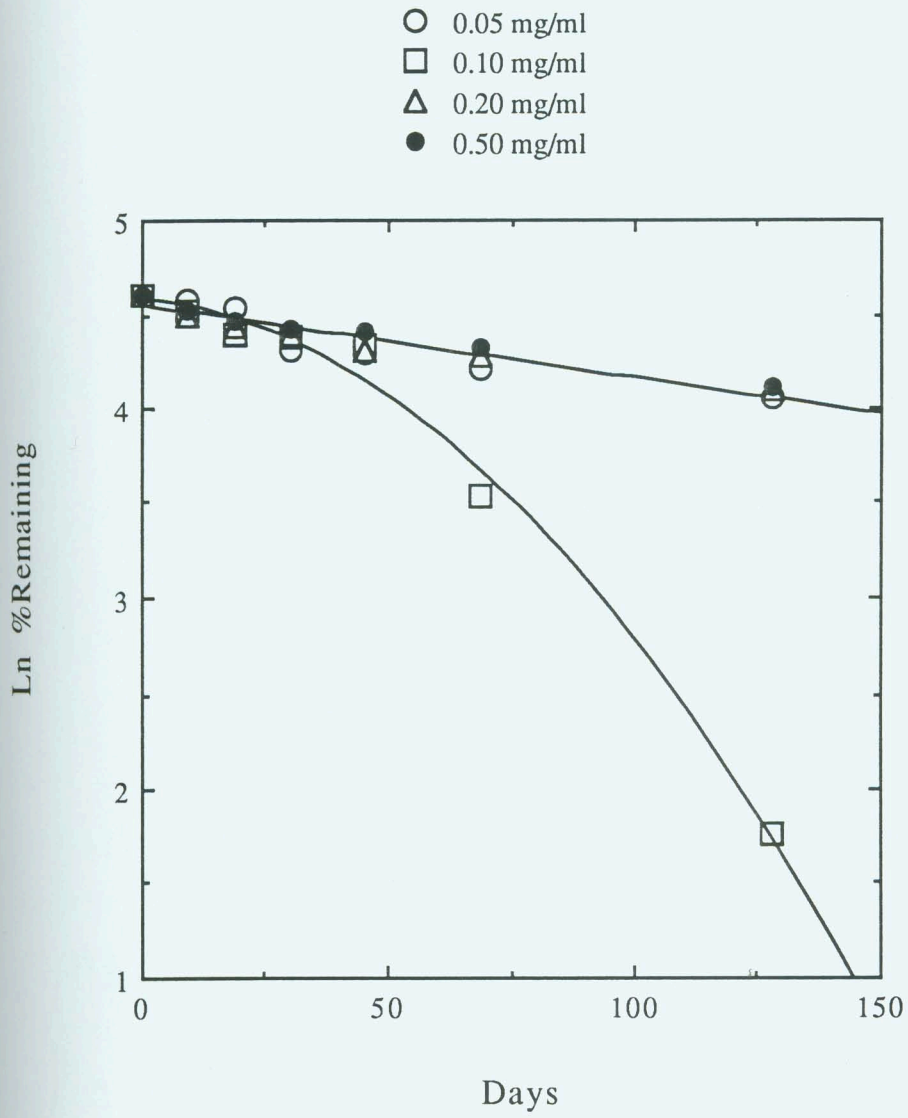
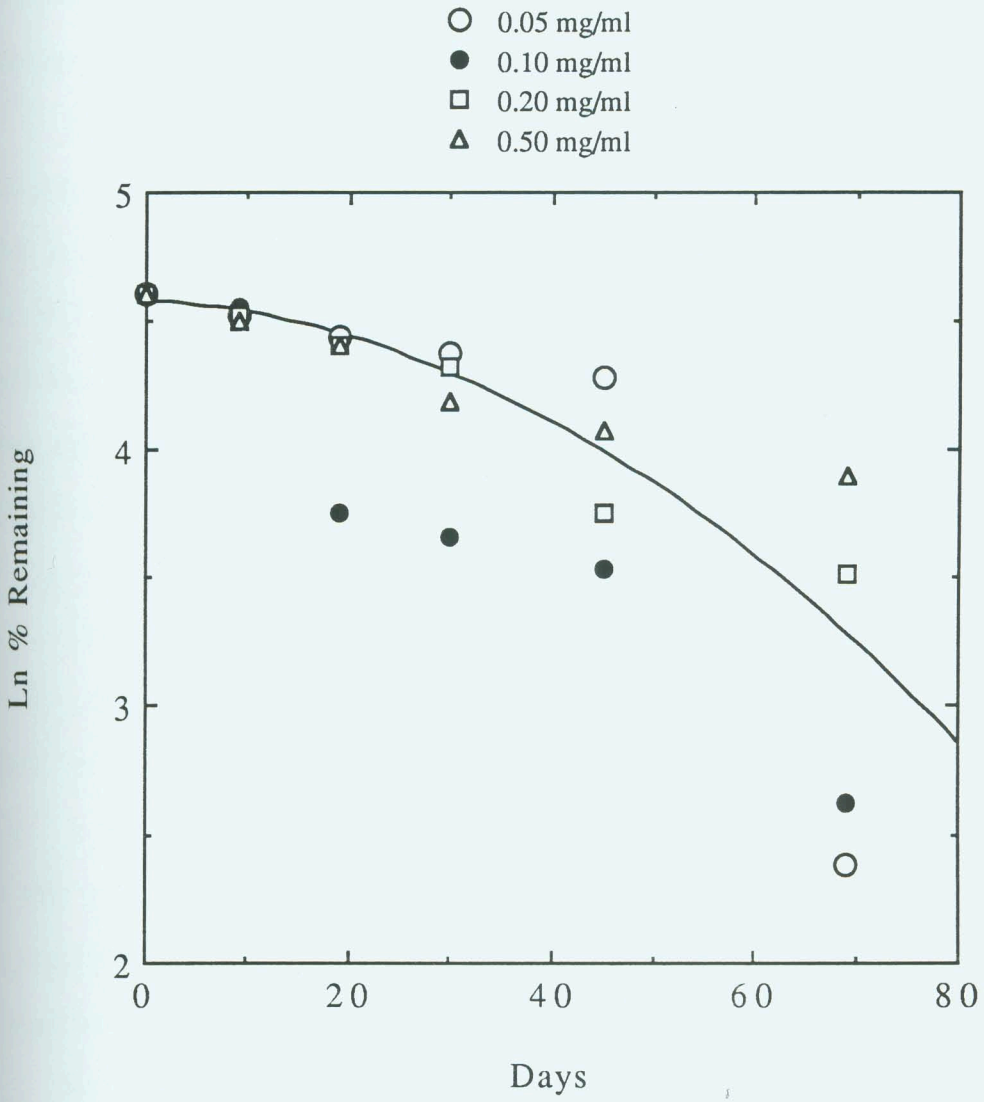


Figure IV-D-7 SKB106203 Kinetics: Study 3, 75°C
Drug Concentration Effect in 0.6 M pH 7 Buffer
μ = 2.2 with KCl
Data from Table III-C-2-a

0.05, 0.20, 0.50 mg/ml: y = 4.57 - 1.94e-3x - 2.44e-4x^2 R^2 = 0.723



IV-E Trace Metal Catalysis

In order to determine if the drug substance, the KCl, or the phosphate buffers used in these studies contributed enough trace metals to catalyze the reaction,[44] the drug concentration studies described in Section IV-D were done in the presence and absence of the metal chelating agent, EDTA.

As may be concluded from Table IV-D-1 and Figure IV-E-1, the reaction rate is much faster in the presence of KCl relative to KCl plus 0.1% EDTA, indicative of catalysis by trace metals contained in the KCl. The rate in plain water is nearly the same as in 0.1% EDTA indicating that at these drug concentrations (1.0-10.0 mg/ml) the drug substance itself is not contributing any trace metals. The statistical analyses are presented in Appendices B-9, B-10, B-11, and B-13.

Trace metal catalysis by phosphate buffer is evident in Figure IV-E-2 as a doubling in the observed degradation rate constant in plain buffer (A) compared to buffer to which 0.1% EDTA was added (D). A similar effect was not noticed in 0.2 M buffer, indicating that at this molarity there is an insufficient concentration of trace metal to catalyze the reaction. In these experiments, the drug concentration was much lower, i.e. 0.2 mg/ml.

Exactly how much trace metal is in these systems is unknown, but an estimate of the contribution from KCl is given in Table IV-E-2.

Table IV-E-1 SKB106203 Kinetics: Study 6, 93°C
Trace Metal Catalysis
Data from Table III-F-1; Appendices B-4, -5, -6, -7, -9, -10, and -11

Mean of Four Drug Concentrations: 1.0, 1.5, 2.0, and 10.0 mg/ml

	$k_{(obs)} \times 10^3$ days^{-1}
A: in H_2O	7
B: in 2.2 M KCl	23
C: in 2.2 M KCl +0.1% EDTA	11
D: in 0.1% EDTA	7

Conclusion: Trace Metal Catalysis by KCl

Table IV-E-2 SKB106203 Kinetics: How much trace metal from KCl?

Worst Case:

0.01M pH 7 buffer:	0.4 μM Pb
	0.9 μM Fe

Best Case:

0.8M pH 7 buffer:	0.08μM Pb
	0.2 μM Fe

Assuming Label Claim of 5 ppm Pb, 3 ppm Fe (Appendix E)

Drug Concentration: 0.4 mM = 0.2 mg/ml

Figure IV-E-1 SKB106203 Kinetics: Study 6, 93°C
Trace Metal Catalysis by KCl
Data from Table III-F-1; Appendix B-10

Mean of Four Drug Concentrations: 1.0, 1.5, 2.0, and 10.0 mg/ml

B: Drug in 2.2 M KCl: y = 4.61 - 2.28e-2x R^2 = 0.950
C: Drug in 2.2 M KCl + 0.1% EDTA: y = 4.58 - 1.10e-2x R^2 = 0.858

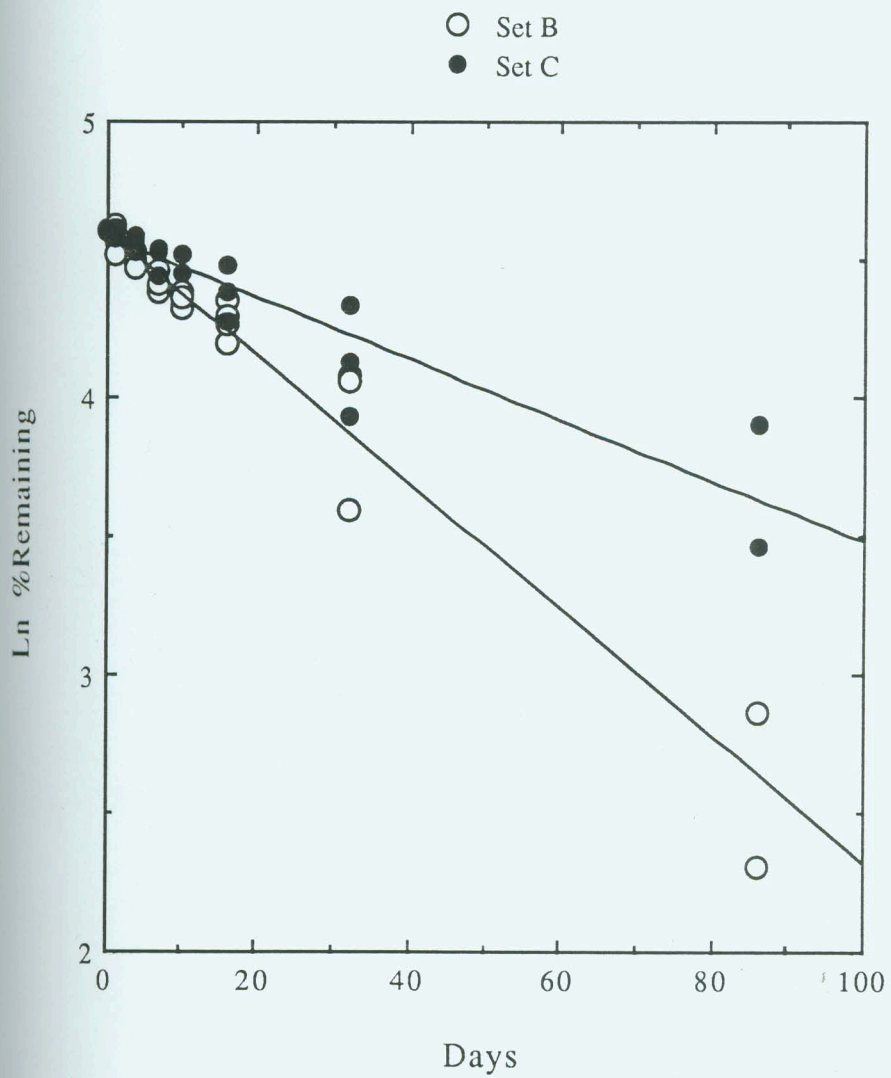
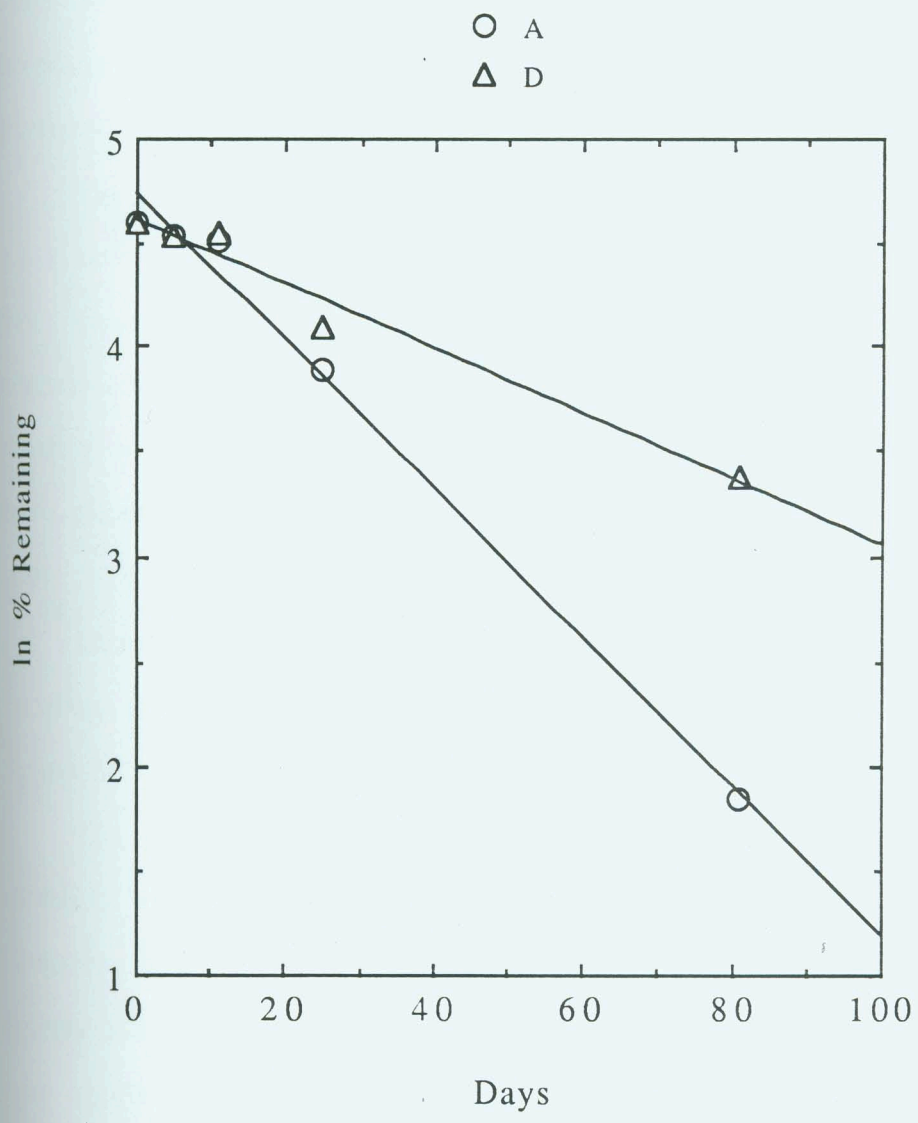


Figure IV-E-2 SKB106203 Kinetics: Study 7, 75°C
Trace Metal Catalysis by Phosphate
Initial Drug Concentration = 0.2 mg/ml
Data from Table III-G

A: Drug in 0.8 M pH 7 Phosphate Buffer, No KCl
D: Drug in 0.8 M pH 7 Phosphate Buffer + 0.1% EDTA

A: y = 4.74 - 3.55e-2x R^2 = 0.992
D: y = 4.62 - 1.56e-2x R^2 = 0.973



IV-F Micellar Properties of the Drug Substance

Although foaming *per se* does not imply that micelles form, it is an interesting observation that solutions of the compound, even in low concentrations, have a tendency to foam.

It is also a visual observation, that as decomposition proceeds, this tendency is lost. This begs the question: is it possible that the compound is surface active, and that it is, indeed, the micellar properties which lead to the apparent discrepancies in kinetic profiles?

The cmc was, at our request, determined at SKB, and a cmc of 6.3×10^{-3} M, or 2.4 mg/ml obtained in H_2O at 21.1°C.[45] Below the cmc, kinetics would probably proceed as normal, however, in a concentration above the cmc, the drug could afford itself either micellar protection or micellar catalysis. This being the case, then there should, broadly, be a difference between kinetics below and above this value.

Some of the variables affecting the cmc and some of the variables affected by a substance having a cmc are given in Appendix G.

The problem lies in that not only the cmc, but also the oxygen solubility are affected in a non-linear fashion by many of the same variables, such as temperature, salt concentration, and drug concentration, and both changes in the cmc over time and oxidation-type reactions may produce downward curvature in the first-order plots.

In light of these possibilities, some of the data collected in this study will be assessed.

As shown in Appendix G, for ionic surfactants below the cmc, gas solubilities are independent of surfactant concentration, and approximate the solubility in pure water. Above the cmc, gas solubilities and concentrations of certain nonpolar solvents have been found to increase linearly with surfactant concentration.[46,47,48,49]

Thus, the micellized form of SKB106203 may be capable of solubilizing a great deal more oxygen than predicted by ionic strength and temperature alone. Based on the chemical structure of the compound, when it forms micelles, it probably does so by orienting its hydrocarbon chains inward; the polar ionic groups to which the reactive sulfur is attached would be oriented outwards toward the bulk aqueous medium. Any additional oxygen dissolved as a result of micellization of the drug substance would most likely be associated with the interior hydrocarbon portion of the micelle since it is relatively nonpolar. This additional oxygen would not be available for reaction. However, the oxygen in solution would probably not all be able to "fit" inside the micelle. Some oxygen would be in the bulk and capable of reacting with the sulfide group. How much oxygen is in the bulk will be affected by temperature and electrolyte concentration.

Conceivably as the drug degrades to below the cmc, the additional oxygen dissolved by the micelles may be released into an oxygen-deficient bulk. At this point, degradation may be

accelerated which would be manifested by a downward curvature in the first-order plots. If the bulk has excess oxygen however, the additional oxygen supplied by the micelles would not affect the kinetics, and no curvature would be noticed. It is also possible that the oxygen released by the micelles may be salted out immediately depending on the nature of the bulk.

Although it has not been established that the cinnamic acid and sulfoxide analogues have cmc's, their chemical structures suggest that they do. It is therefore conceivable that the degrading solution may contain mixed micelles. But because of the degradation products' low solubility in water, this may not be an issue here, except in the presence of high salt concentrations.[50] In samples containing sufficient quantities of KCl and/or EDTA, more of the cinnamic analogue appeared to be solubilized than in samples dissolved in plain water alone (Table IV-E-1). The sulfoxide analogue appears to be salted out by KCl in these same systems. These properties may be useful if one wanted to prepare large quantities of the degradation products.

That the compound forms micelles may explain why the kinetics in high buffer molarity and ionic strength generally show so much more scatter than in low buffer molarity under the same conditions. Without further information, the scatter has been attributed to effects on the chromatography. Suppose, however, that at 0.05 M buffer, the 4 drug concentrations tested (0.05, 0.1, 0.2, 0.5 mg/ml) were below the cmc[51]; then kinetics would be proceed in a normal fashion. In fact one does not notice a drug concentration

effect in 0.05 M buffer, and the individual plots are all first-order, insofar as they have been monitored.

In 0.6 M buffer, however, if the cmc has been lowered by the addition of phosphate and KCl, then it is conceivable that the cmc in these samples may be somewhere between 0.05 and 0.5 mg/ml, the concentration range of the experiment. Since the cmc is not necessarily a sharp transition, and the oxygen solubility as well would be affected by the presence of micelles, it would not be surprising to find scatter in the data in this concentration range, considering the additional variable of ampul-to-ampul variability and the fact that at some point in the experiment, the compound would degrade to below the cmc, at which point another kinetic mechanism would dominate.

Although at pH 7 no drug concentration effect was observed, this may not be the case at other pH's where micellization of the drug substance may affect the degradation rate. From a pharmaceutical standpoint, one might imagine that, possibly, the micellar properties of the compound may be exploited in the formulation of a liquid dosage by formulating the compound in a concentration above or below the cmc, where ever it is most stable.[52] In micellar form, it might be able to solubilize a great deal of oxygen, but this could be salted out with NaCl. When ready to use, it could be diluted with normal saline; this salt concentration may be high enough to salt-out oxygen. Thus it would be stable in packaged form, and stable in immediate use form.

For compounds which do not undergo micellization, making a shelf-product in sufficiently concentrated buffer (to be diluted to isotonicity prior to injection) would salt-out the oxygen without a nitrogen flush being necessary prior to packaging. This would also aid in the manufacture of the drug if it is extremely oxygen sensitive.

To conclude, when dealing an oxygen-sensitive compound in micellar form in a high salt solution, it is probably a delicate balance between oxygen being solubilized by the micelle and salted in or out by the electrolyte and temperature; in order to study the kinetics of SKB106203 properly, it would be necessary to monitor not only the drug and oxygen concentration, but to also determine the cmc in each matrix used. This remains as future work.

Table IV-F-1

Effect of KCl on Solubility of SKB106203 Degradation Products
Sample: 4 days/ 93°C, Initial Drug Concentration = 0.5 mg/ml

	0.05M pH 7, no KCl	0.6M pH 7, μ=2.2 with KCl
assay, mg/ml	0.33	0.27
%Remaining	65.8	53.2
Visual observation	high concentration of white flaky ppt.	very low concentration of white flaky ppt.
Peak Area		
Cinnamic Acid	n.d.	73790
Sulfoxide	1761	n.d

n.d. = none detected

Note: Although the following data are from only 2 samples, the trend shown by these is that found in most other similar samples. From the data it may be seen that the visual observation alone, or the chromatographic data alone may be misleading. By combining the qualitative and quantitative measurements, it is obvious that KCl solubilizes the cinnamic acid analogue to a considerable extent, and although the 0.05 M sample would appear, based on the chromatography to have not degraded to the cinnamic acid, the visual observation shows this not to be the case. The sulfoxide oils out of solution, and its solubility is not appreciably enhanced by the presence of KCl, in fact, it appears to salt it out.

IV-G Changes in pH as a Function of Time in Unbuffered or Slightly Buffered Systems

Some electrode manufacturers suggest increasing the ionic strength of aqueous unbuffered samples with a concentrated salt solution in order to obtain an accurate pH reading. For those unbuffered study samples which were of high ionic strength, the pH should therefore be accurate.

For the samples in unbuffered systems however, ionic strength adjustment was not done in order to maintain sample integrity. Nevertheless, it should be kept in mind that the drug is present as the disodium salt, and increasing the drug concentration in itself imparts an increase in ionic strength. This may be the cause for the differences in pH noted; this difference may therefore be exaggerated, or artifactual. Alternatively, the pH difference in the samples may be related to the fact that the cmc is in this range.

Therefore, although the pH of Study 5 samples (drug in H_20) and Study 6 samples (drug in H_20, in 2.2 M KCl with and without 0.1% EDTA, and drug in 0.1% EDTA) have been monitored, it is acknowledged that the measurements themselves are subject to error owing to the low ionic strength and buffering capacity of the samples, except in the case of EDTA. In the systems which contain EDTA the rise is less rapid (and levels off at a lower pH), because of the buffer capacity of the latter. The 10.0 mg/ml samples generally show fewer pH deviations, again due to the buffer capacity of the drug substance itself.

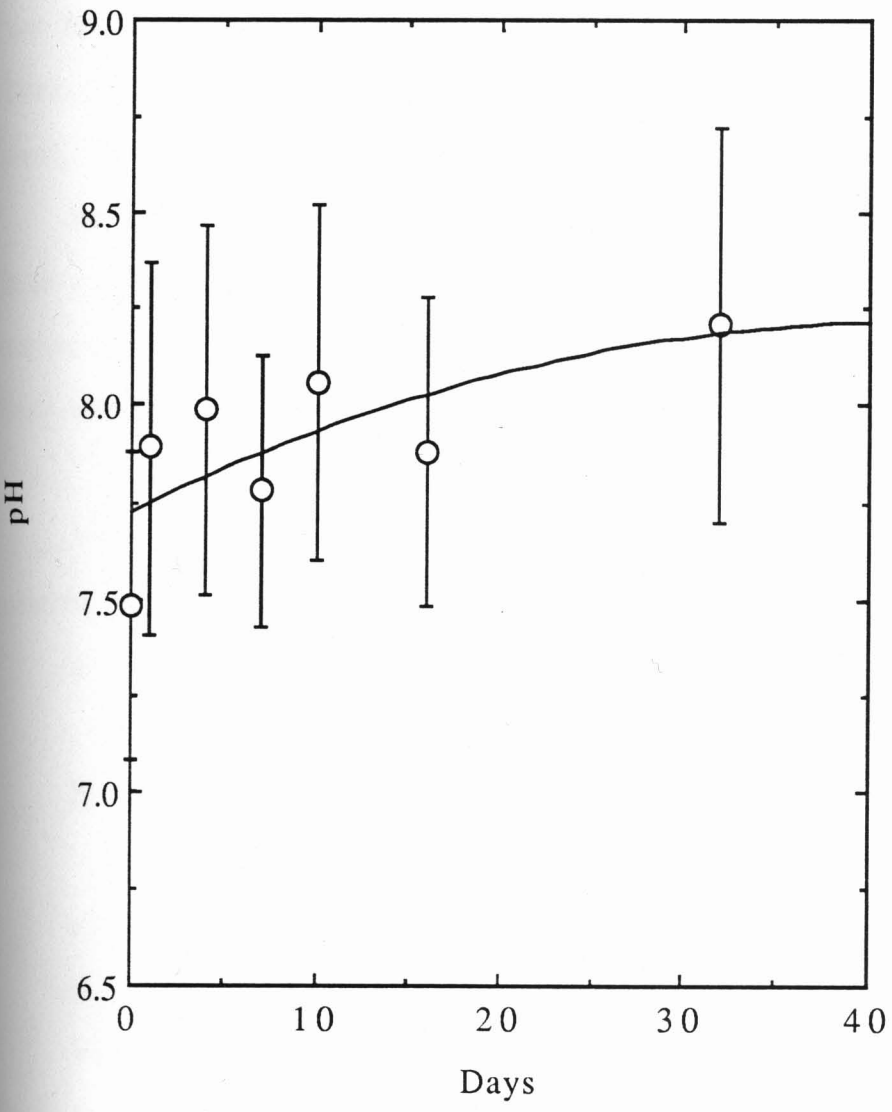
In any event, the data obtained in unbuffered and slightly buffered systems have been treated by essentially ignoring the pH changes[53] . It is noted that the first-order plots of drug in H_20 or KCl are reasonably linear, however this is not an acknowledgement that the rate is not affected by pH. Because of the lack of pH control, it is unwise to overinterpret the results obtained in unbuffered systems.

In all the unbuffered studies, the general pH trend is fairly clear, as may be seen in Figure IV-G-1. A rise in pH occurs fairly rapidly in the case of the systems not containing EDTA, and in these systems, the pH vs time curves are fairly similar.

Of all the systems, water shows the steepest initial pH rise. All the first-order plots of the kinetics of drug in H_20 show an initial nonlinearity corresponding to the initial abrupt increase in pH of these samples. For this reason, any plots involving Set A, drug in H_20, in Study 6 use only the last two or three points in calculating the regression line.

That the pH changes at all, however, may be a useful approach to obtaining a rough estimate of the pH of optimum stability of a given compound. This approach is explored in Appendix D.

Figure IV-G-1 SKB106203 Kinetics: Study 6, 93°C
Mean pH vs Time, +/- Standard Deviation
Average Across 4 Drug Concentrations and 4 Treatments: A, B, C, D
Data from Table III-F-2



IV-H Summary of Variables Which May Affect the Kinetics of Degradation of SKB106203

Variables which might affect the degradation of SKB106203 may be divided into three broad categories: those which affect the concentration of oxygen in solution, those which affect the cmc, and others. Not all of these may cause an effect at a given pH.

In future studies, one might try to keep constant all but one of these variables, although this may be too complex, since they are interrelated. The goal of future work would be to establish which of these variables are truly important, and which may be ignored.

Variables which might affect the concentration of oxygen in solution would be:

a) Temperature: as temperature is increased, one would expect the oxygen concentration in solution to decrease

b) Electrolyte concentration: KCl may salt-in or salt-out O_2, depending on the temperature and concentration of KCl

c) Drug Concentration: above the cmc, more O_2 may be solubilized

Variables which might affect the cmc are:

a) Temperature: cmc as a function of temperature exhibits a broad minimum; the cmc may be higher at 60°C than at 93°C, but be the same at both 25 and 93°C

b) Electrolyte Concentration: KCl may lower the cmc as may buffer salts, although not to the same extent

Other effects on the kinetics of the drug substance are:

a) KCl and/or phosphate trace metals may catalyze the reaction, but KCl itself may inhibit the reaction

b) Drug Concentration: the kinetics may be different at concentrations just above the cmc vs concentrations greatly exceeding the cmc

c) Oxygen Availability: the oxygen concentration in solution may not necessarily reflect the amount available for reaction in a micellar solution

d) As the drug degrades to below the cmc, O_2 may become available for reaction, unless it is salted-out by electrolyte in the bulk

e) The presence of electrolyte may increase the solubility of the degradation products; mixed micelles may form which might in turn affect oxygen solubility etc.

V SUMMARY

The following summarizes the findings of this work:

1. There is no buffer catalysis by phosphate at pH 7, however, there is an ionic strength effect.
2. There is no drug concentration effect between 0 and 0.5 mg/ml in buffer of low molarity and low ionic strength. There also does not appear to be a drug concentration effect between 1 and 10 mg/ml.
3. KCl, used to adjust the ionic strength, appears to affect the kinetics.
4. Some reactions are oxidation reactions at lower temperatures and appear not to be oxygen sensitive at higher temperatures.
5. This phenomenon could be attributed to the higher concentration of oxygen in solution as a consequence of temperature, salting-in, and micellization of the drug substance.
6. Where curvature has been noted, the data have been fairly-well linearized by Equation IV-8.
7. The downward curvature noticed at 60°C may also be described by a global equation of the form $\ln y = a + bt + ct^2$, where the parameters are a function of ionic strength.
8. Trace metal catalysis may play a role in the observed behavior.
9. An equation describing oxygen concentration as a function of temperature and ionic strength for NaCl has been derived.

10. An equation has been developed to roughly predict the pH of optimum stability by following the kinetics of degradation in water.

VI FUTURE WORK

One future goal is to establish Henry's law constants and salting-out constants at the higher temperatures and in matrices commonly encountered in accelerated stability testing of pharmaceuticals.

This may be accomplished directly by an apparatus, designed but not yet tested, in which a solution may be heated to the desired temperature, and the oxygen concentration of the solution, cooled to room temperature without introduction of air, measured using an oxygen electrode. It would be possible to use the same apparatus to run kinetic studies such that kinetic parameters and oxygen concentrations would be obtained concurrently.

Alternately, the oxygen concentrations may be obtained indirectly by monitoring an oxidation of a reaction at lower temperatures, using an oxygen electrode to measure the oxygen concentration, and extrapolating to higher temperatures. Appendix F develops an equation relating oxygen concentration to temperature and electrolyte concentration.

It would also be desirable to determine the cmc of the compound in the various matrices used in the studies. If sufficient quantities of the cinnamic acid and sulfoxide analogues are available, it would be desirable to obtain the cmc's of these as well.

An equation has been proposed in Appendix D to find the pH of maximum stability. This could be ascertained by following the

kinetics in water of several compounds which have a well-characterized pH-rate profile.

The model describing micellar kinetics should be further refined to include the temperature effect, the electrolyte effect, and the drug concentration effect as outlined in IV-H. Experiments in which the amount of oxygen is controlled, and various initial concentrations of oxygen tested are suggested.

A complete pH-rate profile of the compound should be determined once the kinetics at pH 7 are better understood.

Appendix A

Development of Global Equation to Describe Data Obtained in Study 4 at 60°C

The general form of the equation is that of a second-degree polynomial:

$$\ln y = A + Bt + Ct^2 \qquad (A\text{-}1)$$

where y = % Remaining

t = time.

From the plot of ln(-coefficient of t) vs Buffer Molarity one obtains

ln(-coeff. of t) = -5.4727-2.1944M (R^2 =0.982)

coeff. of t = -exp(-5.4727-2.1944M)

= -exp(-5.4727)*-exp(-2.1944M)

B = coeff of t = -0.00420*exp(-2.1944M) (A-2)

Similarly, from the plot of ln(-coeff of t^2) vs Buffer Molarity (Figure A-1), one obtains:

ln(-coeff. of t^2) = -11.125 +2.8718M (R^2 = 0.969)

C = coeff. of t^2 = $-1.474 \times 10^{-5} * \exp(2.8718M)$ (A-3)

A is taken to be ln 100= 4.605.

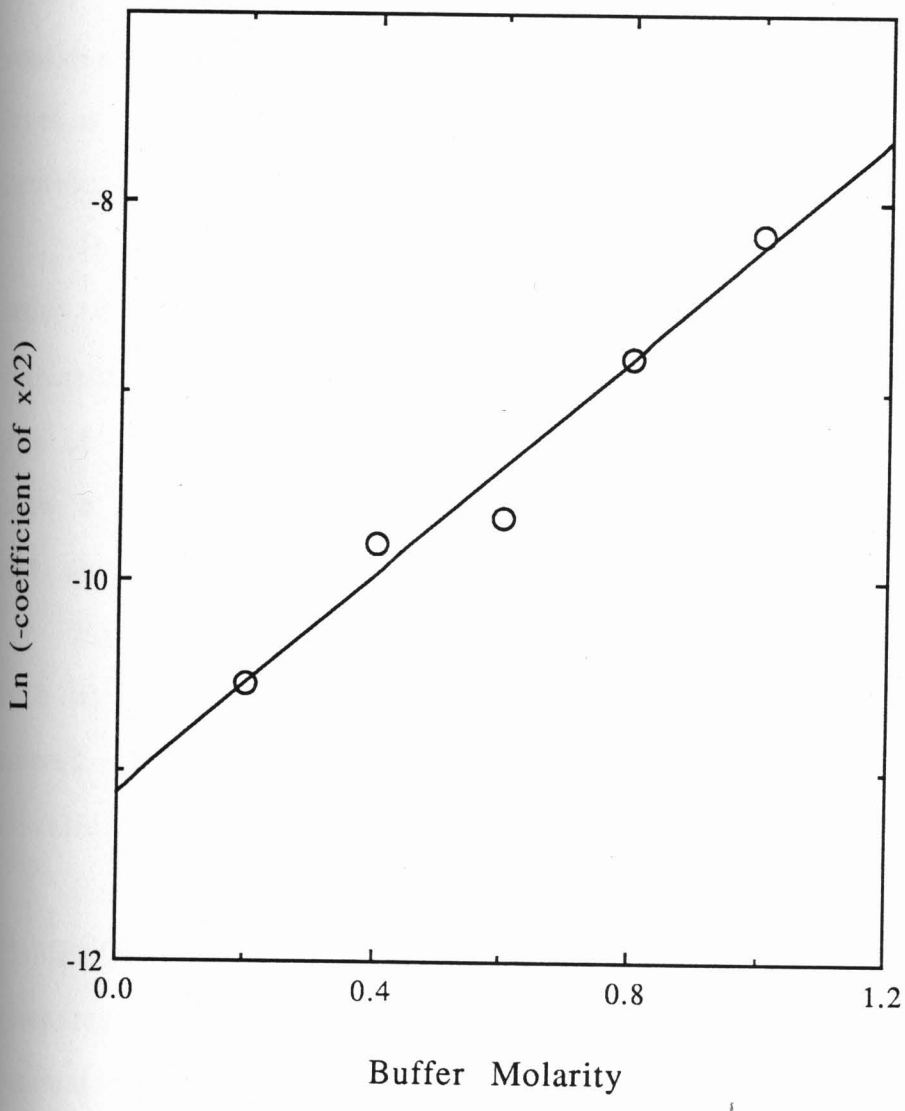
Thus, for a given Buffer Molarity, with no ionic strength adjustment, the coefficients B and C may be calculated, substituted into the Global equation, and predictions made for various time points at 60°C for SKB 106203. The Table A-1 shows the fitted values obtained using the global equation. The predicted values as a function of observed values are also shown in Figure IV-C-2.

Table A-1 SKB106203 Kinetics, Study 4, 60°C:
In pH 7 Buffers with No Ionic Strength Adjustment
Initial Drug Concentration = 0.2 mg/ml
Curve-Fit Results Using Global Equation A-1
with Parameters Described by Equations A-2 and A-3

days	0.2 M, y	0.2M, y^	0.4M , y	0.4M, y^	0.6M , y	0.6M, y^	0.8M, y	0.8M, y^	1.0M	1.0M, y^
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
4	--	---	97.2	99.2	100.1	99.4	99.1	99.5	98.5	99.4
6	100.3	98.3	--	---	--	---	---	---	--	---
25	82.7	91.9	95.0	93.0	95.6	92.3	90.4	89.6	88.1	84.0
45	81.1	84.0	92.4	84.1	91.5	80.4	83.1	71.9	76.0	57.8
69	76.2	73.2	68.2	71.0	57.7	62.4	46.6	47.3	35.5	28.0
91	63.7	62.9	52.1	58.0	42.6	45.6	25.8	27.8	11.8	11.1
110	54.1	54.1	39.0	47.0	31.1	32.5	15.5	15.6	4.8	4.1
147	37.9	38.1	24.3	28.3	16.7	14.2	3.8	3.8	0.4	0.4

Figure A-1 SKB106203 Kinetics: Study 4, 60°C
Semilogarithmic Plot of Coefficient of x^2 vs Buffer Molarity
Data from Figure IV-A-3

y = -11.12 + 2.87x R^2 = 0.969



Appendix B

Statistical Analyses

In all cases, passes means the statistical test fails to show a significant difference at the indicated confidence level; fails means there is a significant difference. Typically, a 95% confidence level is invoked; passing on the 99.5% confidence level is also acceptable, however one must bear in mind that the confidence band is broader.

In many cases, it was desired to compare regression lines in order to determine if the data could be pooled and a single regression line obtained. Statistically, in order to show that the data of two or more regression lines may be pooled, one must fail to show a significant difference in their residual variances, slopes, and elevations.

For two regression lines, a computer program, COMPAREL, has been written in Student MATLAB which allows the raw data to be entered, and returns F statistics for residual variances, slopes, and elevations.

For more than two regression lines, the program COMPARENLINES has been written in Student MATLAB which compares the slopes and elevations. In order to compare the residual variances for two or more regression lines, Bartlett's test is used. The program BARTLETTS has been written in Student MATLAB which accepts as input the degrees of freedom and error Sum of Squares for each regression line, and returns a Chi square statistic. These variables may be easily obtained from the ANOVA

table obtained by running the simple linear regression in STATWORKS. Or, one may use the values generated by COMPARENLINES.

The tables presented in Appendix B contain data calculated using Statworks software as well as from the computer programs COMPAREL, BARTLETTS, and COMPARENLINES. In some cases, columns may not add exactly due to roundoff error; that is, the computer uses all the digits, whereas the table may only report three or four significant digits of the intermediate results. Appendix B-1 provides additional details in order to clarify the procedures.

The computer programs may be found in Appendix C.

Appendix B-1

STUDY 2 Table III-B-1 Figure IV-A-6

This analysis compares the kinetic data (treated as first-order) obtained in buffers of various molarities whose ionic strength has been adjusted to 2.2 with KCl. BARTLETTS compares the residual variances; the intermediate results are presented for clarification of Bartlett's method.

Bartlett's Test

Buffer M	v_i	$v_i s_i^2$	$1/v_i$
0.4	3	0.034969	0.333
0.6	4	0.175799	0.250
0.8	4	0.052796	0.250
0.01	2	0.005427	0.500
0.6*	5	0.041776	0.200
	Σ18	0.31077	1.5333

*data from Study 3

v_i = degrees of freedom = N-2; N=number of time points
s_i = standard error of estimate
(s_i^2)=Mean Squares error
$v_i s_i^2$=error Sum of Squares, from Statworks ANOVA table in Regression, simple; or, from COMAPARENLINES

$s^2=\Sigma v_i s_i^2/\Sigma v_i = 0.31077/18 = 0.017265$
$\Sigma(v_i)\ln s^2 = 18*\ln(0.017265) = -73.064$
$M = \Sigma(v_i)\ln s^2 - \Sigma v_i \ln(s_i^2) = -73.064-(-78.908) = 5.8449$
$C=1 + (1/3(4))*(1.5333-1/18) = 1.1231$

$Chi^2 = M/C = 5.8449/1.1231 = 5.2040 < Chi^2_{crit(95,4)}=9.49$ PASSES

Now, running COMPARENLINES completes the analysis by comparing slopes and elevations:

Within	df	Σx2	Σxy	Σy2	b	df	SS
0.4 M	4	54.8	-10.8	2.18	-0.198	3	0.0350
0.6 M	5	125.3	-27.3	6.11	-0.218	4	0.1758
0.8 M	5	12.5	-25.4	5.21	-0.203	4	0.0528
0.01 M	4	54.8	-18.1	6.16	-0.331	3	0.1660
0.6 M*	6	106.8	-19.6	3.66	-0.184	5	0.0418
		Σ 467.1	-101.3	23.32		19	0.4714
Pooled					-0.217		1.3416
					Difference Between Slopes		0.8703
Within+Between		480.2	-102.5	23.85			0.6336

MS (pooled) 0.0248
MS (W+B) 0.0671

	$F_{crit(95)}$	$F_{crit(99.5)}$
$F_{slopes(4,19)}=0.8703/(0.0248*4)=8.77$	2.90 (Fails)	5.27 (Fails)
$F_{elevation(4,20)}=0.6336/(0.0671*4)=2.36$	2.87 (Passes)	

The data fails the test. Repeat the test, omitting the 0.01 M data, since this has a slope which deviates from the rest:

BARTLETTS Test for Residual Variances:

$Chi^2=3.97$ $Chi^2_{crit(95,3)}=7.81$ Passes

COMPARENLINES:

$F_{slopes(3,16)}=1.1$	$F_{crit(95,3,16)}=3.24$	Passes
$F_{elevation(3,17)}=0.35$	$F_{crit(95,3,17)}=3.20$	Passes

Conclusion: The data may be pooled, excepting the 0.01 M data.

Appendix B-2

Study 3 Table III-C-1-a Figure IV-D-2

BARTLETTS (SSE from Statworks)

	0.05M		0.6M	
	SSE	df	SSE	df
0.05 mg/ml	0.027472	7	0.427574	3
0.10 mg/ml	0.015703	7	0.161896	3
0.20 mg/ml	0.023998	7	0.041776	5
0.50 mg/ml	0.020065	7	0.490762	5
$Chi^2_{(95,3)}$	0.564		6.85	

$Chi^2_{crit(95,3)}$=7.81 Passes

COMPARENLINES

0.05 M pH 7 Phosphate Buffer

Within	df	Σx2	Σxy	Σy2	b	df	SS
0.05 mg/ml	7	258	-18.5	1.36	-0.0718	6	0.0275
0.1 mg/ml	8	338	-25.5	2.13	-0.0754	7	0.2061
0.2 mg/ml	8	338	-23.2	1.61	-0.0685	7	0.0240
0.5 mg/ml	8	338	-22.0	1.45	-0.0651	7	0.0201
		Σ1273	-89.2	6.6		27	0.2776
Pooled					-0.0701		0.2974
			Difference Between Slopes				0.0198
Within+Between		1280	-90.1	6.7			0.3798
Between Adjusted Means							0.0824

MS (Pooled) 0.0106

	$F_{crit(95)}$
$F_{slopes\ (3,27)}$=0.642	2.96 (Passes)
$F_{elevation(3,28)}$=2.59	2.95 (Passes)

Conclusion: There is no significant difference between the four drug concentrations in 0.05M buffer at the 95% confidence level; the data may be pooled.

Appendix B-3

0.6 M pH 7 Phosphate Buffer, Table III-C-1-b, Figure IV-D-2

BARTLETS: see Appendix B-2

COMPARENLINES

Within	df	Σx2	Σxy	Σy2	b	df	SS
0.05 mg/ml	4	58.8	-19.9	7.16	-0.338	3	0.4276
0.1 mg/ml	4	58.8	-20.2	7.11	-0.344	3	0.1619
0.2 mg/ml	7	215.5	-42.6	8.53	-0.198	6	0.0835
0.5 mg/ml	6	106.8	-24.7	6.21	-0.231	5	0.4908
		Σ 440.0	-107.5	29.02		17	1.1637
Pooled					-0.244		2.7477
					Difference Between Slopes		1.5840
Within+Between		459.0	-106.4	29.4			4.7428
Between Adjusted Means							1.9951

MS (Pooled) 0.1526

	$F_{crit(95)}$	$F_{crit(99.5)}$
$F_{slopes\ (3,17)}=7.71$	3.20(Fails)	6.16 (Fails)
$F_{elevation(3,18)}=4.36$	3.16(Fails)	6.03 (Passes)

Repeating, omitting the 0.2 mg/ml:

	$F_{crit(95)}$
$F_{slopes(2,11)}=$ 3.43	3.98 (Passes)
$F_{elevation(2,12)}=$ 3.23	3.89 (Passes)

Conclusion: The data may be pooled, omitting the 0.2 mg/ml data.

Appendix B-4

STUDY 6
Set A (1.0, 1.5, 2.0, 10.0 mg/ml); Table III-F-1
May they be pooled?

Bartlett's Test for Residual Variances

mg/ml	$SS=v_i s_i^2$	v_i=df	s_i^2=Mean Squares	$\ln(s_i^2)$	1/df
1.0	5.343x10^-3	6	8.904x10^-4	-7.024	0.167
1.5	3.962x10^-3	6	6.603x10^-4	-7.323	0.167
2.0	2.01 x10^-2	6	3.349x10^-3	-5.699	0.167
10.0	1.05 x10^-3	5	2.102x10^-4	-8.467	0.2
	Σ 3.046x10^-2	23			0.7

$\Sigma v_i \ln(s_i^2)$ = -42.144-43.939-34.194-42.335 = 162.611
$s^2 = \Sigma v_i s_i^2 / \Sigma v_i$ = 3.046x10^2/23 = 1.324x10^-3
$\Sigma v_i(\ln(s^2))$= 23*ln(1.324x10^-3 = -1.524x10^2
$M=\Sigma v_i(\ln(s^2))-\Sigma v_i \ln(s_i^2)$=-1.524x10^2-(-162.611) =10.188
C=1+(1/3*3)*(0.7001-0.0435)=1.073

Chi^2 = M/C = 9.495
$Chi^2_{crit(97.5,3)}$= 9.348 Fails
$Chi^2_{crit(99,3)}$= 11.345 Passes

Passes at the 99% level
Repeat the analysis, leaving out the 2 mg/ml data

STUDY 6 Set A Without 2 mg/ml data
Bartlett's Test

Σ SS ($=v_i s_i^2$) = 0.010356 ΣΣ v_i(=df) = 17 1/df = 0.534
$\Sigma v_i \ln(s_i^2)$ = 128.42
s^2 = 0.010356/17 = 6.092x10^-4
$\Sigma v_i(\ln(s^2))$= -1.258x10^2
M=-1.258x10^2-(-128.42)= 2.617
C=1+(1/3*2)*(0.5334-0.05882)= 1.0791

Chi^2=M/C=2.4252
$Chi^2_{crit(95,2)}$=5.991 Passes*
*passes even at the 90% level, which is a tighter tolerance level

COMPARENLINES

Set A

Within	df	Σx2	Σxy	Σy2	b	df	SS
1.0 mg/ml	7	5798	-40.18	0.2838	-0.00693	6	0.00535
1.5 mg/ml	7	5798	-37.34	0.2445	-0.00644	6	0.00399
2.0 mg/ml	7	5798	-42.50	0.3317	-0.00733	6	0.02019
10.0 mg/ml	6	5783	-43.72	0.3317	-0.00756	5	0.00109
		23176	-163.74	1.1916		23	0.03062
Pooled					-0.00706		0.03481
				Difference Between Slopes			0.00419
Within+Between		23177	-163.66	1.2429			0.0873
Between Adjusted Means							0.05248

MS (Σ)=0.03062/23=0.00133

MS (pooled)=0.00145

		$F_{crit(95,3,23)}$	$F_{crit(95,3,24)}$
$F_{slopes\ (3,23)}$=	1.05	3.03 (Passes)	
$F_{elevation(3,24)}$=	12.06	3.01 (Fails)	4.72 (Fails)

Since the data fail the test, repeat, omitting the 2 mg/ml data

		$F_{crit(95,2,17)}$
$F_{slopes\ (2,17)}$=	2.97	3.59 (Passes)
$F_{elevation(2,18)}$=	0.21	3.55 (Passes)

CONCLUSION The data from Set A may be pooled, excepting the 2 mg/ml data.

Appendix B-5

STUDY 6 SET B May they be pooled?
Table III-F-1 Figure IV-D-5

Bartlett's Test

mg/ml	$SS=v_i s_i^2$ *	v_i=df	s_i^2=Mean Squares	$\ln(s_i^2)$	1/df
1.0	0.1176	6	0.0196	-3.932	0.167
1.5	1.956x10^-2	6	3.26x10^-3	-5.726	0.167
2.0	6.695x10^-3	5	1.339x10^-3	-6.6158	0.2
10.0	8.663x10^-3	5	1.733x10^-3	-6.3579	0.2
	Σ0.15252	22			0.733

*SS(error) on Statworks

$\Sigma v_i \ln(s_i^2)$= 23.593+34.356+33.079+31.790 = 122.82
$s^2 = \Sigma v_i s_i^2 / \Sigma v_i$ = 0.15252/22 = 6.9327x10^-3
$\Sigma v_i(\ln(s^2))$= -1.0937x10^2
$M=\Sigma v_i(\ln(s^2))-\Sigma v_i \ln(s_i^2)$=-1.0937x10^2+122.818=13.445
C=1+(1/3*3)*(0.7334-0.04545)=1.0764

Chi^2 = M/C = 12.49
$Chi^2_{crit(99.5,3)}$= 12.838 Passes

Redo, omitting the 10 mg/ml data set, and the 86-day point from the 1.0 mg/ml data set:

	df	SSE
1.0 mg/ml	5	1.0383e-2
1.5 mg/ml	6	1.9712e-2
2.0 mg/ml	5	6.6717e-3

Chi^2 = 0.996

$Chi^2_{crit(90,2)}$=4.605 Passes

COMPARENLINES
Set B

Within	df	Σx2	Σxy	Σy2	b	df	SS
1.0 mg/ml	7	5798	-149.4	3.967	-0.0258	6	0.1180
1.5 mg/ml	7	5798	-113.7	2.251	-0.0196	6	0.0197
2.0 mg/ml	6	745	-12.61	0.220	-0.0169	5	0.0067
10.0 mg/ml	6	745	-23.84	0.771	-0.0320	5	0.0084
		13086	-299.6	7.209		22	0.1528
Pooled					-0.0229		0.3511
				Difference Between Slopes			0.1983
Within+Between		13759	-314.4	7.565			0.3797
Between Adjusted Means							0.0286

MS (Σ) = 0.00695
MS (Pooled) = 0.0153

		$F_{crit(99.5)}$
$F_{slopes\ (3,22)}$=	9.56	5.65 (Fails)
$F_{elevation(3,23)}$=	0.62	3.03 (Passes)

Redo, omitting the 2.0 mg/ml data:

		$F_{crit(99.5)}$
$F_{slopes\ (2,17)}$=	9.89	7.35 (Fails)
$F_{elevation(2,18)}$=	0.64	7.21 (Passes)

Redo, omitting the 10 mg/ml data:

		$F_{crit(99.5)}$
$F_{slopes\ (2,17)}$=	7.83	7.35 (Fails)
$F_{elevation(2,18)}$=	0.56	7.21 (Passes)

Redo, omitting the 86-day data point from the 1.0 mg/ml data:

		$F_{crit(99.5)}$
$F_{slopes\ (3,21)}$=	21.36	5.73 (Fails)
$F_{elevation(3,22)}$=	0.70	5.65 (Passes)

Redo, omitting the 10 mg/ml data and the 86-day point from the 1.0 mg/ml data set:

		$F_{crit(95)}$
$F_{slopes\ (2,16)}$=	4 34	4.69 (Passes)
$F_{elevation(2,17)}$=	0.68	7.62 (Passes)

Conclusion: The 1.0, 1.5, and 2.0 mg/ml data may be pooled, omitting the last data point from the 1.0 mg/ml data set.

Appendix B-6

STUDY 6 Set C Table III-F-1

May they be pooled?
May have to omit 2 mg/ml

mg/ml	$SS=v_i s_i^2$ *	v_i=df	s_i^2=Mean Squares	$\ln(s_i^2)$	1/df
1.0	0.1315	6	0.02191	-3.821	0.167
1.5	9.157x10^-3	4	0.002289	-6.080	0.250
2.0	0.02664	5	0.005328	-5.235	0.200
10.0	1.806x10^2	5	0.003612	-5.623	0.200
	Σ0.18536	20			0.817

*SS(error) on Statworks

$\Sigma v_i \ln(s_i^2)$ = -22.924-24.318-26.174-28.117 = -101.533
$s^2 = \Sigma v_i s_i^2 / \Sigma v_i$ = 9.268x10^-3
$\Sigma v_i(\ln(s^2))$= -93.624
$M=\Sigma v_i(\ln(s^2))-\Sigma v_i \ln(s_i^2)$=-93.624+101.533=7.909
C=1+(1/3*3)*(0.8167-0.05) =1.085

Chi^2 = M/C = 7.28
$Chi^2_{crit(95,3)}$= 7.82 Passes, even without omitting 2.0 mg/ml data

Note: if the test is repeated, omitting the 2.0 mg/ml data:
Chi^2 = M/C = 6.532
$Chi^2_{crit(95,2)}$= 5.991
$Chi^2_{crit(97.5,2)}$= 7.378

Passes at the 97.5% level

COMPARENLINES

Set C

Within	df	Σx2	Σxy	Σy2	b	df	SS
1.0 mg/ml	7	5798	-50.2	0.5662	-8.66e-3	6	0.132
1.5 mg/ml	5	745	- 11.6	0.1889	-1.55e-2	4	9.16e-3
2.0 mg/ml	6	745	-26.1	0.9423	-3.50e-2	5	2.66e-2
10.0 mg/ml	6	5406	-72.4	0.9865	-1.34e-2	6	1.81e-2
		12694	-160.2	2.6840		20	0.186
Pooled					-1.26e-2		0.661
				Difference Between Slopes			0.475
Within+Between		13522	-160.0	2.7419			0.849
Between Adjusted Means							0.188

MS(Σ) = 9.28e-3

MS(pooled) = 3.15e-2

		$F_{crit(99.5)}$
$F_{slopes\ (3,20)}$=	17.09	5.82 (Fails)
$F_{elevation(3,21)}$=	1.99	5.73 (Fails)

Repeat, omitting 2.0 mg/ml data:

		$F_{crit(95)}$
$F_{slopes\ (2,15)}$=	3.64	3.68 (Passes)
$F_{elevation(2,16)}$=	0.46	3.63 (Passes)

The data may be pooled, omitting the 2.0 mg/ml data; the 2.0 mg/ml data are significantly different from the 1.0, 1.5, and 10.0 mg/ml data on the 95% confidence level.

Appendix B-7
Study 6 Set D Table III-F-1 Figure IV-D-3
May they be pooled?

mg/ml	$SS=v_i s_i^2$ *	v_i=df	1/df	$v_i \ln(s_i^2)$
1.0	0.00401	6	0.167	-43.87
1.5	0.00367	6	0.167	-44.392
2.0	0.00584	6	0.167	-41.604
10.0	0.00436	6	0.167	-43.363
Σ	0.01788	24	0.668	-173.229

*SS(error) on Statworks
$s^2 = \Sigma v_i s_i^2 / \Sigma v_i$ = 7.451 x 10^-4
$\Sigma v_i(\ln(s^2))$= -172.847
$M=\Sigma v_i(\ln(s^2))-\Sigma v_i \ln(s_i^2)$=-172.847+173.229 =0.3820
C=1+(1/3*3)*(0.6667-0.04167)=1.0694

Chi^2 = M/C = 0.3572
$Chi^2_{crit(95,3)}$= 7.815 Passes (even at 90% level)

COMPARENLINES
Set D

Within	df	Σx2	Σxy	Σy2	b	df	SS
1.0 mg/ml	7	5798	-37.8	0.2505	-6.52e-3	6	4.047e-3
1.5 mg/ml	7	5798	-38.0	0.2526	-6.55e-3	6	3.674e-3
2.0 mg/ml	7	5798	-46.1	0.3724	-7.95e-3	6	5.864e-3
10.0 mg/ml	7	5798	-35.3	0.2197	-6.09e-3	6	4.426e-3
		23190	-157.2	1.0953		24	1.801e-2
Pooled					-6.78e-3		2.938e-2
					Difference Between Slopes		1.137e-2
Within+Between		23190	-157.2	1.1060			
Between Adjusted Means							4.011e-2

MS(Σ) = 7.505e-4
MS(pooled) = 1.175e-3

		$F_{crit(95)}$	$F_{crit(99.5)}$
$F_{slopes\ (3,24)}$=	5.05	3.01 (Fails)	5.52 (Passes)
$F_{elevation(3,25)}$=	3.04	2.99 (Fails)	5.46 (Passes)

Conclusion: All the data from Set D may be pooled; it is not significantly different on the 99.5% Level.

Appendix B-8
STUDY 5 Table III-E-1 Figure IV-D-4
BARTLETTS (SSE from Statworks)

	df	SSE
1 mg/ml	4	0.003608
2 mg/ml	4	0.001510
5 mg/ml	5	0.001650
10 mg/ml	5	0.000398

$Chi^2 = 5.42$
Chi^2 (without 1 mg/ml) = 2.66
$Chi^2_{crit(95,3)} = 7.81$ Passes

COMPARENLINES

Within	df	Σx2	Σxy	Σy2	b	df	SS
1 mg/ml	5	56.2	-2.52	0.116	-0.045	4	0.0036
2 mg/ml	5	40.8	-1.16	0.035	-0.029	4	0.0015
5 mg/ml	6	61.3	-1.61	0.044	-0.026	5	0.0016
10 mg/ml	6	61.3	-1.39	0.032	-0.023	5	0.0004
		219.6	-6.69	0.227		18	0.0072
	Pooled				-0.030		0.0237
			Difference Between Slopes				0.0165
Within+Between		221.8	-6.62	0.266			0.0681
Between Adjusted Means							0.044
MS(Σ) =		3.98e-4					
MS(pooled)=		1.25e-3					

		$F_{crit(95)}$	$F_{crit(99)}$
$F_{slopes\ (3,18)}$=	13.83	3.16 (Fails)	5.09 (Fails)
$F_{elevation(3,19)}$=	11.90	3.13 (Fails)	5.01 (Fails)

Repeat, omitting 1 mg/ml data:

		$F_{crit(95)}$	$F_{crit(99)}$
$F_{slopes(2,14)}$=	1.80	3.74 (Passes	
$F_{elevation(2,15)}$=	6.26	3.68 (Fails)	6.36 (Passes)

Conclusion: The 2, 5, and 10 mg/ml data may be pooled.

Appendix B-9

STUDY 6, 93°C Table III-F-1

The data for Sets A and D of Study 6 (93°C) agree quite well at 1.0 and 1.5 mg/ml, however become more scattered at 2.0 and 10.0 mg/ml. Sets A and D are shown to be equivalent, if one ignores the 2 mg/ml data of Set A. Thus, it may be concluded that drug in 0.1% EDTA serves as a control, that is, it is not different than drug in water, and EDTA itself does not accelerate the degradation of the drug, nor protect it in any way. These sets of experiments also show that the drug itself does not contain any trace metals which may catalyze the reaction.

Set A vs D Statistical Comparison for each concentration

A: Drug in H20

D: Drug in 0.1% EDTA

1.0 mg/ml

	Found*	$F_{critical}$**	Conclusion
$F_{residuals}$	1.3215	(95,6,6) =4.28	Passes
F_{slopes}	0.6232	(95,1,12)=4.75	Passes
$F_{elevation}$	2.0296	(95,1,13)=4.67	Passes

*using COMPAREL
**from F-Tables

1.5 mg/ml

	Found*	$F_{critical}$**	Conclusion
$F_{residuals}$	1.0861	(95,6,6) =4.28	Passes
F_{slopes}	0.0568	(95,1,12)=4.75	Passes

$F_{elevation}$	0.4561	(95,1,13)=4.67	Passes

2.0 mg/ml

	Found*	$F_{critical}$**	Conclusion
$F_{residuals}$	3.4427	(95,6,6) =4.28	Passes
F_{slopes}	0.5156	(95,1,12)=4.75	Passes
$F_{elevation}$	24.8876	(95,1,13)=4.67	Fails***

***at all confidence levels

10 mg/ml

	Found*	$F_{critical}$**	Conclusion
$F_{residuals}$	3.3795	(95,5,6) =4.39	Passes
F_{slopes}	12.4097	(95,1,11)=4.84	Fails
		(99.5,1,11)=12.23	Barely Fails
$F_{elevation}$	10.0168	(95,1,12)=4.75	Fails
		(99.5,1,12)=11.75	Passes

Conclusion: Sets A and D not significantly different at low concentrations; slightly different at higher concentrations.

Appendix B-10

STUDY 6, 93°C Table III-F-1 Figure IV-E-1

Data from Sets B and C at each concentration are shown to be significantly different where sufficient data has been collected, namely at the 1.0 and 1.0 mg/ml levels. When all the data from Sets B and C are pooled, they are shown not to be equivalent to each other,[54] and show degradation rates which are 3 and 1.6 times greater than the control (Sets A and D), respectively.

Compare Sets B and C for each concentration, then pool all concentrations in each set.

B: Drug in 2.2 M KCl
C Drug in 2.2 M KCl + 0.1% EDTA

Calculations done using COMPAREL (Matlab)

1.0 mg/ml

		F_{crit}	
F_{resid}=	1.1158	(95,6,6)= 4.28	Passes
F_{slope}=	40.8733	(95,1,12)= 4.75	Fails
F_{elev}=	2.5399	(95,1,13)= 4.67	Passes

1.0 mg/ml (omit last point)

		F_{crit}	
F_{resid}=	10.571	(95,5,6)= 4.39	Fails
		(99.5,5,6)=11.46	Passes
F_{slope}=	1.7759	(95,1,11)= 4.84	Passes
F_{elev}=	0.9221	(95,1,12)= 4.75	Passes

Passes, at the 99.5% Level, omitting the last data point

1.5 mg/ml

		F_{crit}	
F_{resid}=	1.4342	(95,6,4)= 6.16	Passes
F_{slope}=	3.8272	(95,1,10)=4.96	Passes
F_{elev}=	2.8559	(95,1,11)=4.84	Passes

Passes, at the 95% Level, but no 86-day point for C; last data point at 32 days

2.0 mg/ml

		F_{crit}	
F_{resid}=	3.986	(95,5,5)= 5.05	Passes
F_{slope}=	36.8199	(95,1,10)=4.96	Fails
F_{elev}=	2.9277	(95,1,11)=4.84	Passes

Fails

10.0 mg/ml

		F_{crit}	
F_{resid}=	2.0960	(95,5,5)= 5.05	Passes
F_{slope}=	85.5941	(95,1,10)=4.96	Fails
F_{elev}=	6.1003	(95,1,11)=4.84	Fails
		(97.5,1,11)=6.72	Passes

Fails

Compare Set B and C, pooling all concentrations

		F_{crit}	
F_{resid}=	2.3992	(95,28,26)= 1.92	Fails
		(99,28,26)= 2.53	Passes
F_{slope}=	36.3733	(99.5,1,54)= 8.83	Fails
F_{elev}=	4.8218	(97.5,1,55)= 5.39	Passes

Fails; there is a significant difference between B and C if all the data are considered; at the lower concentrations, 1 and 1.5 mg/ml, there does not appear to be a significant difference, however this is most likely due to the absence of 86-day data points which may have revealed the difference.

Appendix B-11

STUDY 6 Table III-F-1
All A (N=31) vs All D (N = 32)

Using Program COMPARE.L (MATLAB) obtain $F_{residuals}$, F_{slope}, and $F_{elevation}$

	Found	F-statistic	Fcrit*		Conclusion
F_{resid}	= 2.2513	(95,29,30)	(95,30,30)	=1.84	Fails
F_{slopes}	= 0.4251	(95,1,59)	(95,1,60)	= 4.00	Passes
$F_{elev.}$	= 17.7574	(95,1,60)	(95,1,60)	= 4.00	Fails

*from F-Table

Conclusion: Fails at the 95% Level; repeat the comparison, leaving out the 0.2 mg/ml data of Set A.

	Found	F-statistic	Fcrit*		Conclusion
F_{resid}	= 1.9489	(95,21,30)	(95,21,30)	= 1.98	Passes
F_{slopes}	= 0.3592	(95,1,51)	(95,1,51)	= 4.04	Passes
$F_{elev.}$	= 7.8805	(95,1,52)	(95,1,52)	= 4.04	Fails
			(99.5,1,52)	= 8.63	Passes

*from Table

Conclusion: Passes on the 99.5% level, omitting the 0.2 mg/ml data in Set A; this omission is justified based on the disparity in pH

Appendix B-12

Study 7 Table III-G

Data from COMPAREL (Matlab)

Set A 0.2M (N=4) vs 0.8M (N=5) A=no ionic strength control

		F_{crit}		
F_{resid}=	1.0873	(95,2,3)=	9.55	Passes
F_{slope}=	9.3882	(95,1,5)=	6.61	Fails
		(97.5,1,6)=	10.01	Passes
F_{elev}=	0.1414	(95,1,6)=	5.99	Passes

Set B 0.2M (N=5) vs 0.8M (N=5) B=2.23 M KCl

		F_{crit}		
F_{resid}=	1.077	(95,3,3)=	9.28	Passes
F_{slope}=	2.4846	(95,1,6)=	5.99	Passes
F_{elev}=	2.0510	(95,1,7)=	5.59	Passes

Set C 0.2M (N=5) vs 0.8M (N=5) C=2.23 M KCl + 0.1% EDTA

		F_{crit}		
F_{resid}=	1.040	(95,3,3)=	9.28	Passes
F_{slope}=	1.0509	(95,1,6)=	5.99	Passes
F_{elev}=	0.8087	(95,1,7)=	5.59	Passes

Set D 0.2M (N=5) vs 0.8M (N=5) D=0.1 %EDTA control

		F_{crit}		
F_{resid}=	1.3867	(95,3,3)=	9.28	Passes
F_{slope}=	1.5637	(95,1,6)=	5.99	Passes
F_{elev}=	0.4199	(95,1,7)=	5.59	Passes

Conclusion: No difference at the 95% level between 0.2 and 0.8 M in Sets B, C, and D. Therefore, pool all the data and compare B vs C, B vs D, C vs D

Appendix B-13

STUDY 7 Table III-G

Statistical Analysis using COMPAREL

all B vs all C

		F_{crit}		
F_{resid}=	2.319	(95,3,3)=	9.28	Passes
F_{slope}=	3.5461	(95,1,6)=	5.99	Passes
F_{elev}=	2.4844	(95,1,7)=	5.59	Passes

all B vs all D

		F_{crit}		
F_{resid}=	3.0762	(95,3,3)=	9.28	Passes
F_{slope}=	0.7797	(95,1,6)=	5.99	Passes
F_{elev}=	0.7430	(95,1,7)=	5.59	Passes

all C vs all D

		F_{crit}		
F_{resid}=	1.3265	(95,3,3)=	9.28	Passes
F_{slope}=	2.0213	(95,1,6)=	5.99	Passes
F_{elev}=	1.3392	(95,1,7)=	5.59	Passes

Conclusion: Statistically, no difference between B, C, D, even at the 90% level. In this case, the sparsity of the data may be leading to statistically invalid conclusions.

Appendix C MATLAB Computer Programs

These programs were written in Student MATLAB follow the procedures outlined in Statistical Methods, 7th edition, by Snedecor, G.W. and Cochran, W.G. The programs have been validated by inputting data from Snedecor and Cochran examples and obtaining the same results as those published. In the case of comparing multiple slopes, COMPARETEST, an abbreviated version of COMPARENLINES was written in order to validate the program against the published data, since only one example was given in the text, Example 18.9.1, and does not include raw data.

Appendix C-1

COMPAREL

```
% This program determines if 2 regressions may be pooled using
% the method outlined in Snedocor, G.W. and Cochran, W.G.,
% Statistical Methods7th ed., The Iowa State University Press,
% section 18.9, p. 385-88

% User must have data entered on worksheet; label x1, y1, x2, and
y2
% the 1xN matrices containing the x and y values for the 2
regression
% lines to be compared. If comparing >2 lines use COMPARENLINES.
% Note: COMPARENLINES will return the same values as COMPAREL
for Fslopes and Felevations, however does not calculate Fresiduals.

% This section calculates variables for the first regression line.

MX1=mean(x1);
S1=(MX1-x1);
S2=S1.^2;
SX1=sum(S2);
MY1=mean(y1);
S3=(MY1-y1);
S4=S3.^2;
SY1=sum(S4);
N1=size(x1);
N1=N1(2);
LAST1=sum(x1.*y1)-sum(y1)*sum(x1)/N1;
SLOPE1=LAST1/SX1;
SS1=SY1-(SLOPE1)^2*SX1;

%This section calculates variables for the 2nd regression line.

MX2=mean(x2);
S1=(MX2-x2);
S2=S1.^2;
SX2=sum(S2);
MY2=mean(y2);
S3=(MY2-y2);
S4=S3.^2;
```

```
SY2=sum(S4);
N2=size(x2);
N2=N2(2);
LAST2=sum(x2.*y2)-sum(y2)*sum(x2)/N2;
SLOPE2=LAST2/SX2;
SS2=SY2-(SLOPE2)^2*SX2;

% This section calculates pooled and summed variables

SUMSS=SS1+SS2;
DF1=N1-2;
DF2=N2-2;
SUMDF=DF1+DF2;

% ARE RESIDUAL VARIANCES THE SAME?


MS1=SS1/DF1;
MS2=SS2/DF2;
FRS=MS1/MS2
'IF F(RESIDUAL VARIANCES) < 1, TAKE RECIPROCAL'
pause

%ARE SLOPES DIFFERENT?

SUMY=SY1+SY2;
SUMX=SX1+SX2;
SUMLASTS=LAST1+LAST2;
SUMSLOPES=(SUMLASTS)/SUMX;
POOLEDSS=SUMY-((SUMSLOPES^2)*SUMX);

%DIFFERENCE IN SLOPES IS DIFFERENCE IN SS

DELSS=abs(POOLEDSS-SUMSS);
MSTOTAL=SUMSS/SUMDF;

FS=DELSS/MSTOTAL
'THIS IS F(SLOPES)'
pause

%F-TEST OF THE ADJUSTED MEANS
```

```
ALLX=[x1 x2];
MX3=mean(ALLX);
S1=(MX3-ALLX);
S2=S1.^2;
SX3=sum(S2);
ALLY=[y1 y2];
MY3=mean(ALLY);
S3=(MY3-ALLY);
S4=S3.^2;
SY3=sum(S4);

N3=size(ALLX);
N3=N3(2);
LAST3=sum(ALLX.*ALLY)-sum(ALLY)*sum(ALLX)/N3;

SLOPE3=LAST3/SX3;
SS3=SY3-(SLOPE3)^2*SX3;
SSB=SS3-POOLEDSS;
MSPOOLED=POOLEDSS/(N3-3);
FELEV=SSB/MSPOOLED
'F(COMPARISON OF ELEVATIONS)'
```

Appendix C-2

BARTLETTS

```
% Bartlett's Test For Variances (when >2 regression lines are being
%compared)

% User must be prepared to enter at the prompts the degrees of
%freedom (N-2) and error Sum of Squares. These may be easily
%obtained from the Simple Regression ANOVA table on Statworks.


Sumv=0;
SumSSE=0;
SumINVv=0;
Sumr=0;
n=input('How many regression lines do you wish to compare? ')
for i=1:n
i
v=input('Degrees of Freedom, N-2 ');
INVv=1/v;
SumINVv=SumINVv+INVv;                          %Σ1/v


SSE=input('Error Sum of Squares ');
Sumv=Sumv+v;
SumSSE=SumSSE+SSE;

MS=SSE/v;
q=log(MS);
r=v*q;                                         %Σv_i ln(s_i^2)
Sumr=Sumr+r;
end

ssquared=SumSSE/Sumv;
s=Sumv*(log(ssquared));                        %(Σv_i)(lns^2)
M=s-Sumr;
C=1+(1/((n-1)*3))*(SumINVv-(1/Sumv));

CHIsquared=M/C
```

Appendix C-3

COMPARENLINES

```
% This program determines if >2 regressions may be pooled using
% the method outlined in Snedocor, G.W. and Cochran, W.G.,
% Statistical Methods 7th ed., The Iowa State University Press,
% section 18.9, p. 385-88
% User may have data entered on worksheet: label x1=[1 2 3...n],
% y1, x2, y2 etc. the 1xN matrices containing the x and y values
% for the n regression lines to be compared; input them at the
% prompts i.e. input x1, etc. Or, at the prompt, input the individual
% value separated by a space.
%
% The residual variances must be calculated separately using
% Bartlett's Test.  If comparing only 2 lines, use COMPAREL.


SUMSS=0;
SUMDF=0;              %for deviations from regression, N-2
SUMY=0;
SUMX=0;
SUMXY=0;
sumdf=0;              %N-1
ALLX=[];
ALLY=[];

n=input('How many regression lines do you want to compare?    ')
     for i=1:n
RegressionNo=i
x=input('input x values for line  ');
ALLX=[ALLX x];
y=input('input y values for line  ');
ALLY=[ALLY y];

MX=mean(x);
S1=(MX-x);
S2=S1.^2;
SX=sum(S2);
MY=mean(y);
S3=(MY-y);
S4=S3.^2;
```

```
SY=sum(S4);
N=size(x);

N=N(2);
df=N-1;
sumdf=sumdf+df;

%DF is degrees of freedom for deviations from regression, N-2

DF=N-2;
SXY=sum(x.*y)-sum(y)*sum(x)/N;

SLOPE=SXY/SX;
dfSxSxySymb=[df SX SXY SY SLOPE]
pause
SS=SY-(SXY^2)/SX;
DevFromRegDFSS=[DF SS]
SUMSS=SUMSS+SS;
SUMDF=SUMDF+DF;
          if i==n
dfss=sumdf-n;
MSTOTAL=SUMSS/dfss;
          SumdfSumSSsumMS=[dfss SUMSS MSTOTAL]

          end
pause
%ARE SLOPES DIFFERENT?
SUMY=SUMY+SY;
SUMX=SUMX+SX;
SUMXY=SXY+SUMXY;
    end
PooledSumXxyY=[SUMX SUMXY SUMY]
pause

MEANSLOPE=SUMXY/SUMX;
POOLEDSS=SUMY-(MEANSLOPE^2)*SUMX;

PooledbSS=[MEANSLOPE POOLEDSS]
pause

%DIFFERENCE IN SLOPES IS DIFFERENCE IN SS
```

```
DELSS=abs(POOLEDSS-SUMSS)

FS=DELSS/(MSTOTAL*(n-1))

pause
'THIS IS F(SLOPES)'
dfa=n-1;

df=[dfa  dfss]
pause

%F-TEST OF THE ADJUSTED MEANS

MX3=mean(ALLX);
S1=(MX3-ALLX);
S2=S1.^2;
SX3=sum(S2);

MY3=mean(ALLY);
S3=(MY3-ALLY);
S4=S3.^2;
SY3=sum(S4);

N3=size(ALLX);
N3=N3(2);
SUMXY3=sum(ALLX.*ALLY)-sum(ALLY)*sum(ALLX)/N3;

SLOPE3=SUMXY3/SX3;
WandBSS3=SY3-(SUMXY3^2)/SX3
WandBsXsXYsY=[SX3 SUMXY3 SY3]
pause
SSB=WandBSS3-POOLEDSS;
df=SUMDF+1;
MSPOOLED=POOLEDSS/df;
SSbetwMSpooled=[SSB  MSPOOLED]

pause
FELEV=SSB/(MSPOOLED*(n-1))

'This is F, COMPARISON OF ELEVATIONS'

df=[dfa  df]
```

Appendix C-4

COMPARETEST

```
% This program determines if >2 regressions have the same slope
% according to the procedure outlined in Snedocor, G.W. and
% Cochran,W.G., Statistical Methods7th ed., The Iowa State
% University Press, section 18.9, p. 385-88.  This is an abbreviated
% version of COMPARENLINES in order to validate the comparison
% of slopes section in the program COMPARENLINES by inputting
% the data from example and obtaining the same F statistic.
% User must have calculated the degrees of freedom, the Σx^2, Σxy,
% Σy^2, and the slope; input them at the prompts.  If the user has
% data, then COMPARENLINES should be used.

SUMSS=0;
SUMDF=0;                %for deviations from regression, N-2
SUMY=0;
SUMX=0;
SUMXY=0;
sumdf=0;                %N-1

n=input('How many regression lines do you want to compare?    ')
     for i=1:n
RegressionNo=i

df=input('enter  df');
SX=input('enter  sumx');
SXY=input('enter  sumxy');
SY=input('enter  sumy');

sumdf=sumdf+df;

b=input('enter  slope');

SS=SY-(SXY^2)/SX

SUMSS=SUMSS+SS;

          if i==n
dfss=sumdf-n
MSTOTAL=SUMSS/dfss
```

```
        end

pause
%ARE SLOPES DIFFERENT?

SUMY=SUMY+SY;
SUMX=SUMX+SX;
SUMXY=SXY+SUMXY;

    end
PooledSumXxyY=[SUMX SUMXY SUMY]
pause

MEANSLOPE=SUMXY/SUMX;
POOLEDSS=SUMY-(MEANSLOPE^2)*SUMX;
PooledbSS=[MEANSLOPE POOLEDSS]
pause

%DIFFERENCE IN SLOPES IS DIFFERENCE IN SS

DELSS=abs(POOLEDSS-SUMSS)

FS=DELSS/(MSTOTAL*(n-1))
'THIS IS F(SLOPES)'
dfa=n-1;

df=[dfa  dfss]
pause
```

Appendix D

Pinpointing Optimum pH in Pharmaceutical Solution Systems, A Theoretical Approach

Knowing the effect of pH is necessary in order to optimize stability. This information must frequently be obtained at an early point in product development (e.g. in preformulation), where minimal amounts of drug are available. The procedure to follow shows that it may be possible to get a rough estimate of the pH of optimum stability of water-soluble compounds by simply monitoring the degradation in water and the corresponding pH values, without doing a complete pH-rate profile.

This has been done with SKB106203, a dicarboxylic acid, in Studies 5 and 6 at 93°C; the drug concentrations were 1.0, 1.5, 2.0, 5.0, and 10.0 mg/ml.

In an unbuffered system, the decomposition is such that a monoacid is formed from a dicarboxylic acid, and the pH increases as a function of time. This is shown in Figures III-E-2 and III-F-6.

If the true pH profile is such that there is a minimum in the rate constant at a given pH which is attained during the period where the unbuffered drug substance is tested, then the minimum k-value will be reached at some point, t_m, and at points before t_m, the rate will be decreasing with time, and at timepoints beyond k will increase with time. This can empirically be expressed as:

$$k = a - bt + ct^2 \qquad (1)$$

where the minimum occurs at t_m when

$$dk/dt = -b + 2ct_m^2 = 0 \qquad (2)$$

i.e.

$$t_m = b/2c \qquad (3)$$

Inserting the expression in a common (pseudo-) first order decomposition equation yields:

$$dC/C = -kdt = -(a - bt + ct^2)dt \qquad (4)$$

which, if C is expressed as fraction retained, integrates to:

$$\ln[C] = - at + (b/2)t^2 - (c/3)t^3] + \text{Constant} \qquad (5)$$

This type of graph fits nicely, as shown in Fig. A-1. Fitting the 1.0 mg/ml data from Study 6, Set A (Table III-F-1) to a third-degree polynomial gives the following least squares fit values:

$$\text{Constant} = 0.012 \qquad (6)$$

$$a = 0.0174 \qquad (7)$$

$$b = 2 \times 4.30 \times 10^{-4} = 8.6 \times 10^{-4} \quad (8)$$

$$c = 3 \times 3.64 \times 10^{-6} = 1.09 \times 10^{-5} \quad (9)$$

and the value of R^2 is 0.999. The minimum value of k occurs at

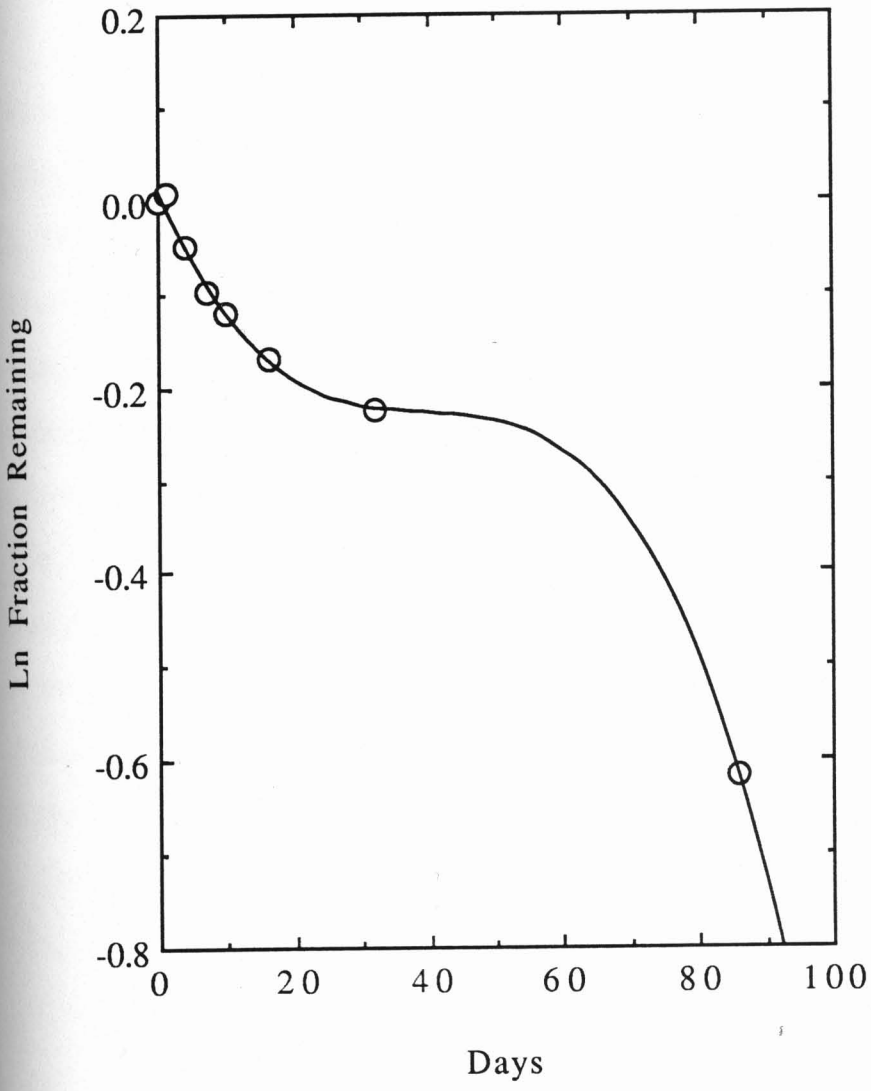
$$t_m = b/2c = (8.6/2*1.09) \times 10 = 39 \text{ days} \quad (9)$$

From Figure III-F-6 it can be seen that this corresponds roughly to a pH of 8.75.

As future work, the theory should be tested with a variety of compounds whose pH-rate profiles are well-established.

Figure A-D-1 SKB106203 Kinetics: Study 6, 93°C
1.0 mg/ml in H20

y = 1.15e-2 - 1.74e-2x + 4.30e-4x^2 - 3.64e-6x^3 R^2 = 0.999



Appendix E

Chemical Analysis of KCl (MCB, Reagent Grade)

Analysis Listed on Bottle:

	Maximum Impurities and Specification
Barium	~0.001%
Bromide	~0.01%
Ca, Mg, R_2O_3 ppt.	0.005%
Chlorate and Nitrate (NO_3)	0.003%
Heavy Metals as Pb	5 ppm
Insoluble Matter	0.005%
Iodide	0.002%
Iron	3 ppm
Nitrogen Compounds (as N)	0.001%
pH of 5% Solution at 25°C	5.4-8.6
Phosphate	5 ppm
Sodium	0.005%
Sulfate	0.001%

Appendix F

Relationship Between Henry's Law and the Setchenow Equation; the Description of a Global Equation to Predict Oxygen Solubility as a Function of Temperature and Ionic Strength; and Outlines of Experiments to Determine Oxygen Solubility

With knowledge of Henry's Law constants and salting-out constants, the amount of oxygen in solution cannot be calculated, and
the true rate constant for an oxidation reaction determined.

Oxidation of a compound in solution[55] may be described by

$$A + O_2 \rightarrow D \qquad (1)$$

or

$$A + O_2 \rightarrow [AO_2] \rightarrow D \qquad (2)$$

The symbol D here denotes decomposition product, and the bracket in Equation 2 is used to denote a more or less stable transition product.

If the oxidation is a simple oxidation, that is, not auto-oxidative, then the decomposition will result in the following equations:

$$d[A]/dt = -k_2[O_2][A] \qquad (3)$$

or, if the oxygen is in abundance:

$$\ln[A/A_o] = -k''t = -k_2[O_2]t \qquad (4)$$

where the apparent first order rate constant, k", is related to the second order rate constant, k_2, by

$$k'' = k_2[O_2] \qquad (5)$$

where brackets denote concentrations. Henry's law dictates that

$$[O_2] = KP_{o2} \qquad (6)$$

This inserted in Equation 5 gives:

$$k'' = k_2KP_{o2} \qquad (7)$$

where K is the Henry's Law-constant in units of atm^{-1}, and P_{ox} is the oxygen pressure. Thus, knowing the partial pressure of oxygen or the Henry's law constant allows determination of the other from the rate constant.

The problem is complicated by the fact that some oxidative processes are auto-oxidative, and in such cases, Equation 8 applies

$$k_2 = k^*\{[A_o]-[A]\} \qquad (8)$$

i.e. the rate constant increases with increasing amount of decomposition. The reaction equation now becomes:

$$dA/dt = -k_2P_{o2}K[A] = k^*P_{o2}K\{[A_o]-[A]\}[A] \qquad (9)$$

The resulting kinetic equation for this situation, as described in Section IV-B, is

$$\ln[x/(1-x)] = -kt \qquad (10)$$

where

$$k = k^*A_o\ K\ P_{o2} \qquad (11)$$

Three experiments are now outlined which would allow determination of Henry's law constants and oxygen solubilities:

(1) For a simple oxidation, carrying out the reaction at different oxygen pressures, which may be measured, allows the quantity k_2K to be obtained as the slope of the apparent rate constant (k") plotted versus partial oxygen pressure (P_{o2}), as predicted by Equation 7.

(2) The oxidation reaction is then followed say at 4 temperatures below 50°C. The rate constants are obtained from the first-order plots, and the oxygen concentration measured with an electrode directly.

From the observed rate constant and the oxygen concentration, the true rate constant, k_2 may be obtained from equation 5. K can now be calculated.

The reaction is then monitored at, say, 4 temperatures higher than 50°C, and the observed rate constants, k", obtained. From an Arrhenius plot of the four lower temperatures, one predicts the k_2's for higher temperatures. Then, although the oxygen concentrations may not be measured directly at the high temperatures due to the thermal instability of the oxygen electrode, they may be calculated from the ratio of the observed rate constant and the predicted rate constant at a given temperature:

$$[O_2]_{unknown}=k"/k_2$$

P_{o2} is calculated from Equation 18, assuming P_o to be 0.22 atm. The Henry's Law constant may then be obtained from Equation 6.

(3) One could use buffers of different molarities to alter the oxygen concentration in solution at a given temperature. The exact concentration of oxygen in each solution could be measured with an oxygen electrode, and the degradation of an oxidizable substance monitored in each buffer. From a plot of rate constant vs oxygen concentration, extrapolation to zero oxygen concentration would yield the degradation rate in the absence of oxygen. From such a plot, one might observe a minimum buffer or salt concentration which would salt-out oxygen without the need to nitrogen purge or to add an oxygen scavenger. The kinetic salt effect would be

determined as well. This method of controlling oxygen concentration is technically less complicated than bubbling gas into the reaction mixture. An apparatus has been designed, but not yet tested, which should allow the oxygen concentration to be determined with an oxygen electrode in any solution at any temperature. Once this has been determined in the apparatus, the reaction may be run in ampuls in a routine manner.

It is noted that $[O_2]$ is the amount of oxygen dissolved in solution, which is affected by many variables, for example partial pressure of oxygen above the solution, as dictated by Henry's Law (Equation 6), temperature, and concentration of electrolyte in solution, as governed by the Setchenow equation (Equation 12).

Between 0 and 30°C, it may be seen from Figure A-F-1 that the Henry's law constant does not follow an Arrhenius equation.[56]

The Setchenow equation states, that for a given temperature,

$$\log(S°/S)=k_S C_S \qquad (12)$$

where S° and S are solubilities of oxygen, in cubic centimeters at STP per liter, in the solvent and in the ionic solution respectively, and C_S is the concentration of the electrolyte in equivalents per liter.[57, 58]

Henry's Law and the Setchenow equation may be related as follows. If one were to measure, at a given partial pressure of oxygen, e.g. 1 atmosphere, and at a given temperature, the oxygen

concentration in H_20 and in a salt solution of known concentration , then a ratio of Henry's Law constants would be obtained:

$$[O_2]_{H20}/[O_2]_{salt\ soln.}=S°/S=(K_{H20}*P_{O2})/(K_{salt\ soln.}*P_{O2}) \qquad (13)$$

Taking logarithms of both sides produces the Setchenow equation:

$$\log[(K_{H20}*P_{O2})/(K_{salt\ soln.}*P_{O2})]=\log(S°/S)=k_sC_s \qquad (14)$$

which relates Henry's Law constants and electrolyte concentration at a given temperature.

However, it may be desired to predict the solubility of oxygen as a function of both temperature and ionic strength. One approach might be as follows.

As regards the effect of ionic strength on the oxygen solubility, Figure A-F-2 is constructed from data[58] published in International Critical Tables. The plots are linear in good agreement with the Setchenow equation and the findings for barium and sodium chlorides reported by Harned and Owen[59] and for sodium chloride and hydrochloric acid.[60]

It is noted that the linearity is good, but that the curves shift according to the ionic strength. When slopes and intercepts are plotted versus ionic strength, μ, (or sodium chloride normality), then Figure A-F-3 results.

If the least squares fit equations shown in the Figure A-F-3 are introduced into a Van't Hoff form, then

$$\ln(1000S) = \{1000(1.67-0.185\mu)/T\} - 2.27 + 0.302\mu =$$
$$-2.27+ (1{,}670/T) - 185(\mu/T) + 0.302\mu \qquad (15)$$

One may multiply regress oxygen solubility versus the three variables: (1000/T), (1000μ/T) and μ, and if this is done the relation becomes:

$$\ln(1000S) = -2.264 + (1{,}666/T) - (184.5\mu/T)+ 0.300\mu \qquad (16)$$

which is a statistically superior representation, but still quite close to Equation 15. The correlation coefficient for Equation 16 is 0.9993.

With Equation 16 it is now possible, at any temperature and ionic strength, to roughly predict the solubility of oxygen in solution at 1 atm oxygen pressure; by means of Henry's law this can then be proportionated down to the actual oxygen pressure. This equation has been used to roughly estimate the oxygen concentration in some of the solutions used in this study. These results may be found in Table F-1; it is noted that the oxygen concentrations were proportioned by multiplying by 0.22 = P_o.

If equation 12 is used to predict oxygen concentration in other than sodium chloride solutions or water, however, it is assumed that the solute behaves in a manner similar to sodium chloride, and that at temperatures above 30°C the equation still holds. This may be a good approximation, when it becomes impossible to measure the oxygen concentration directly, however, it

would be desirable to directly measure the oxygen concentration in these cases.

At a given value of μ, Equation 12 reduces to a conventional Van't Hoff type relation:

$$\ln S = (-\Delta H/RT) + W \qquad (17)$$

where ΔH is the heat of solution of oxygen, R is the gas constant and W is a constant.

If a solution is filled into an ampul, and if the amount of oxygen in the head-space is assumed to be the significantly larger than that in solution, then, approximately, the vapor pressure of oxygen at temperature T will be

$$P_{o2} = P_o \ (T/298) \qquad (18)$$

where P_o is ~0.22, the oxygen pressure at the temperature of preparation (assumed to be 298°K)[61]. The logarithmic form of Equation 18 is:

$$\ln P_{o2} = \ln(P_o/298) + \ln T = a + \ln T \qquad (19)$$

where

$$a = \ln(P_o/298) \qquad (20)$$

The term S in the above is the solubility at an oxygen pressure of one atmosphere. At an oxygen pressure of P_{O2} it would be proportionate thereto (by Henry's law) i.e. in the nomenclature of Equation 6:

$$[O_2] = P_{o2}S \qquad (21)$$

The rate constant in Equation 7 is

$$k'' = k_2[O_2] = k_2P_{O2}S \qquad (22)$$

Inserting Equations 16 and19 into the logarithmic form of Equation 22 then gives:

$$\ln(k'')=\ln(k_2)+\ln(P_{O2})+\ln S=\{(E_a-\Delta H)/RT\}+\ln T+W^* \qquad (23)$$

where W* is a constant, E_a is the energy of activation for Reaction 1, and ΔH is the heat of solution of oxygen in the system. Plotting (lnk-lnT) vs 1/T should result in a straight line with a slope of (-Ea-ΔH)/R.

Fig. A-F-1 Arrhenius type plot of Henry's law constant for oxygen in water. Lack of linearity is noted.

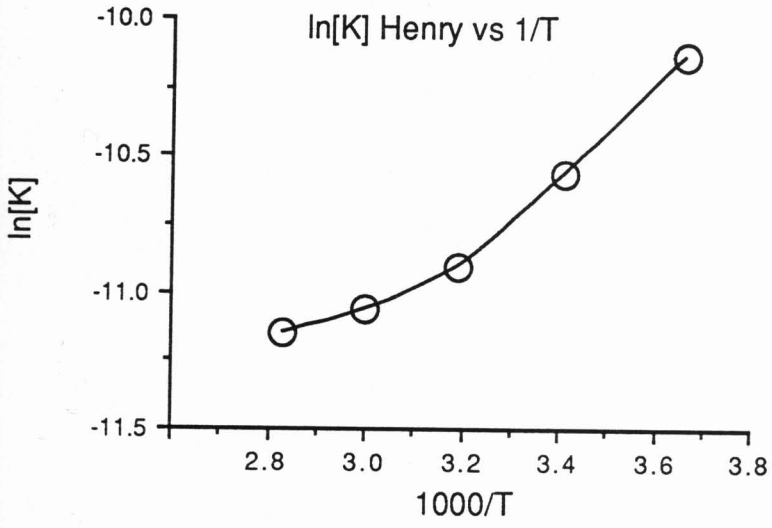


Figure A-F-2. Solubility of oxygen, in volume of gas reduced to 0°C and 1 atm, dissolved in one volume of solution when the partial gas pressure is 1 atm. Data plotted as ln[S] versus 1/T.

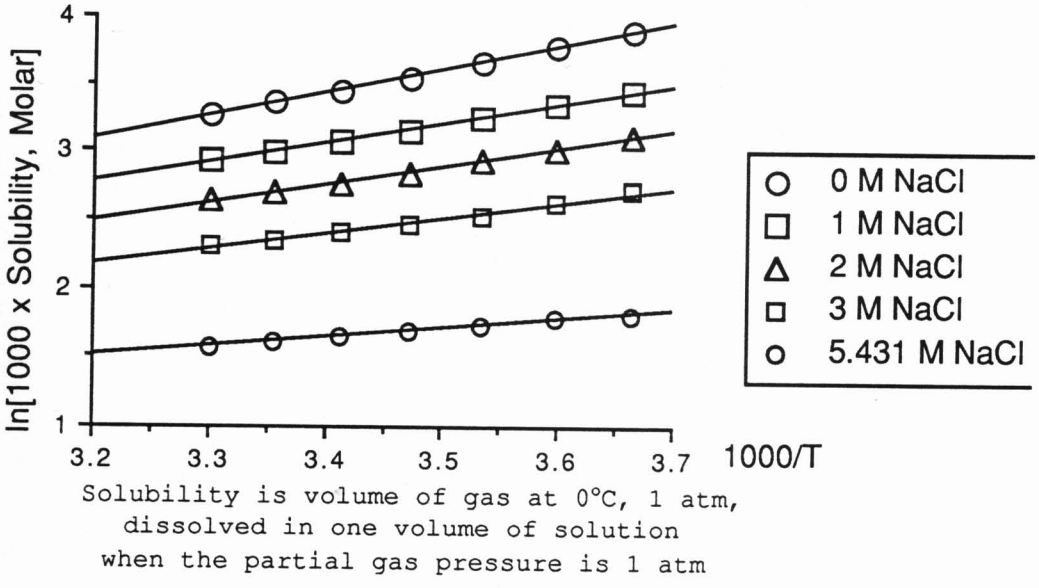


Figure A-F-3 Slopes and Intercepts from Figure A-H-2 plotted versus ionic strength.

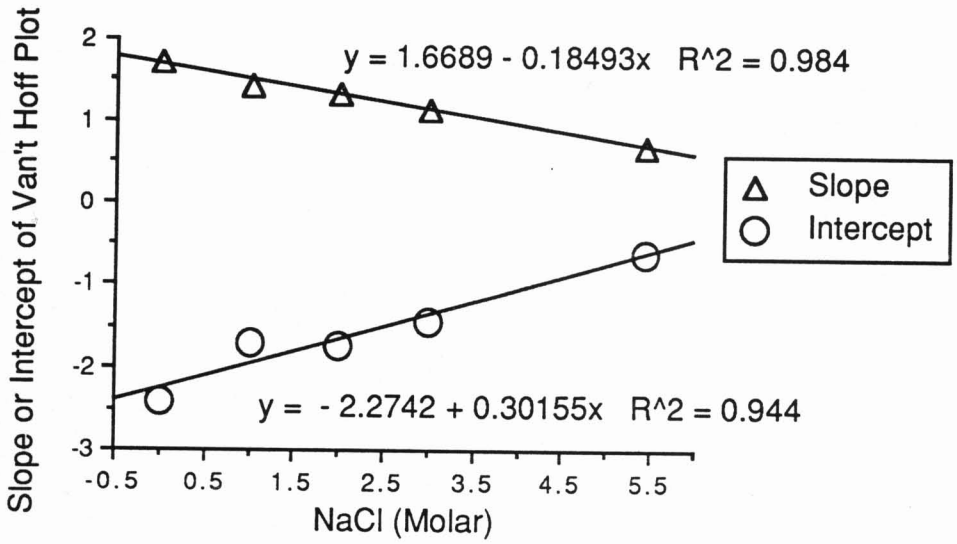


Table F-1 Estimates of Oxygen Concentrations (mM) as a function of T and μ as Predicted by Equation 16

T, °C	μ=0*	μ=0.11**	μ=2.2	%H_2O
3°C	2.2	2.1	1.4	64
75°C	2.8	2.7	1.7	61
60°C	3.4	3.3	2.0	59
5°C	6.1	5.4	3.0	49

* (H_2O)
**(0.05M)

At 93°C, in μ=2.2 KCl, [O_2] is (1.4/6.1) x 100 = 23% of that at 25°C in H_2O

[Drug] = 0.2 mg/ml = 0.41 mM

Appendix G Variables Affecting the cmc, and Variables Which are Affected by a cmc

G-1. Dependence of cmc on Electrolyte Concentration

Generally, an increase in salt concentration decreases the cmc as may be seen from Table A-G-1. The data are plotted in Figures A-G-1 and -2.

Figure A-G-1 normalizes the data obtained for the different substances for the purpose of observing the general % reduction in cmc regardless of the identity of the compound. Figure A-G-2 linearizes the SDS data according to the equation

$$\log \text{cmc} = k_1 - \log([\text{salt}] + \text{cmc}) \qquad \text{(A-G-1)}$$

where k_1 is an experimental constant.[62] As may be seen in Figure A-G-1, increasing the molarity of the salt concentration from 0 to 0.1M caused the cmc of SDS to be lowered to ~20 % of its original value. It should be noted that although Equation A-G-1 linearizes the SDS data, other surfactants have been known to show concave curvature and negative deviations from linearity at salt concentrations greater than 0.4M.[63]

In the present study, therefore, all the buffers containing KCl could have reduced the cmc of the drug substance from 2.4 to at least 0.5 mg/ml (=0.2 x 2.4 mg/ml), since when present, KCl is at least 0.4M (Table A-G-2). Due to the deviations in high salt environment, the cmc of the drug substance may be even lower than 0.1 mg/ml in systems containing KCl.

Table A-G-1 Dependence of cmc on Electrolyte Concentration

Surfactant	Solution	cmc, mmole/liter
Sodium Dodecyl Sulfate[64]	Water	8.1
	0.02 M NaCl	3.82
	0.03 M NaCl	3.09
	0.10 M NaCl	1.39
	0.20 M NaCl	0.83
	0.40 M NaCl	0.52
Dodecylamine HCl[64]	Water	13.1
	0.0157 M NaCl	10.4
	0.0237 M NaCl	9.25
	0.0460 M NaCl	7.23
Decyl Trimethyl Ammonium Bromide[64]	Water	68.0
	0.013 M NaCl	10.7
Tetradecyl trimethyl Ammonium Bromide[64]	Water	3.02
	0.013 M NaCl	1.80
Chlorpromazine HCl[65]	Water	22.0
	0.100 M NaCl	7.0
	0.165 M NaCl	5.2
	0.200 M NaCl	4.1
	0.250 M NaCl	3.5
	0.400 M NaCl	2.8
	0.600 M NaCl	2.3
Sodium Dodecanoate[66]	0.001N NaOH	27.4
	0.018 M NaCl	19.4
	0.0394 M NaCl	15.8
	0.0855 M NaCl	11.3
Potassium Dodecanoate[66]	0.001N NaOH	27.7
	0.0516 M NaCl	16.3
	0.0853 M NaCl	11.4
	0.1813 M NaCl	7.8

Table A-G-2 Molarity of KCl in pH 7 Buffers Whose Ionic Strength Has Been Adjusted to 2.2 with KCl

Buffer Molarity	g KCl/100 ml Buffer	Molarity of KCl
0.01	16.469	2.2
0.05	15.804	2.1
0.40	9.982	1.3
0.60	6.654	0.9
0.80	3.327	0.4

Figure A-G-1 Reduction in cmc (Moles/Liter) of Surfactants as a Function of Salt Concentration, Normalized to 1 = cmc in H_20
Data from Table A-G-1

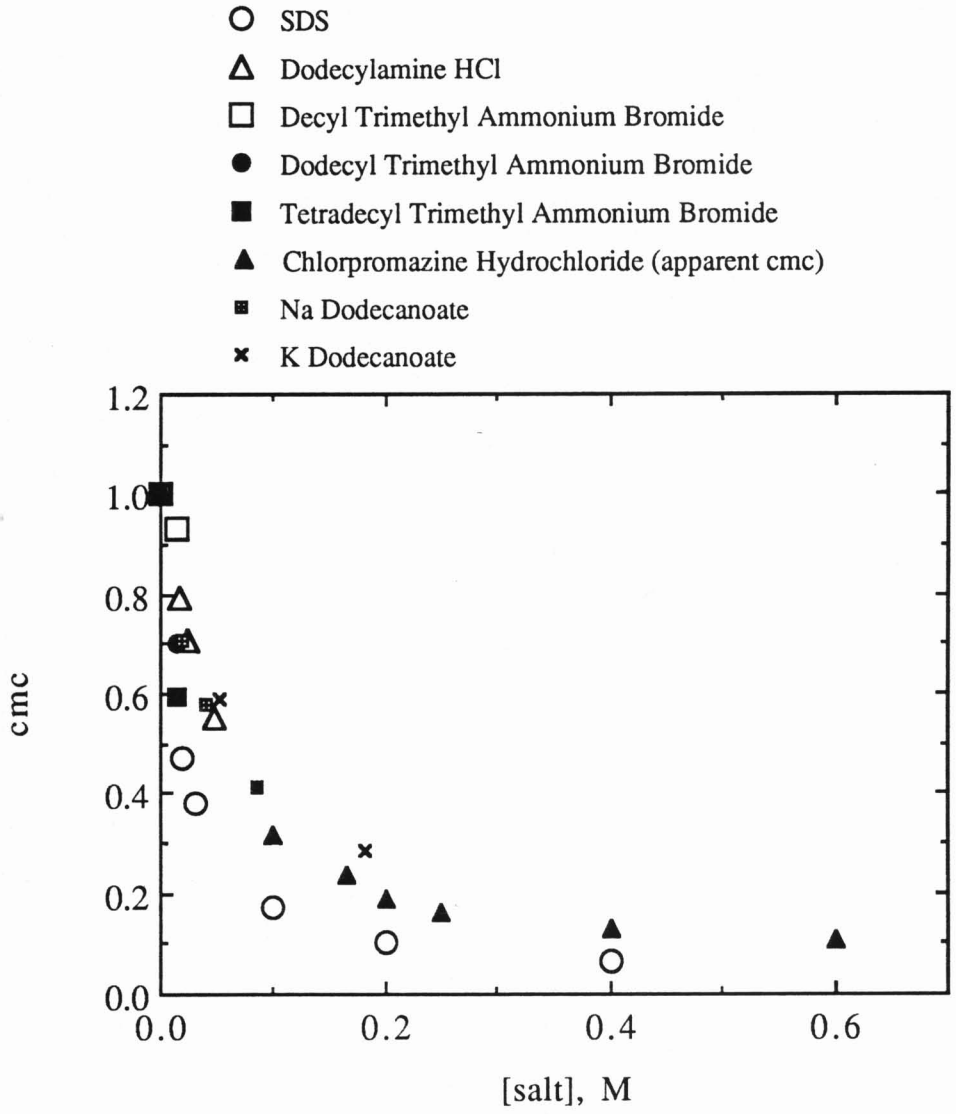
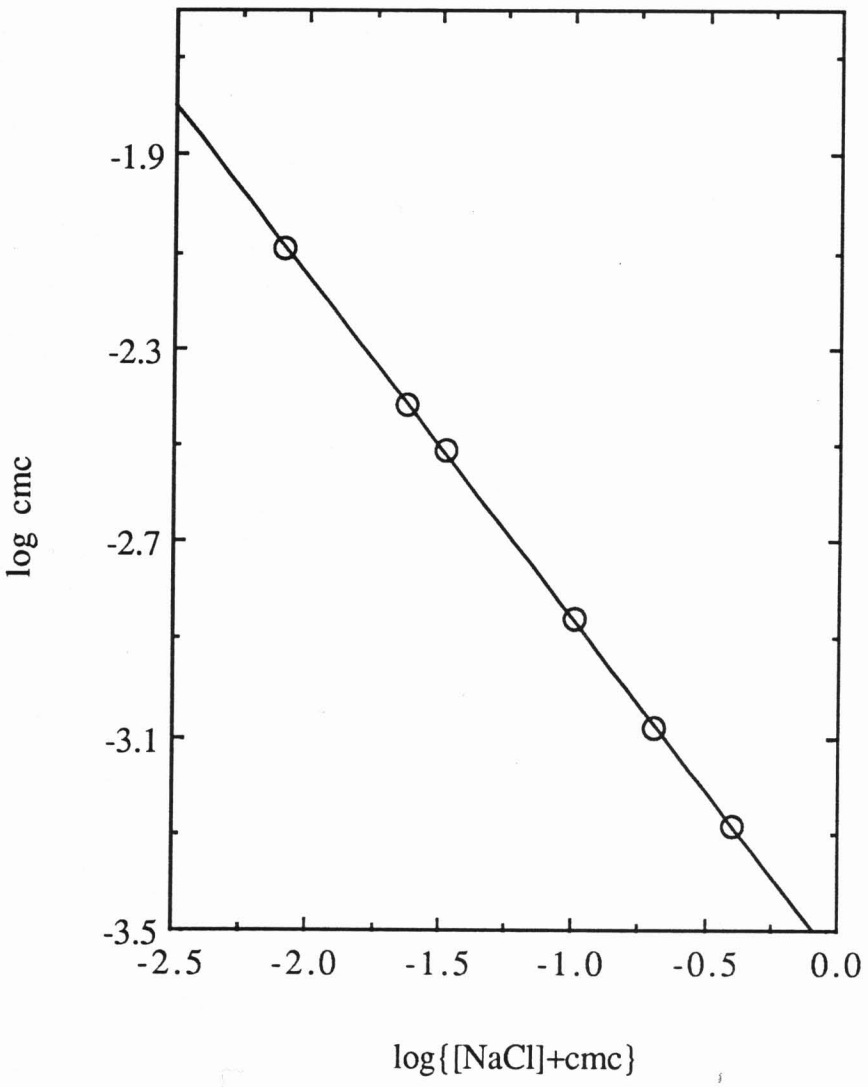


Figure A-G-2 Sodium Dodecyl Sulfate
Effect of Electrolyte Concentration on cmc
Data from Table A-G-1

y = -3.56 - 0.71x R^2 = 1.000



A-G-2. Dependence of Observed Rate Constant on Electrolyte Concentration; Dependence of cmc on Temperature

Electrolytes are known to inhibit the rates of micellar-catalyzed reactions. Graphs from Garnett *et al* [67] are reproduced in Figures A-G-3, -4, and -5 showing the inhibitory effect of chloride salts on the hydrolysis of the surfactant sodium dodecyl sulfate (SDS) in low and high concentrations SDS. The cmc of SDS is 8.1 x 10-3 M; at 90°C, the reaction is monitored at an SDS concentration just slightly above the cmc, and at 70°C at a tenfold greater concentration.

The inhibitory effect of added NaCl is much less pronounced when the surfactant concentration is well above the cmc. It is noted that at 90°C the potassium salt has a more inhibitory effect than the sodium salt, and the k(observed)'s are higher in potassium salt than in the sodium salt. It is also of interest that at 70°C and at SDS levels well above the cmc, the potassium salt increases the rate of the reaction, whereas the sodium salt does not.

In these graphs, k2(relative) is the ratio of the observed second-order rate constants obtained in the presence of added electrolytes to the observed second-order constants in their absence.

With respect to the present study, the addition of KCl may have had a pronounced effect on the degradation rate in those samples with drug concentrations less than 0.5 mg/ml, since in these samples, the cmc may be somewhere in the range of the initial drug concentration used in the study.

In general, the temperature effect on cmc exhibits a broad minimum between 10 and 90°C; some surfactants have been found to be more temperature-sensitive than others.[68] It is therefore difficult to predict the effect of temperature on the cmc of the drug substance, except that it may be lower at 60 than at 93°C.

Figure A-G-3 Sodium Dodecyl Sulfate: 90°C
Effect of Chloride Salts on k_2(relative)
for Hydrolysis in 0.02 M Perchloric Acid;
Initial Surfactant Concentration = 0.035 M
(from Garnett, *et al* [70])

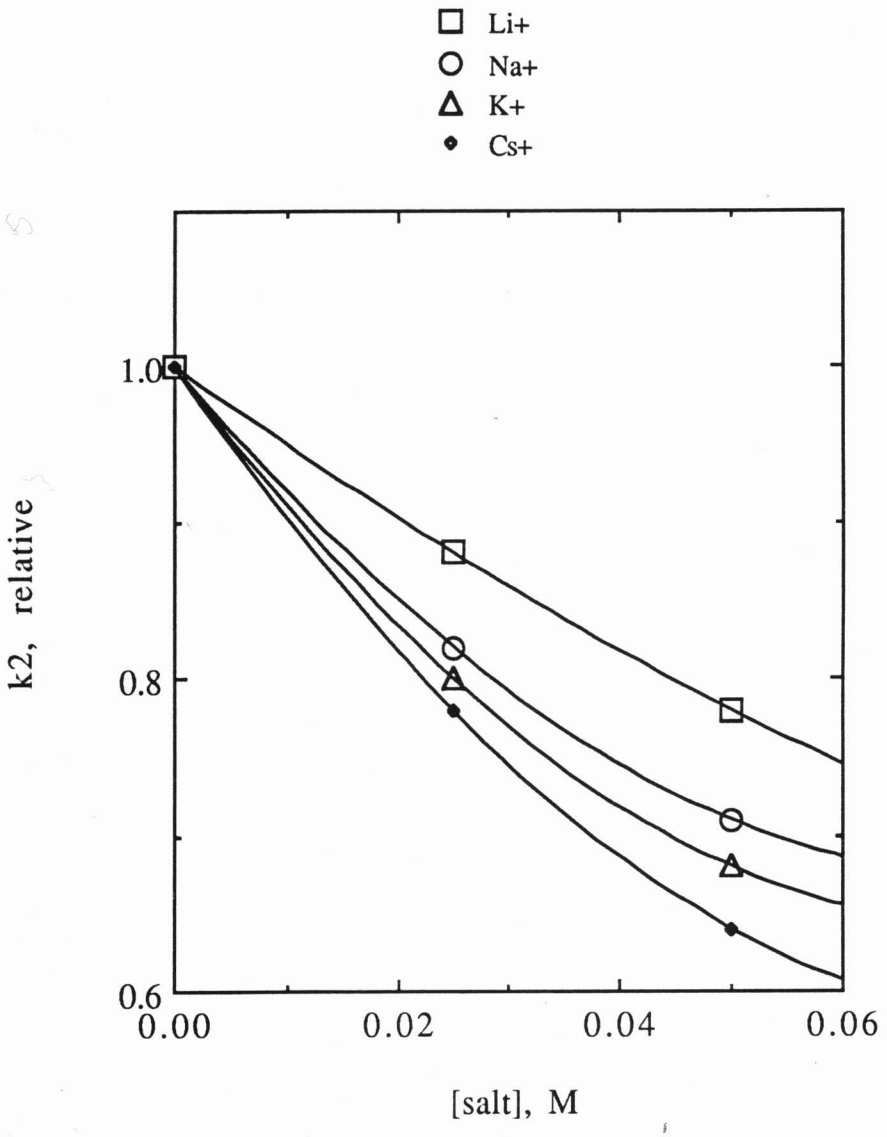


Figure A-G-4 Sodium Dodecyl Sulfate: 90°C
Plots of Reciprocal Observed Rate Constants Against Chloride Salt Concentrations for Hydrolysis in 0.02 M Perchloric Acid; Initial Surfactant Concentration = 0.35 M (reconstructed from Garnett *et al* [70])

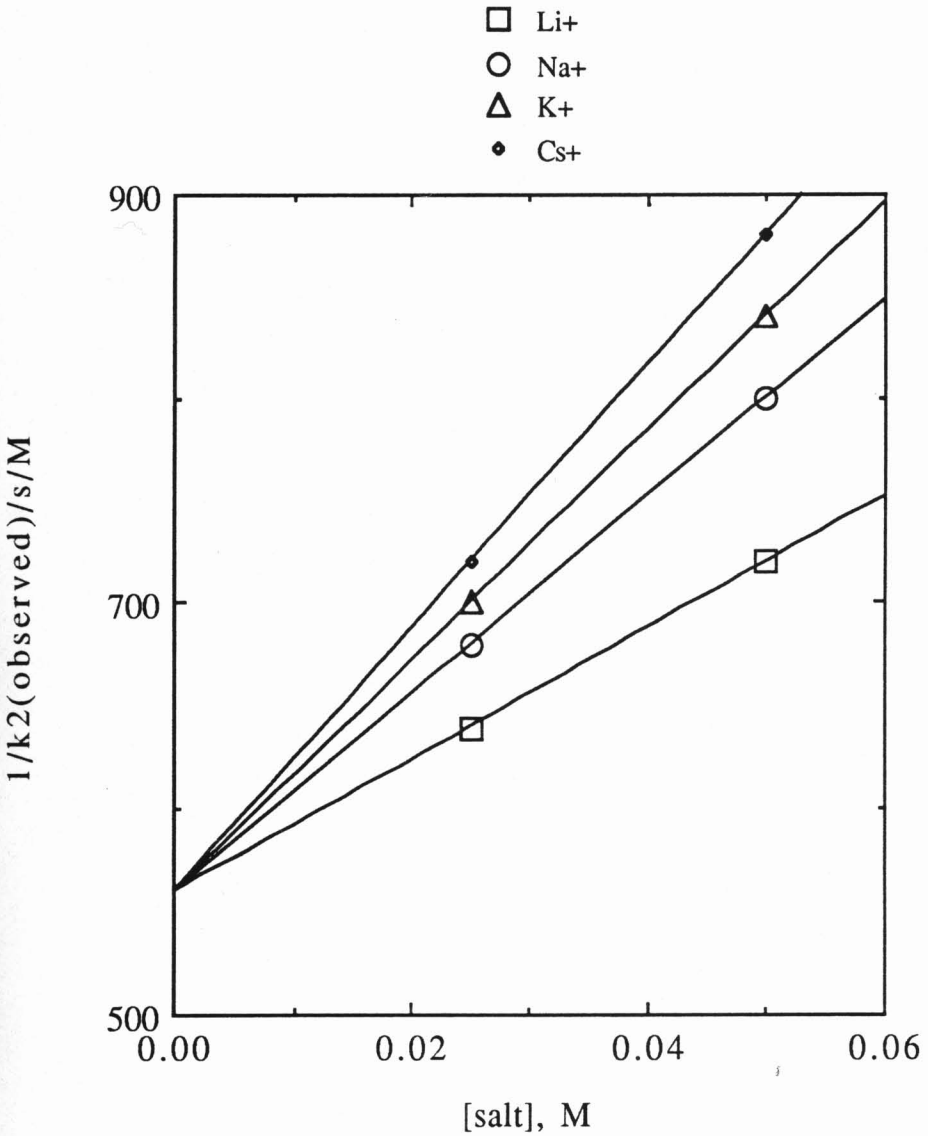
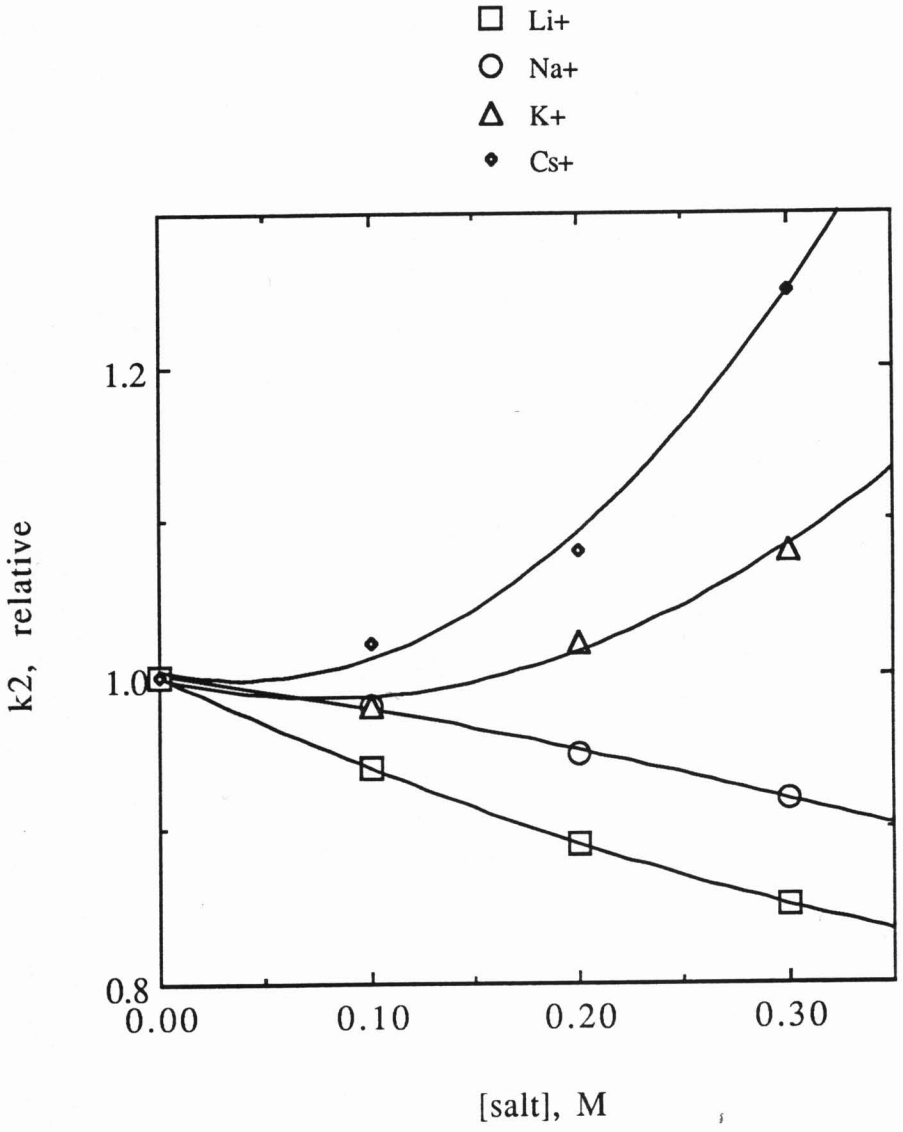


Figure A-G-5 Sodium Dodecyl Sulfate, 70°C
Effect of Chloride Salts on the Relative Rate Constant for Hydrolysis in 0.0425 M Perchloric Acid; Initial Surfactant Concentration = 0.35 M (reconstructed from Garnett *et al* [70])



A-G-3. Dependence of Oxygen Solubility on Surfactant Concentration

The following is taken from Prapaitrakul and King[69] who followed the solubilities of oxygen, argon, methane, ethane, propane, and carbon tetrachloride at elevated pressures in aqueous solutions of surfactants. The surfactants examined were decyltrimethyl- and cetyltrimethylammonium bromide (DTAB and CTAB)[70], however, anionic surfactants of the alkyl sulfate class were shown to exhibit similar behavior, according to the authors.

Below the cmc, the gas solubilities are independent of surfactant concentration. However, regardless of the nature of the surfactant, ionic or not, above the cmc, the gas solubilities are seen to increase linearly with surfactant concentration, indicating micellar solubilization.

With regards to oxygen, its solubility at 26°C and 1 atmosphere was only increased very slightly as the surfactant concentration was increased from 0.1M to 0.5M relative to the other gases studied which were more nonpolar in nature. However, the solubility of oxygen was found to be an order of magnitude higher in micelles than in water.

In the present research, oxygen solubility may be affected by the drug concentration, since in many instances, the drug concentration may have been above the cmc.

Appendix H Critical Micelle Concentration of SKB106203

SUBJECT: **Determination of Critical Micelle Concentration of SK&F 106203-Z2**

INTRODUCTION:

SK&F 106203-Z2; (S)-beta-[(2-carboxyethyl)thio]-2-(8-phenyloctyl)benzenepropranoic acid is an oral leukotriene antagonist and is being developed for the treatment of asthma. Due to the surfactant like nature of the compound a study was carried out to determine the critical micelle concentration, (cmc), of the compound. Surface tension determinations are routinely used for the determination of cmc. The break point in the plot of surface tension vs. log concentration determines the cmc.

METHOD:

The Kruss Processor Tensiometer K-12, a fully automatic instrument for the measurement of surface and interfacial tension of liquids, was used to carry out the measurements. A 10% w/w solution of SK&F 106203-Z2, (lot # CTP-H-08C), was added to a cell containing 50 mls of water. This stock solution was added in increments of 0.0276 mls and measurements were made at a temperature of 21.1^{o}C. After each addition of the stock solution the surface tension was measured for a total of 154 readings covering a concentration range of $1.14x10^{-4}$ M to $1.5x10^{-2}$M. The raw data is tabulated in Appendix 1. A plot of surface tension versus log concentration is used to determine the cmc, Figure 1. The surface concentration, area per molecule at the interface, and the efficiency of adsorption are also calculated using derivations of the Gibbs Adsorption equation, Table 1.

RESULTS:

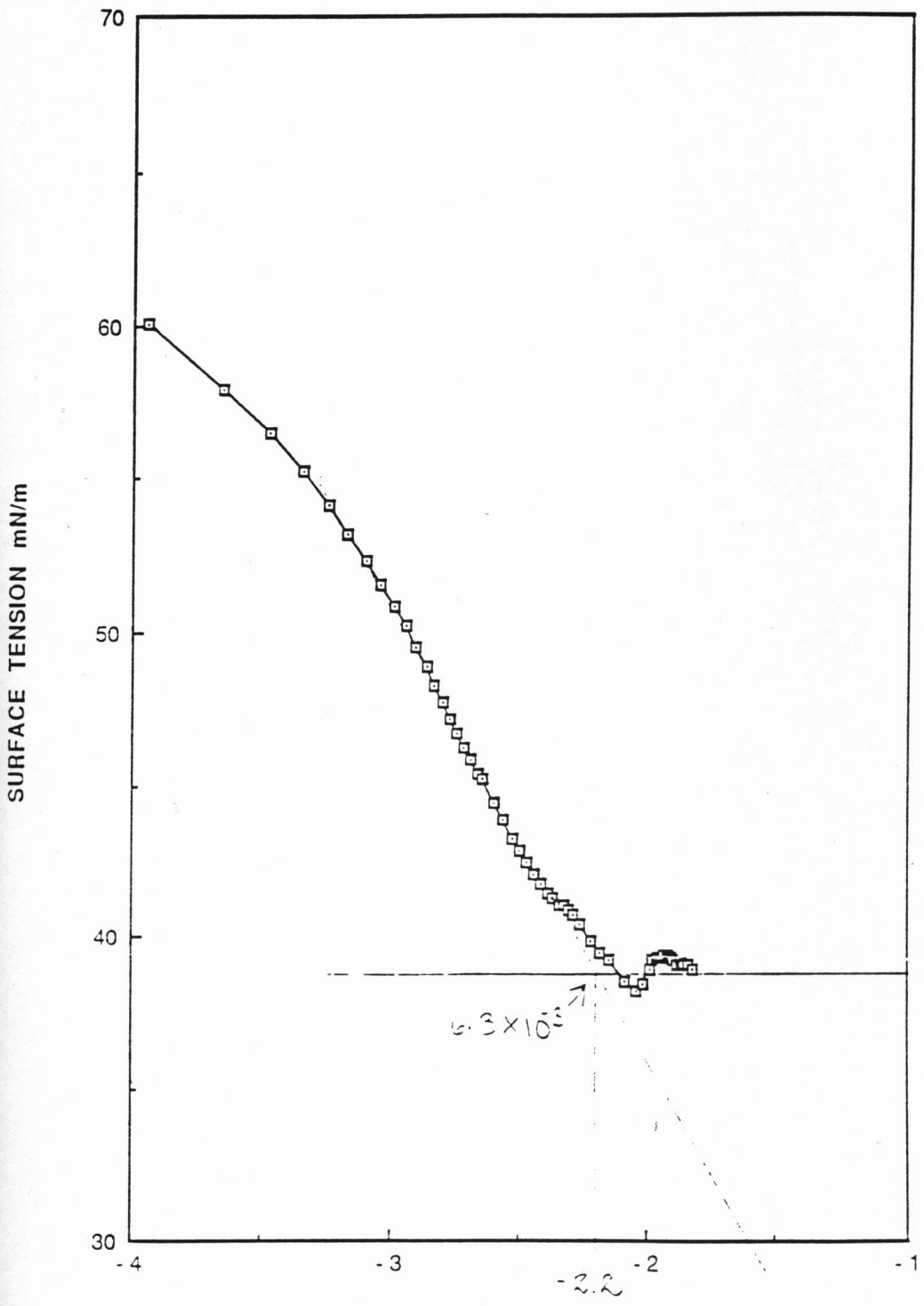
TABLE 1

Surface Concentration = $1.02x10^{-3}$ moles/$1000m^{2}$

Area Per Molecule At The Interface = 162.1 angtroms squared

Efficiency of Adsorption = 2.24

Critical Micelle Concentration (CMC) = $6.3x10^{-3}$M.


70
60
50
40
30
SURFACE TENSION mN/m
- 4
- 3
- 2
- 1
CONCENTRATION (m)

	A	B	C	D	E	F	G	H
1	Surface Tension data for SK&F 106203-Z2							
2	Measured using a Kruss K-12 tensiometer.							
3	A 10% w/w solution of SK&F 106203-Z2 was added to a cell containing 50 ml of water.							
4	Surface tension was measured after each addition. pH at end = 7.6.							
5	Temperature = 21.1 C.							
6	Value for water = 71.95 mN/m							
7								
8	Number	Tot. Vol. Cell	Vol of Addition	Wt. 106203	Wt. 106203	Molarity	Log C. (Mol)	Surface
9					per litre			Tension mN/m
10								
11	1	50.0276	0.0276	0.00276	0.05516955	0.00011339	-3.94543699	60.04
12	2	50.0552	0.0552	0.00552	0.11027825	0.00022665	-3.64464653	57.92
13	3	50.0828	0.0828	0.00828	0.16532622	0.00033979	-3.46879467	56.52
14	4	50.1104	0.1104	0.01104	0.22031355	0.0004528	-3.3440952	55.27
15	5	50.138	0.138	0.0138	0.27524034	0.00056569	-3.24742432	54.17
16	6	50.1656	0.1656	0.01656	0.33010669	0.00067845	-3.16848208	53.22
17	7	50.1932	0.1932	0.01932	0.3849127	0.00079109	-3.10177417	52.38
18	8	50.2208	0.2208	0.02208	0.43965847	0.00090361	-3.04402096	51.6
19	9	50.2484	0.2484	0.02484	0.4943441	0.001016	-2.99310705	50.9
20	10	50.276	0.276	0.0276	0.54896969	0.00112827	-2.94758804	50.21
21	11	50.3036	0.3036	0.03036	0.60353533	0.00124041	-2.9064337	49.55
22	12	50.3312	0.3312	0.03312	0.65804114	0.00135244	-2.86888336	48.92
23	13	50.3588	0.3588	0.03588	0.71248719	0.00146434	-2.83435934	48.31
24	14	50.3864	0.3864	0.03864	0.7668736	0.00157611	-2.80241262	47.76
25	15	50.414	0.414	0.0414	0.82120046	0.00168777	-2.77268722	47.17
26	16	50.4416	0.4416	0.04416	0.87546787	0.0017993	-2.74489619	46.72
27	17	50.4692	0.4692	0.04692	0.92967592	0.00191071	-2.71880482	46.27
28	18	50.4968	0.4968	0.04968	0.98382472	0.002022	-2.69421867	45.81
29	19	50.5244	0.5244	0.05244	1.03791435	0.00213317	-2.67097488	45.39
30	20	50.552	0.552	0.0552	1.09194493	0.00224421	-2.64893567	45.21
31	21	50.5796	0.5796	0.05796	1.14591654	0.00235514	-2.62798342	44.83
32	22	50.6072	0.6072	0.06072	1.19982927	0.00246594	-2.60801695	44.45
33	23	50.6348	0.6348	0.06348	1.25368324	0.00257663	-2.58894858	44.13
34	24	50.6624	0.6624	0.06624	1.30747852	0.00268719	-2.57070184	43.87
35	25	50.69	0.69	0.069	1.36121523	0.00279763	-2.5532096	43.58
36	26	50.7176	0.7176	0.07176	1.41489345	0.00290795	-2.53641267	43.28
37	27	50.7452	0.7452	0.07452	1.46851328	0.00301815	-2.52025853	43.05
38	28	50.7728	0.7728	0.07728	1.52207481	0.00312824	-2.5047004	42.83
39	29	50.8004	0.8004	0.08004	1.57557815	0.0032382	-2.48969645	42.6
40	30	50.828	0.828	0.0828	1.62902337	0.00334804	-2.47520909	42.44
41	31	50.8556	0.8556	0.08556	1.68241059	0.00345777	-2.46120441	42.25
42	32	50.8832	0.8832	0.08832	1.73573989	0.00356737	-2.44765176	42.05
43	33	50.9108	0.9108	0.09108	1.78901137	0.00367686	-2.4345233	41.92
44	34	50.9384	0.9384	0.09384	1.84222512	0.00378622	-2.4217937	41.75
45	35	50.966	0.966	0.0966	1.89538123	0.00389547	-2.40943983	41.59
46	36	50.9936	0.9936	0.09936	1.94847981	0.0040046	-2.39744049	41.46
47	37	51.0212	1.0212	0.10212	2.00152094	0.00411362	-2.38577627	41.47
48	38	51.0488	1.0488	0.10488	2.05450471	0.00422251	-2.37442926	41.31
49	39	51.0764	1.0764	0.10764	2.10743122	0.00433129	-2.36338299	41.25
50	40	51.104	1.104	0.1104	2.16030056	0.00443995	-2.35262222	41.09
51	41	51.1316	1.1316	0.11316	2.21311283	0.00454849	-2.34213285	41.12
52	42	51.1592	1.1592	0.11592	2.26586811	0.00465691	-2.33190177	41.06
53	43	51.1868	1.1868	0.11868	2.31856651	0.00476522	-2.32191685	41.06
54	44	51.2144	1.2144	0.12144	2.3712081	0.00487341	-2.31216673	40.94
55	45	51.242	1.242	0.1242	2.42379298	0.00498149	-2.30264088	40.85
56	46	51.2696	1.2696	0.12696	2.47632125	0.00508945	-2.29332942	40.72
57	47	51.2972	1.2972	0.12972	2.52879299	0.00519729	-2.28422312	40.62

Mixture cap C

	A	B	C	D	E	F	G	H
58	48	51.3248	1.3248	0.13248	2.5812083	0.00530502	-2.27531335	40.53
59	49	51.3524	1.3524	0.13524	2.63356727	0.00541263	-2.26659199	40.43
60	50	51.38	1.38	0.138	2.68586999	0.00552012	-2.25805142	40.33
61	51	51.4076	1.4076	0.14076	2.73811654	0.0056275	-2.24968447	40.22
62	52	51.4352	1.4352	0.14352	2.79030703	0.00573476	-2.24148441	40.13
63	53	51.4628	1.4628	0.14628	2.84244153	0.00584191	-2.23344486	40.02
64	54	51.4904	1.4904	0.14904	2.89452014	0.00594895	-2.22555983	39.92
65	55	51.518	1.518	0.1518	2.94654296	0.00605587	-2.21782363	39.02
66	56	51.5456	1.5456	0.15456	2.99851006	0.00616267	-2.21023089	39.71
67	57	51.5732	1.5732	0.15732	3.05042154	0.00626936	-2.20277654	39.63
68	58	51.6008	1.6008	0.16008	3.10227748	0.00637594	-2.19545576	39.56
69	59	51.6284	1.6284	0.16284	3.15407799	0.0064824	-2.18826398	39.48
70	60	51.656	1.656	0.1656	3.20582314	0.00658875	-2.18119684	39.37
71	61	51.6836	1.6836	0.16836	3.25751302	0.00669499	-2.17425024	39.3
72	62	51.7112	1.7112	0.17112	3.30914773	0.00680111	-2.16742025	39.23
73	63	51.7388	1.7388	0.17388	3.36072735	0.00690712	-2.16070312	39.3
74	64	51.7664	1.7664	0.17664	3.41225196	0.00701301	-2.15409531	39.24
75	65	51.794	1.794	0.1794	3.46372167	0.0071188	-2.14759342	39.15
76	66	51.8216	1.8216	0.18216	3.51513655	0.00722447	-2.1411942	39.11
77	67	51.8492	1.8492	0.18492	3.56649669	0.00733002	-2.13489458	39
78	68	51.8768	1.8768	0.18768	3.61780218	0.00743547	-2.12869159	38.97
79	69	51.9044	1.9044	0.19044	3.66905311	0.0075408	-2.12258241	38.88
80	70	51.932	1.932	0.1932	3.72024956	0.00764602	-2.11656433	38.83
81	71	51.9596	1.9596	0.19596	3.77139162	0.00775113	-2.11063477	38.75
82	72	51.9872	1.9872	0.19872	3.82247938	0.00785613	-2.10479125	38.7
83	73	52.0148	2.0148	0.20148	3.87351292	0.00796102	-2.09903139	38.61
84	74	52.0424	2.0424	0.20424	3.92449234	0.00806579	-2.09335292	38.58
85	75	52.07	2.07	0.207	3.97541771	0.00817046	-2.08775364	38.49
86	76	52.0976	2.0976	0.20976	4.02628912	0.00827501	-2.08223145	38.42
87	77	52.1252	2.1252	0.21252	4.07710666	0.00837945	-2.07678433	38.41
88	78	52.1528	2.1528	0.21528	4.12787041	0.00848378	-2.07141035	38.35
89	79	52.1804	2.1804	0.21804	4.17858046	0.00858801	-2.06610763	38.34
90	80	52.208	2.208	0.2208	4.2292369	0.00869212	-2.06087439	38.26
91	81	52.2356	2.2356	0.22356	4.2798398	0.00879612	-2.05570889	38.23
92	82	52.2632	2.2632	0.22632	4.33038926	0.00890001	-2.05060947	38.35
93	83	52.2908	2.2908	0.22908	4.38088536	0.00900379	-2.04557451	38.28
94	84	52.3184	2.3184	0.23184	4.43132818	0.00910747	-2.04060249	38.26
95	85	52.346	2.346	0.2346	4.4817178	0.00921103	-2.0356919	38.39
96	86	52.3736	2.3736	0.23736	4.53205432	0.00931448	-2.0308413	38.39
97	87	52.4012	2.4012	0.24012	4.58233781	0.00941783	-2.0260493	38.31
98	88	52.4288	2.4288	0.24288	4.63256836	0.00952106	-2.02131457	38.47
99	89	52.4564	2.4564	0.24564	4.68274605	0.00962419	-2.0166358	38.49
100	90	52.484	2.484	0.2484	4.73287097	0.00972721	-2.01201174	38.69
101	91	52.5116	2.5116	0.25116	4.7829432	0.00983012	-2.00744118	38.73
102	92	52.5392	2.5392	0.25392	4.83296282	0.00993292	-2.00292295	38.79
103	93	52.5668	2.5668	0.25668	4.88292991	0.01003562	-1.99845591	38.92
104	94	52.5944	2.5944	0.25944	4.93284456	0.0101382	-1.99403897	38.98
105	95	52.622	2.622	0.2622	4.98270685	0.01024068	-1.98967107	39.05
106	96	52.6496	2.6496	0.26496	5.03251687	0.01034306	-1.98535116	39.3
107	97	52.6772	2.6772	0.26772	5.08227468	0.01044532	-1.98107827	39.25
108	98	52.7048	2.7048	0.27048	5.13198039	0.01054748	-1.97685141	39.31
109	99	52.7324	2.7324	0.27324	5.18163406	0.01064953	-1.97266966	39.32
110	100	52.76	2.76	0.276	5.23123578	0.01075147	-1.96853211	39.35
111	101	52.7876	2.7876	0.27876	5.28078564	0.01085331	-1.96443786	39.32
112	102	52.8152	2.8152	0.28152	5.33028371	0.01095504	-1.96038608	39.37
113	103	52.8428	2.8428	0.28428	5.37973007	0.01105666	-1.95637592	39.33
114	104	52.8704	2.8704	0.28704	5.4291248	0.01115818	-1.95240658	39.32

	A	B	C	D	E	F	G	H
115	105	52.898	2.898	0.2898	5.478468	0.01125959	-1.94847727	39.31
116	106	52.9256	2.9256	0.29256	5.52775972	0.0113609	-1.94458725	39.31
117	107	52.9532	2.9532	0.29532	5.57700007	0.0114621	-1.94073575	39.43
118	108	52.9808	2.9808	0.29808	5.62618911	0.0115632	-1.93692208	39.42
119	109	53.0084	3.0084	0.30084	5.67532693	0.01166419	-1.93314552	39.42
120	110	53.036	3.036	0.3036	5.72441361	0.01176507	-1.9294054	39.44
121	111	53.0636	3.0636	0.30636	5.77344922	0.01186585	-1.92570105	39.44
122	112	53.0912	3.0912	0.30912	5.82243385	0.01196653	-1.92203184	39.4
123	113	53.1188	3.1188	0.31188	5.87136758	0.0120671	-1.91839713	39.38
124	114	53.1464	3.1464	0.31464	5.92025048	0.01216757	-1.91479632	39.37
125	115	53.174	3.174	0.3174	5.96908263	0.01226793	-1.91122881	39.37
126	116	53.2016	3.2016	0.32016	6.01786412	0.01236819	-1.90769403	39.32
127	117	53.2292	3.2292	0.32292	6.06659503	0.01246834	-1.9041914	39.31
128	118	53.2568	3.2568	0.32568	6.11527542	0.01256839	-1.90072038	39.31
129	119	53.2844	3.2844	0.32844	6.16390538	0.01266834	-1.89728044	39.26
130	120	53.312	3.312	0.3312	6.21248499	0.01276818	-1.89387105	39.29
131	121	53.3396	3.3396	0.33396	6.26101433	0.01286792	-1.8904917	39.28
132	122	53.3672	3.3672	0.33672	6.30949347	0.01296755	-1.88714191	39.19
133	123	53.3948	3.3948	0.33948	6.35792249	0.01306709	-1.88382117	39.16
134	124	53.4224	3.4224	0.34224	6.40630148	0.01316652	-1.88052903	39.13
135	125	53.45	3.45	0.345	6.4546305	0.01326585	-1.87726502	39.19
136	126	53.4776	3.4776	0.34776	6.50290963	0.01336507	-1.87402868	39.24
137	127	53.5052	3.5052	0.35052	6.55113895	0.0134642	-1.87081959	39.24
138	128	53.5328	3.5328	0.35328	6.59931855	0.01356322	-1.86763731	39.19
139	129	53.5604	3.5604	0.35604	6.64744849	0.01366214	-1.86448142	39.15
140	130	53.588	3.588	0.3588	6.69552885	0.01376095	-1.86135152	39.15
141	131	53.6156	3.6156	0.36156	6.74355971	0.01385967	-1.8582472	39.16
142	132	53.6432	3.6432	0.36432	6.79154115	0.01395828	-1.85516807	39.09
143	133	53.6708	3.6708	0.36708	6.83947323	0.01405679	-1.85211375	39.07
144	134	53.6984	3.6984	0.36984	6.88735605	0.0141552	-1.84908387	39.06
145	135	53.726	3.726	0.3726	6.93518967	0.01425351	-1.84607806	39.03
146	136	53.7536	3.7536	0.37536	6.98297416	0.01435172	-1.84309597	39.02
147	137	53.7812	3.7812	0.37812	7.03070962	0.01444983	-1.84013724	39.08
148	138	53.8088	3.8088	0.38088	7.0783961	0.01454784	-1.83720154	39.1
149	139	53.8364	3.8364	0.38364	7.12603369	0.01464575	-1.83428853	39.02
150	140	53.864	3.864	0.3864	7.17362246	0.01474355	-1.83139789	39.03
151	141	53.8916	3.8916	0.38916	7.22116248	0.01484126	-1.82852929	39.05
152	142	53.9192	3.9192	0.39192	7.26865384	0.01493886	-1.82568242	38.97
153	143	53.9468	3.9468	0.39468	7.3160966	0.01503637	-1.82285697	38.94
154	144	53.9744	3.9744	0.39744	7.36349084	0.01513378	-1.82005265	38.97

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9. Salting Out is the decrease in solubility of a non-electrolyte in ionic solutions; salting-in is the corresponding increase. Both are described empirically by the Setchenow equation: $\log(S°/S)=k_S C_S$ (see Appendix F) where S° and S are the equilibrium solubilities in cubic centimeters at STP per liter of the nonelectrolyte molecules in the solvent and in the ionic solution, respectively, and C_S is the concentration of the electrolyte in equivalents per liter; ks, the salting out constant measures the sensitivity of,for example, the oxygen activity coefficient toward a particular salt. If ks is positive, the activity coefficient is increased, and the solubility is decreased by the particular salt.(Setchenow, A.S., Z. Physik. Chem. (Leipzig), 4:117 (1889); also Jenks, W.P. Catalysis in Chemistry and Enzymology, McGraw Hill Series in Advanced Chemistry, McGraw Hill, NY 1969

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24. Khomutov, Reference 7

25. Appendix G summarizes these findings.

26. Carstensen, J.T., J. Pharm. Sci., 59(8):1140-1143 (1970)

27. It is noted in Figure IV-A-6 that the 0.01M data show a slightly higher degradation rate, and these data are not included in the regression. At this concentration of buffer, a far greater amount of KCl must be used to bring the ionic strength to 2.2 than for the other buffers. It is theorized that the higher degradation rate observed is not due to a buffer effect, but rather an indication of trace metal catalysis from the KCl. This is further explored in Section IV-C.

28. At 75°C, in the absence of KCl, the semilogarithmic plots are linear insofar as they have been monitored, in some cases, for two or three halflives. In Table III-C-2-a (75°C, no KCl), first-order rate constants are reported, however, it is possible that if these reactions were monitored beyond one half-life, some curvature may be noticed.

29. See Principles of Colloid and Surface Chemistry, 2nd edition, by Paul Hiemenz, Marcel Dekker, Inc., NY, 1986, Chapter 8.2

30. Hiemenz, Paul, Principles of Colloid and Surface Chemistry, 2nd edition, Marcel Dekker, Inc., NY 1986, Chapter 8.2

31. Khomutov, References 7 and 8

32. Drug Stability, Principles and Practices, J. T. Carstensen, Marcel Decker, New York, 1990, Chapter 2, Section 12

33. or ionic strength, since in this case they are multiples of each other

34. Patel, R.M. and Zografi, G., J. Pharm. Sci., 55(12):1345-1349 (December 1966)

35. Garnett, C.J., Lambie, A.J., Beck, W.H., and Liler, M., J. Chem. Soc., Faraday Trans. 1, 79:953-964 (1983)

36. Garnett, C.J., Lambie, A.J., Beck, W.H., and Liler, M., J. Chem. Soc., Faraday Trans. 1, 79:965-973 (1983)

37. Ong, J.T. and Kostenbauder, H.B., J. Pharm. Sci., 64(8):1378-1380 (August 1975)

38. see Appendix G

39. There are several examples in the literature where kinetics have been used to determine the cmc of compounds, e.g. Kurz, J.L., J. Phys. Chem.11: 2239-2246 (1962)

40. Appendix B-6

41. Appendix B-7

42. Appendix B-5

43. It is noted that the 1.0 mg/ml data shows downward curvature typical of an oxidation reaction, however, without a timepoint between 32 and 86 days, it is difficult to judge whether or not this is real.

44. Appendix E

45. The cmc raw data was collected by SKB using a Kruss Processor Tensiometer K-12; the report is reproduced in Appendix H.

46. Prapaitrakul, W. and King, A.D., J. Colloid Interf. Sci., 106(1):186-193 (July 1985)

47. Although not in great abundance, the published literature suggests that for a given molarity of electrolyte, oxygen concentration decreases then increases slightly as the temperature is varied between 10 and 85°C in aqueous solutions of electrolytes. The exact minimum in the curve varies depending on the electrolyte, but is on average at about 62°C. This has been shown only for LiCl, not NaCl.

48. Dumkowski, Ph.D. Thesis, U. Wisconsin-Madison, 1986

49. see Appendix G.

50. Also, they most likely are more soluble at higher temperatures than at room temperature. Many samples removed from the oven had little or no precipitate, but upon standing developed one. The cinnamic acid analogue is a white flocculant precipitate, whereas the sulfoxide analogue oils out (yellow-brown). This compound may be a good model to study crystallization phenomena. That the compound degrades primarily to cinnamic acid and sulfoxide analogues has been substantiated in random samples during the study, where mass balance has been checked. The sum of sulfoxide and cinnamic acid analogues has, in all cases checked, been insignificantly different from the beginning molar amounts of the compound.

51. a reasonable assumption, since the cmc in water is ~ 2.4 mg/ml.

52. This was found in the case of Penicillin V, which does not have a cmc, but forms aggregates at high concentrations; Ong J. T. H. and Kostenbauder, Reference 37

53. Preliminary kinetic results obtained from SKB did not indicate a pH-dependence on degradation rate in the pH region 6.5-10. Thus, at the time the present experiments were initiated, the pH fluctuations expected in an unbuffered kinetic experiment were not considered to be as critical as they might have been with a compound which might undergo base hydrolysis.

54. Appendix B-13

55. J.T.Carstensen: Drug Stability, 1990, Marcel Dekker, N.Y., pg. 91-94

56. Maron, S.M. and Prutton, C.F., Principles of Physical Chemistry, 4th Edition, Macmillan, London, 1965, pg. 297

57. Setchenov, A.S., Z. Physik. Chem. (Leipzig), 4:117 (1889)

58. Washburn, E.W., Ed.: International Critical Tables, vol. III, pg. 272 McGraw-Hill N.Y., 1928

59. Harned, H.S. and Owen, B.B., The Physical Chemistry of Electrolytic Solutions, Reinhold, N.Y., 1950, pg. 401 and 565

60. M.Randall and C.F.Failey, Chem. Rev., 4:271 and 285 (1927)

61. This is an approximation, because it assumes that no oxygen dissolves in the liquid, so that the pre-requisite for this is that the main amount of oxygen is in the head space, a fairly reasonable assumption.

62. Chan, Chun Chiu, M.S. Thesis, U. Wisconsin-Madison, 1985

63. Chan, Chun Chiu, Ph.D Thesis in progress, U. Wisconsin-Madison,1985

64. J.N. Phillips, Trans. Faraday Soc., 51:561 (1955) in Principles of Colloid and Surface Chemistry, 2nd edition, by Paul Hiemenz, Marcel Dekker, Inc., NY, 1986, Chapter 8.2

65. D. Attwood, R. Natarajan, J. Pharm. Pharmacol., 35:317-319 (1983)

66. Chan, Chun Chiu, M.S. Thesis, U. Wisconsin-Madison, 1985

67. Garnett, Reference 36

68. Mukerjee, P., and Mysels, K.J., Critical Micelle Concentrations of Aqueous Surfactant Systems, Nat. Stand. Ref. Data Series, Nat. Bur. Stand. (U.S.) NSRD S-NBS 36, Feb. 1961

69. Prapaitrakul, Reference 46

70. cmc for DTAB = 0.055 M; cmc for CTAB = 0.0009 M