Kinetics of Aerobic Oxidation of Ascorbic Acid in the Presence of Nonionic Surfactants

S. M. BLAUG[x] and B. HAJRATWALA*

Abstract □ The effect of two nonionic surfactants, poloxalkol and polysorbate 80, on the aerobic oxidation of ascorbic acid was studied at 67° and pH 4.55 and 6.60. Results show a relationship between surfactant concentration and oxidation rate, the rate increasing at surfactant concentrations well below the surfactant's CMC and decreasing as the surfactant concentration approaches its CMC. The thermodynamic parameters were obtained for ascorbic acid solutions containing 1% surfactant.

Keyphrases □ Ascorbic acid—aerobic oxidation, effects of nonionic surfactants poloxalkol and polysorbate 80, kinetic parameters □ Surfactants—effects of poloxalkol and polysorbate 80 on ascorbic acid aerobic oxidation kinetics □ Poloxalkol—effects on aerobic oxidation of ascorbic acid, kinetic parameters □ Polysorbate 80—effects on aerobic oxidation of ascorbic acid, kinetic parameters □ Oxidation—ascorbic acid in presence of nonionic surfactants, above and below CMC

As early as 1918, McBain and Bolam (1) found that soap solutions protected materials against hydrolysis. Nogami *et al.* (2) reported that the hydrolysis of aspirin was suppressed by cationic, anionic, and nonionic surfactants, and they also reported the stabilization of various other drugs through the use of surfactants (3, 4). Other investigators (5, 6) showed that vitamin A solubilized in aqueous solutions of nonionic surfactants was more resistant to oxidation than vitamin A in an oil solution. However, Patel *et al.* (7) reported that solubilized vitamin A decomposed faster than nonsolubilized vitamin A.

Carless and coworkers (8–13), using benzaldehyde oxidation as a model system, studied the role of surfactants in retarding oxidation. They found that solubilized systems were oxidized at lower rates and were less susceptible to the catalytic action of metallic ions than emulsified systems (10).

The stability of ascorbic acid in various pharmaceutical vehicles has been reported. Giral (14) attributed the stability of ascorbic acid in simple syrup to the viscosity of the syrup. Aqueous solutions of sucrose, sorbitol, glycerin, and propylene glycol were found to stabilize ascorbic acid (15), and similar findings were reported by other workers (16). Viscous solutions containing natural hydrophilic colloids appeared to accelerate the rate of oxidation of ascorbic acid. This was attributed to the presence of metal ion or impurities in the colloids (15).

Two recent studies (17, 18) of the copper-catalyzed oxidation of ascorbic acid in the presence of polysorbates 20 and 80 showed interesting results. When using saturated solutions of ascorbic acid containing varying concentrations of polysorbate 20 (17), the oxidation rate of ascorbic acid declined rapidly between 40 and 75% surfactant. The decrease in the rate at high concentrations of surfactant was attributed to a decrease in the diffusion of oxygen to the site of oxidation due to the high viscosity of the solution.

In the presence of polysorbate 80 (19), a rapid decline in the rate of oxidation of ascorbic acid was reported in solutions containing 3% ascorbic acid and varying concentrations of polysorbate 80. The rate declined in solutions containing up to 10% surfactant and remained constant at surfactant concentrations between 10 and 30%. The decrease in the oxidation rate of ascorbic acid was attributed to an increase in both micelle concentration and micelle aggregation number in solutions containing up to 10% polysorbate 80. The increase in viscosity produced at high surfactant concentration had no significant effect on the rate of oxidation of ascorbic acid.

A previous investigation (19) concerned the aerobic degradation of ascorbic acid in aqueous solutions over the 3.52–7.22 pH range. The purpose of the present investigation was to study the aerobic degradation of ascorbic acid in the presence of two nonionic surfactants at pH 4.55 and 6.60. Surfactants used in internal preparations must be nontoxic and effective as stabilizers in relatively low concentrations. The agents used in this study were polysorbate 80[1] and poloxalkol (polyoxyethylene–polyoxypropylene polymer)[2].

EXPERIMENTAL[3]

Materials—All reagents used were of analytically pure grade as previously reported (19). Surfactants were used as received without further purification.

Method of Analysis—Kinetic runs were carried out as previously described (19) using the 2,6-dichloroindophenol method.

Determination of Critical Micelle Concentration—The critical micelle concentrations (CMC) of polysorbate 80 and poloxalkol were determined at 25° in acetate buffers (pH 3.52, 4.55 and 5.45) and phosphate buffer (pH 6.60). The buffers were adjusted to ionic strength 0.4 by the addition of potassium chloride. The CMC's were determined from plots of surface tension *versus* concentration, using the point of intersection obtained by extrapolation of the straight-line portions of the curve as the CMC value.

Oxidation in Presence of Surfactants—To determine the effect of the surfactants on the analytical procedure previously described, ascorbic acid solutions containing 0.01 mole liter^{-1} of ascorbic acid and 5% polysorbate 80 or poloxalkol were prepared in acetate buffers (pH 3.52, 4.55, and 5.45) and phosphate buffer (pH 6.60). The solutions were analyzed for ascorbic acid using the 2,6-dichloroindophenol method.

The effect of polysorbate 80 and poloxalkol on the rate of oxidation of ascorbic acid was studied in acetate buffer (pH 4.55) and

[1] Tween 80, Atlas Chemical Co., Wilmington, Del.

[2] Pluronic F-68, Wyandotte Chemicals Corp., Wyandotte, Mich.

[3] Surface tension measurements were obtained using a Fisher surface tensiometer model 21 equipped with a 6-cm platinum–iridium ring. Viscosities were determined with an Ostwald-Fenske viscometer (size 100). A constant-temperature bath with a Haake thermoregulator series "ED" and a thermometer calibrated to 0.1° was used for all studies.

Table I—Analysis of Ascorbic Acid at Various pH's and Ionic Strength 0.4 in the Presence of Polysorbate 80 and Poloxalkol Using the 2,6-Dichloroindophenol Volumetric Method

Buffer	pH	Ascorbic Acid Added, moles liter^{-1} × 10^3	Ascorbic Acid Found, moles liter^{-1} × 10^3
	Polysorbate 80, 5% (w/v)		
Acetate	3.52	10.00	10.10
	4.55	10.00	10.02
	5.45	10.00	10.06
Phosphate	6.60	10.00	10.15
	Poloxalkol, 5% (w/v)		
Acetate	3.52	10.00	10.02
	4.55	10.00	10.15
	5.45	10.00	10.10
Phosphate	6.60	10.00	10.06

phosphate buffer (pH 6.60) at 67° and ionic strength 0.4. The concentrations of polysorbate 80 and of poloxalkol used were 0.0001, 0.001, 0.01, 0.05, 0.10, 0.50, 1.00, and 2.00% and 0.001, 0.01, 0.10, 0.50, 1.00, and 2.00%, respectively. Surfactant solutions containing 0.50% or more surfactant were prepared by dissolving accurately weighed quantities of surfactant in appropriate buffers. Solutions containing less than 0.50% surfactant were prepared by a dilution technique. The contents were analyzed for ascorbic acid using the previously described method (19).

Effect of Surfactant Concentration on Viscosity—Since high viscosity may contribute to the stability of a drug solution, the viscosities of aqueous solutions containing various concentrations of polysorbate 80 and poloxalkol were determined at 30 and 67°. Densities of the solutions were determined at the temperatures of the study using a pycnometer.

Effect of pH and Temperature on Stability of Ascorbic Acid Solutions Containing 1% Surfactant—The reactions were carried out in acetate buffer (pH 4.55) and phosphate buffer (pH 6.60) in the presence of 1% polysorbate 80 or poloxalkol at 50, 55, 60, and 67°. All buffers are adjusted to ionic strength 0.4 using potassium chloride. The concentration of ascorbic acid used was 0.01 mole liter^{-1}. Reactions were carried out for a maximum of 50 hr, and all aliquots were analyzed using the 2,6-dichloroindophenol method. Activation energies and $\Delta H\ddagger$, $\Delta S\ddagger$, and $\Delta F\ddagger$ values were determined from Arrhenius plots obtained by plotting log k *versus* $1/T$.

DISCUSSION

All rate constants were calculated from the first-order rate equation. The slopes of the lines were calculated by regression analysis (20, 21) on an electronic calculator[4]. All rate constants were calculated with n (number of points on the line) equal to 5–10. The correlation coefficient of all of the calculations was between −0.950 and −1.000. The thermodynamic parameters were calculated from Arrhenius plots using specially programmed cards.

The CMC of the two surfactants used in the study was determined at 25° using the surface tension lowering method. The CMC's were not significantly different at pH 3.52, 4.55, 5.45, and 6.60. The CMC's were approximately 10 mg/100 ml for polysorbate 80 and 100 mg/100 ml for poloxalkol at 25°. A typical plot of the effect of various surfactant concentrations on the surface tension of ascorbic acid solutions at pH 3.5 is shown in Fig. 1. The data shown in Table I indicate that the surfactants used did not interfere with the 2,6-dichloroindophenol method of analysis for ascorbic acid.

The effect of surfactant concentration on the rate of oxidation of ascorbic acid is shown in Fig. 2 and summarized in Tables II and III. The oxidation of ascorbic acid increased in dilute surfactant solutions containing a quantity of surfactant equal to 0.01 of the respective CMC values determined at 25°. Since this is an oxidation process, the magnitude of increase was approximately the same at pH 4.55 and 6.60 for both surfactants; *i.e.*, for polysorbate 80 at pH 4.55 and 6.60, the rate increased 1.53- and 1.60-fold over the rate obtained in surfactant-free buffer. In a dilute solution of poloxalkol, the increase in rate was 1.13- (pH 4.55) and 1.25- (pH 6.60) fold over the rate obtained in surfactant-free buffer. At surfactant concentrations equal to 0.1 of the respective CMC values, the rate was approximately the same as that obtained in surfactant-free buffer.

The increase observed in the oxidation rate of ascorbic acid in very dilute surfactant solutions may be due to the adsorption of

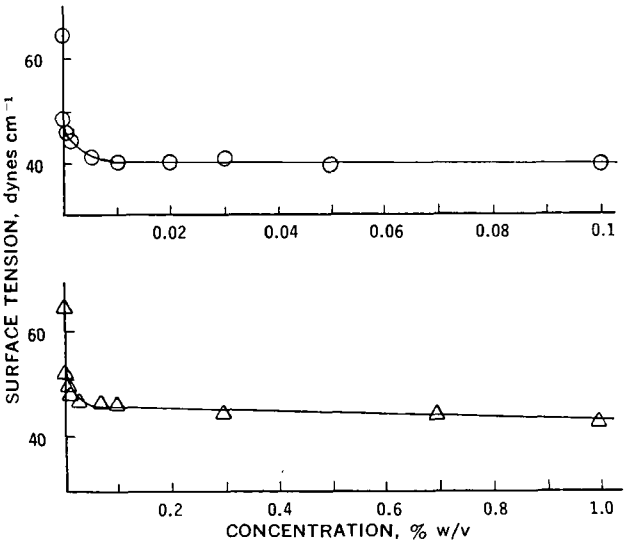

Figure 1—*Effect of concentration of surfactant on the surface tension of ascorbic acid (0.01 mole liter^{-1}) solution at 25° in acetate buffer pH 3.52 and ionic strength 0.4. Key: ○, polysorbate 80; and △, poloxalkol.*

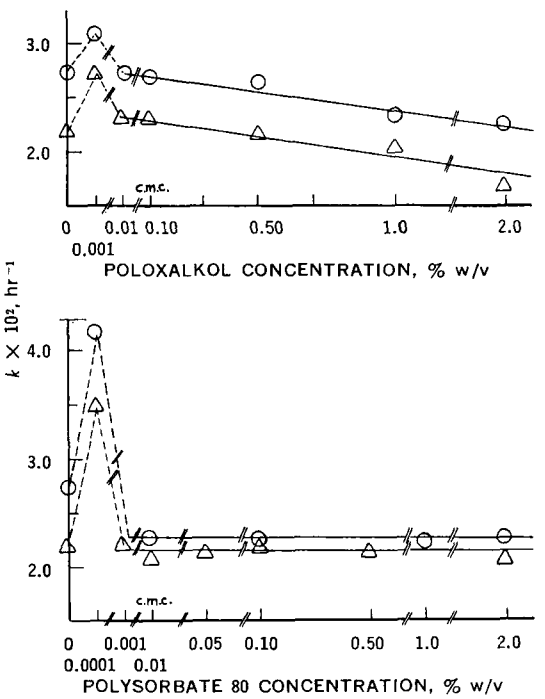

Figure 2—*Effect of various concentrations of surfactants on the rate of oxidation of ascorbic acid at 67° and ionic strength 0.4. Key: △, pH 4.55 (acetate buffer); and ○, pH 6.60 (phosphate buffer).*

[4] Wang model 360K/362K card programmer CP-1 with specially programmed cards.

Table II—Specific Rate Constants for the Oxidation of Ascorbic Acid at 67°, pH 4.55 and 6.60, and Ionic Strength 0.4 in the Presence of Polysorbate 80 and Poloxalkol

Buffer	pH	Polysorbate 80, % w/v	$k \times 10^2$, hr^{-1}	Poloxalkol, % w/v	$k \times 10^2$, hr^{-1}
Acetate	4.55	0.0	2.74	0.0	2.74
		0.0001	4.18	0.001	3.10
		0.01	2.26	0.01	2.74
		0.10	2.26	0.10	2.68
		1.00	2.23	0.50	2.64
		2.00	2.26	1.00	2.35
				2.00	2.25
Phosphate	6.60	0.0	2.20	0.0	2.20
		0.0001	3.52	0.001	2.74
		0.001	2.21	0.01	2.31
		0.01	2.12	0.10	2.31
		0.05	2.15	0.50	2.16
		0.10	2.20	1.00	2.07
		0.50	2.16	2.00	1.69
		2.00	2.08	—	—

Table III—Specific Rate Constants for the Oxidation of Ascorbic Acid at Various Temperatures and pH's in the Presence of 1% Surfactant

Surfactant	Buffer[a]	pH	Temperature	$k \times 10^2$, hr^{-1}
Polysorbate 80	Acetate	4.55	50.0°	1.03
			55.0°	1.42
			60.0°	1.96
			67.0°	2.23
	Phosphate	6.60	50.0°	2.36
			55.0°	2.59
			60.0°	2.30
Poloxalkol	Acetate	4.55	50.0°	1.07
			55.0°	1.34
			60.0°	1.92
			67.0°	2.35
	Phosphate	6.60	50.0°	2.49
			55.0°	2.21
			60.0°	1.70
			67.0°	2.07

[a] Ionic strength 0.4.

ascorbic acid molecules on the surface of the surfactant molecules, making them more susceptible to oxidative attack through surface catalysis. The increase in the oxidation rate might also be due to the formation of an association complex between ascorbic acid and surfactant molecules in which ascorbic acid is made more susceptible to oxidative degradation. These findings are in agreement with reported observations (17, 18).

Since the CMC's of nonionic surfactants decrease with increasing temperature, the CMC's determined at 25° were higher than those at the temperature of this study (67°). At surfactant concentrations equal to 0.1 of the CMC values determined at 25°, the oxidation rates of ascorbic acid in polysorbate 80 and poloxalkol solutions at 67° were approximately the same as the rates determined in surfactant-free buffer at the same temperature. Therefore, the CMC's of the two surfactants at 67° are probably approximately equal to 0.1 of their CMC's at 25°.

For polysorbate 80, no effect on the oxidation rate was observed at surfactant concentrations ranging from 0.1 of its CMC value (0.001%) up to 2% (w/v) surfactant; for poloxalkol, the oxidation rate of ascorbic acid decreased over the same surfactant concentration range. The effect can be shown by the following equations. For polysorbate 80 at pH 4.55:

$$k = 0.0225 \qquad \text{(Eq. 1)}$$

and at pH 6.60:

$$k = 0.0217 \qquad \text{(Eq. 2)}$$

For poloxalkol at pH 4.55:

$$k = 0.0272 - 0.0025(C) \qquad \text{(Eq. 3)}$$

and at pH 6.60:

$$k = 0.0233 - 0.0031(C) \qquad \text{(Eq. 4)}$$

where k is in $hour^{-1}$ and (C) is surfactant concentration from 0.01 to 2.00%. Equations 1–4 were obtained by regression analysis.

It is difficult to correlate the results (17, 18) of previous studies with those obtained in this study. Nixon and Chawla (17) studied saturated solutions of ascorbic acid containing high concentrations of polysorbate 20 and reported a sharp decrease in the copper-catalyzed oxidation rate in solutions containing about 40% surfactant. The decrease in the rate was attributed to a decrease in oxygen diffusion due to the high viscosity of the surfactant solutions. The decrease was also explained on the basis that, at very high polysorbate 20 concentrations, water was enclosed as a discontinuous pseudophase inside the micelles; the ascorbic acid dissolved in the water was protected from oxygen by the surfactant micelles.

In one study (18), solutions containing 3% ascorbic acid and varying amounts of polysorbate 80 were used. The solutions were not buffered and their pH varied from 2.5 to 4.0. Polysorbate 80 concentrations varied from 0.015 to 70%. The oxidation rate of ascorbic acid decreased in solutions containing 0.05–10% surfactant. The decrease in rate was attributed to an increase in micelle size.

An increase in the viscosity of a surfactant solution with increasing surfactant concentration could cause a decrease in the diffusion rate of oxygen, thereby decreasing the oxidative degradation rate of ascorbic acid. The viscosities of dispersions containing polysorbate 80 and poloxalkol were determined over the range of surfactant concentrations used (Fig. 3). The maximum increases in viscosity were 0.04 cps for polysorbate 80 and 0.09 cps for poloxalkol. These increases are not significant enough to affect markedly the diffusion rate of oxygen.

The differences in the behavior of polysorbate 80 (mol. wt. approximately 1270) and poloxalkol (mol. wt. approximately 8350) in stabilizing solutions containing ascorbic acid may be related to differences in the aggregation number and size of the micelles formed in each surfactant solution or to a difference in the number of available sites in the surfactant molecules at which ascorbic acid may bind. It is difficult to picture a geometrical arrangement for poloxalkol molecules that would produce large micelles with high aggregation numbers. This surfactant has very small lipophilic groups interrupted by a large number of polar ether oxygens. The ether oxygens do represent a large number of potential binding sites for ascorbic acid, so the decrease in the oxida-

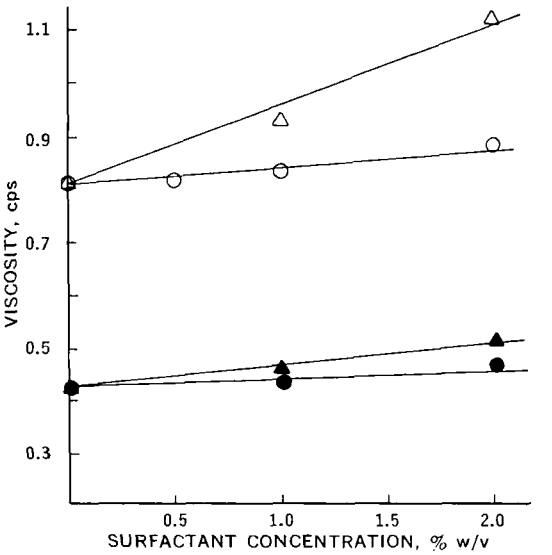

Figure 3—*Effect of various concentrations of surfactant on the viscosity. Key:* ○, *polysorbate 80 at 30°;* ●, *polysorbate 80 at 67°;* △, *poloxalkol at 30°; and* ▲, *poloxalkol at 67°.*

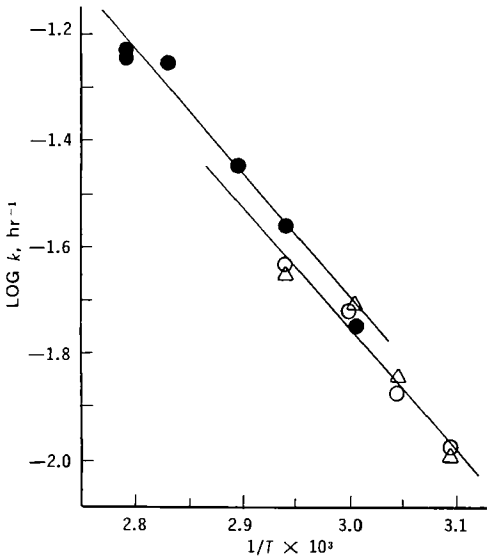

Figure 4—*Arrhenius plots showing the temperature dependence of ascorbic acid oxidation at pH 4.55 (acetate buffer) and ionic strength 0.4 in the presence of 1.0% surfactant. Key: ●, without surfactant; △, polysorbate 80; and ○, poloxalkol.*

tion rate of ascorbic acid may be due to interaction with these sites. Polysorbate 80 has a more favorable hydrophilic–lipophilic balance for the formation of spherical micelles. However, the polysorbate 80 molecules contain fewer potential binding sites than do the poloxalkol molecules. Also, ascorbic acid may be more sterically hindered from interacting with the binding sites in polysorbate 80 than with those in the straight chain poloxalkol.

The effects of pH and temperature on the aerobic oxidation of ascorbic acid are summarized in Table III. Figure 4 shows the plot of log k *versus* $1/T$ at pH 4.55. The thermodynamic parameters together with statistical parameters are shown in Table IV for ascorbic acid oxidation at 67° in pH 4.55 buffer and in buffer containing 1% surfactant. The large negative values of entropy of activation, $\Delta S\ddagger$, indicate that the degree of disorder in the activated complex and in the reaction is great.

At pH 6.60 the plot of log k *versus* $1/T$ obtained in the presence of either surfactant was not linear, so it was not possible to calculate the thermodynamic parameters at pH 6.60. The nonlinearity at pH 6.60 in solutions containing 1% surfactant was probably due to a complex mechanism. Either the mechanism proposed earlier (19) was not followed or additional reactions involving the surfactant molecules play a role in the rate-determining steps in the oxidative degradation of ascorbic acid.

CONCLUSIONS

Two nonionic surfactants, polysorbate 80 and poloxalkol, affected the oxidation rate of ascorbic acid. The rate increased sharply at surfactant concentrations equal to 0.01 of the CMC values. At surfactant concentrations equal to 0.1 of the CMC values, the rate was approximately the same as that obtained in surfactant-free water. For polysorbate 80, no effect on the oxidation rate was observed at surfactant concentrations ranging from 0.1 of the CMC value up to 2% surfactant, whereas for poloxalkol the oxidation rate decreased over the same surfactant concentration range.

The increase observed in the oxidation rate of ascorbic acid in very dilute surfactant solutions may be due to adsorption of ascorbic acid molecules on the surface of the surfactant molecules, making them more susceptible to oxidative attack through surface catalysis. The increase could also be due to the formation of an association complex between ascorbic acid and surfactant molecules.

The difference in the behavior of polysorbate 80 and poloxalkol in stabilizing solutions containing ascorbic acid may be related to differences in aggregation number and size of the micelles formed in surfactant solution or to a difference in the number of available sites in the surfactant molecules at which ascorbic acid may bind.

Table IV—Thermodynamic and Statistical Parameters Obtained from Arrhenius Plots for Ascorbic Acid Oxidation at 67°, pH 4.55, and Ionic Strength 0.4 in the Presence of 1% Surfactant

Parameter	No Surfactant[a]	Polysorbate 80	Poloxalkol
Thermodynamic			
Ea, kcal mole^{-1}	10.9	10.2	10.5
$H^{\ddagger}$, kcal mole^{-1}	10.3	9.5	9.8
$S^{\ddagger}$, cal mole^{-1} deg^{-1}	−52.1	−54.5	−53.6
$F^{\ddagger}$, kcal mole^{-1}	28.0	28.0	28.0
Statistical			
r (correlation coefficient)	−0.989	−0.968	−0.987
S_E (standard error of estimate)	0.035	0.046	0.030

[a] From Ref. 19, Table III.

Plots of log k *versus* $1/T$ yielded a straight-line relationship with a negative slope at pH 4.55 in buffer and in buffer containing 1% surfactant. The specific rate constants and half-life periods at 25° can be obtained from the plots by extrapolation. The nonlinearity of the Arrhenius plots at pH 6.60 in surfactant-containing solutions prevented the calculation of activation energies or other thermodynamic parameters.

REFERENCES

(1) J. W. McBain and T. R. Bolam, *J. Chem. Soc.*, **113**, 825(1918).

(2) H. Nogami, S. Awazu, and N. Nakajima, *Chem. Pharm. Bull.*, **10**, 503(1962).

(3) H. Nogami, S. Awazu, K. Watanake, and K. Sata, *ibid.*, **8**, 1136(1960).

(4) H. Nogami and T. Suzuki, *ibid.*, **9**, 646(1961).

(5) C. J. Kern and T. Antoshkiw, *Ind. Eng. Chem.*, **42**, 709(1950).

(6) C. L. J. Coles and D. F. W. Thomas, *J. Pharm. Pharmacol.*, **4**, 898(1952).

(7) F. S. M. Patel, V. S. Gupta, and M. V. Radhakrishna, *J. Sci. Ind. Res., India*, **14c**, 17(1955).

(8) J. E. Carless and J. R. Nixon, *J. Pharm. Pharmacol.*, **9**, 963(1957).

(9) *Ibid.*, **12**, 348(1960).

(10) J. E. Carless and A. G. Mitchell, *J. Pharm. Pharmacol.*, **14**, 46(1962).

(11) J. E. Carless and J. Swarbrick, *ibid.*, **14**, 977(1962).

(12) J. Swarbrick and J. E. Carless, *ibid.*, **16**, 596(1964).

(13) *Ibid.*, **16**, 670(1964).

(14) F. Giral, *J. Amer. Pharm. Ass., Sci. Ed.*, **36**, 82(1947).

(15) F. J. Bandelin and J. V. Tuschoff, *ibid.*, **44**, 241(1955).

(16) A. Bartilucci and N. E. Foss, *ibid.*, **43**, 159(1954).

(17) J. R. Nixon and B. P. S. Chawla, *J. Pharm. Pharmacol.*, **17**, 558(1965).

(18) R. I. Poust and J. L. Colaizzi, *J. Pharm. Sci.*, **57**, 2119(1968).

(19) S. M. Blaug and B. Hajratwala, *ibid.*, **61**, 556(1972).

(20) S. S. Wilks, "Elementary Statistical Analysis," Princeton University Press, Princeton, N.J., 1964, p. 236.

(21) I. Guttman and S. S. Wilks, "Introductory Engineering Statistics," Wiley, New York, N.Y., 1965, p. 241.

ACKNOWLEDGMENTS AND ADDRESSES

Received January 8, 1974, from the *College of Pharmacy, University of Iowa, Iowa City, IA 52242*

Accepted for publication March 25, 1974.

* Present address: Department of Pharmacy, University of Otago, Dunedin, New Zealand.

ˣ To whom inquiries should be directed.