by cupric ions in aqueous solutions at neutral pH can be demonstrated only in the presence of phosphate ions.

It has been demonstrated in this study that 21-dehydroprednisolone interacts with histones. This is in accord with the report of Kripalani and Sorby (10) that the degradative products of hydrocortisone interact strongly with human serum albumin. The different binding characteristics of histones with prednisolone and 21-dehydroprednisolone (Fig. 6) suggest that they interact with different loci on the histone molecule. The binding characteristics of these two steroids with transcortin and serum albumin were distinctly different (13). Preliminary studies on the interaction of 21-dehydrocorticosteroids with amino acids in this laboratory suggests that the reaction occurs between the 21-aldehyde and the α-amino group to form Schiff bases.

The possibility that prednisolone (21-dehydrocortisol, is formed under physiological conditions is suggested by the demonstration of 21-hydroxysteroid dehydrogenase in rat and sheep liver and bovine adrenal gland (14–16) although the reaction is strongly in the direction of the alcohol formation. It is noteworthy that the 21-dehydrosteroids are less active or inactive in biological systems in comparison to the parent corticosteroids (17, 18), suggesting that they need to be converted to the alcohol form to be biologically active. Since the biologically active prednisolone did not bind appreciably with histones in comparison with prednisolone, the mechanism of action of prednisolone probably does not depend on its interaction with histones.

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Keyphrases

Prednisolone—degradation products
EDTA, cupric ions effect—prednisolone degradation
21-Dehydroprednisolone binding—calf thymus histones
TLC—separation, identity

Copper-Catalyzed Oxidation of Ascorbic Acid in Gels and Aqueous Solutions of Polysorbate 80

By ROLLAND I. POUST and JOHN L. COLAIZZI

The stability of ascorbic acid in aqueous solution has been studied as a function of polysorbate 80 concentration. The range of polysorbate concentrations studied was sufficiently wide to provide systems ranging from solutions of low viscosity to hydrogels. The study has been conducted at 30, 40, 50, and 60°. First-order rate constants for the oxidative reaction were calculated, and they appear to decrease as a function of polysorbate 80 concentration up to 10 percent, except at 30°, in which case the rate constants decreased up to 30 percent polysorbate 80. The rate generally leveled off at higher surfactant concentrations. It appears that this phenomenon is probably a result of micelle formation, or, more specifically, the increases in micellar aggregation number and micellar concentration which occur up to about 30 percent polysorbate 80. It does not appear that increased viscosity has a significant influence on the rate of ascorbic acid oxidation in these systems.

THE POSSIBILITY OF ENHANCING the stability of drugs by incorporating them into structured vehicles, such as thixotropic gels, has been suggested. Drugs such as vitamins, which are susceptible to decomposition, have been found to be stable for longer periods of time in thixotropic preparations (1). Macek (2) noted that a vehicle of peanut or sesame oil gelled with 2%

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aluminum monostearate reduces the rate of solution (and thus the hydrolytic tendency) of penicillin due to a physical entrapment of the penicillin within the cells of the hydrophobic structural network of the gel. However, relatively little experimental evidence exists describing specific studies performed to investigate this potential means of drug stabilization.

Several studies have indicated the stabilizing effects of nonionic surfactants on various drugs. Kern and Antoshkiw (3) found that aqueous dispersions containing 20% (w/v) polyoxyethylene (20) sorbitan monolaurate[1] stabilized vitamin A to a greater extent than did cottonseed oil containing 2% tocopherol and 4% lecithin, the optimum antioxidant combination for the oil. Swarbrick and Rhodes (4), in a study on the oxidation of linoleic acid in aqueous solutions of polyoxyethylene (23) lauryl ether,[2] found that increasing the surfactant concentration significantly decreased the rate of oxidation. In a series of studies, Carless *et al.* (5–9) found that the rate of oxidation of certain aldehydes and oils was decreased by increasing the concentration of nonionic surfactants used (cetomacrogol[3] and betaine[4]). In a similar study, Mitchell and Wan (10) also found that for a fixed concentration of aldehyde, an increase in surfactant concentration caused a decrease in the rate of oxidation. Swarbrick (11) has reviewed most of these studies in a review article dealing with solubilized pharmaceutical systems.

With such observations in mind, the authors have investigated the stability pattern of ascorbic acid at several temperatures in a range of concentrations of polysorbate 80[5] sufficiently wide to provide vehicles ranging from aqueous solutions to hydrogels. Thus it was possible to investigate relative effects of surfactant concentration, viscosity, and gelation on the stability of this particular drug.

Several attempts have been made to stabilize aqueous solutions of ascorbic acid against oxidation to dehydroascorbic acid by employing various pharmaceutical vehicles and additives, all of which tend to cause slight increases in the viscosity of the solution. Giral (12) hints that the viscosity of a pharmaceutical vehicle such as simple syrup might be a protective factor in stabilizing ascorbic acid. Bandelin and Tuschhoff (13) found that natural hydrophilic colloids (methylcellulose, carboxymethylcellulose, tragacanth, and pectin) which were added to produce solutions of greater viscosity, seemed to accelerate oxidation. This could have been due to trace metals or other impurities introduced by these gums. They also stated that aqueous solutions of sucrose, sorbitol, glycerin, and propylene glycol stabilized ascorbic acid. In a similar study, Bartilucci and Foss (14) found that ascorbic acid was most stable in high concentrations of propylene glycol with either (*a*) water and glycerin or sorbitol, or (*b*) a mixture of glycerin and sorbitol. In a study similar to this one, Nixon and Chawla (15) determined the effects of a wide range of polysorbate 20 concentrations on the oxidation rate of ascorbic acid in an aqueous solution buffered at pH 3.4. They found that the oxidation rates for the copper-catalyzed oxidation rise slightly in aqueous solutions of polysorbate 20 up to a concentration of about 40% (w/w). From that concentration, the oxidation rate then decreased quite rapidly until a concentration of 75% surfactant was reached. Here a leveling off of the rate of oxidation was observed up to about 93% where a slight decrease occurred. At the same time they noted that the aqueous systems showed a fairly rapid increase in viscosity from about 30 to 60% polysorbate 20. From the maximum, which occurred at 62.8%, a gradual decrease in viscosity was noted as the concentration of surfactant approached 100%. In all cases, the rheological properties of these systems were found to be Newtonian in nature. From these results the authors concluded that incorporation of ascorbic acid within the micelle appeared to be responsible for an increase in the oxidation rate at low surfactant concentrations, but that the high viscosity of the concentrated polysorbate 20 solutions caused a much larger reduction in oxidation rate due to a decrease in the diffusion of oxygen to the reaction site.

The viscosity-building and gelation characteristics of aqueous polysorbate 80 mixtures have been reported by Atlas Chemical Industries, Inc. (16). For example, it was reported that the viscosity of aqueous polysorbate 80 systems increased to 740 cps. at a surfactant concentration of 40% (w/w). Actual gel formation occurred from 45 to 65% surfactant concentrations. The viscosity then decreased from 4,700 to 500 cps. between 70 and 95% polysorbate 80. Polysorbate 80 was chosen for this study because of its nontoxic nature (17), gelling characteristics, viscosity-building effects, and the apparent physical, chemical, and microbial stability of the systems.

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[1] Marketed as Tween 20, Atlas Chemical Industries, Inc., Wilmington, Del.
[2] Marketed as Brij 35, Atlas Chemical Industries, Inc., Wilmington, Del.
[3] Polyethylene glycol 1000 monocetyl ether.
[4] *N*-Alkyl, *N*,*N*-dimethylglycines.
[5] Marketed as Tween 80 by Atlas Chemical Industries, Inc., Wilmington, Del.

MATERIALS AND METHODS

Ascorbic acid was well suited for this study because its fairly rapid copper-catalyzed oxidation can be followed by a simple UV spectrophotometric assay (18). This method enables one to distinguish between ascorbic acid, which absorbs UV light in acidified solutions at 245 mμ, and its main product of oxidation, dehydroascorbic acid, which shows virtually no absorbance in this region of the spectrum.

Preparation and Storage of Test Systems—Exactly 3.00 g. of ascorbic acid[6] was weighed and dissolved in a small quantity of deionized water.[7] The remaining water, enough to give a total mass of 100 g., was then added. Polysorbate 80 USP XVII[5] in sufficient quantities to make concentrations of 0, 0.015, 0.05, 5, 10, 30, 40, 50, 60, and 70%[8] was then gradually stirred into the aqueous solution (using a Fisher Fultork Labmotor[9]) until a homogeneous mixture was obtained. For concentrations of polysorbate 80 above 40%, the mixture was heated on a steam bath to facilitate the escape of air which became entrapped as gels were formed in this concentration range. Initial readings indicated that this heating was not deleterious to the stability of the drug. Finally, 1.0 ml. of a 1×10^{-2} *M* cupric sulfate[10] solution was pipeted into the mixture to give a concentration of 1×10^{-4} *M* in cupric ions. Each test system was assayed immediately upon the completion of its preparation, and was then poured into a 4-oz. amber glass jar, sealed, and stored at either 30, 40, 50, or 60°. Temperature control was achieved by a water bath (30°) Sargent Thermonitor[11] or one of two ovens employed.[12] Temperatures were kept within ±0.5°.

Periodic Assays of Test Systems—All test systems were assayed immediately upon completion of their preparation at various predetermined time intervals. This was accomplished by accurately weighing a 1-g. sample of the test system, quantitatively transferring it to a 100-ml. volumetric flask, and diluting to volume with deionized water. Then 5.0 ml. of this solution was pipeted into another 100-ml. volumetric flask, 10.0 ml. of a 0.2 *N* hydrochloric acid solution was added, and the solution diluted to volume with deionized water. The absorbance of this solution was then determined at 245 mμ on a spectrophotometer (Beckman DB-G)[13] against a suitable reagent blank. The concentration of ascorbic acid remaining was determined from a Beer's law plot[14] of absorbance as a function of ascorbic acid concentration. From these values, the percentage of ascorbic acid remaining was calculated.

Table I—Viscosity Values of Various Concentrations of Polysorbate 80 in Water at 30°

Concn. of Polysorbate 80, % w/w	Viscosity, cps.
40	1304
50	— [a]
60	— [a]
70	4154
100	609

[a] Gel formation.

RESULTS

The physical appearance of the test systems in the concentration range studied was somewhat varied. Those systems containing no polysorbate 80 were clear, colorless solutions at all temperatures. The systems containing 0.015 and 0.05% polysorbate 80 were clear, faintly yellow solutions. The systems containing 5, 10, 30, and 40% surfactant were all clear solutions of increasing viscosity and increasing intensity of yellow color, due to the increasing concentrations of polysorbate 80. The 50% polysorbate 80 system was a translucent yellow gel at 30 and 40°, and a clear, yellow, highly viscous liquid at 50 and 60°. The 60% polysorbate 80 system was a clear yellow gel at 30° and a clear, yellow, highly viscous liquid at the other temperatures. The systems containing 70% surfactant were clear, yellow, viscous liquids at all temperatures. The liquid systems exhibited Newtonian rheological properties, and the gels behaved as non-Newtonian systems when analyzed in the rotational viscosimeter (Stormer). Viscosity values for some of the Newtonian liquids, as determined at 30°, are summarized in Table I.

The results of the stability studies at 30, 40, 50, and 60° are summarized in Tables II–V, showing the percentage concentrations of ascorbic acid remaining at various times in each concentration of polysorbate 80 used. At certain concentrations of surfactant, some protection from oxidation was afforded the ascorbic acid. In the cases of 60 and 70% polysorbate 80 at 30 and 40°, the reaction seemed to stop after a few days. The logarithms of the percentages of ascorbic acid remaining were plotted as a function of time for all concentrations of polysorbate 80 used at all temperatures. Four such plots representing the reaction in water, 10, 40, and 60% polysorbate 80 appear as Figs. 1–4. From these plots it appeared that the kinetics of these reactions is first-order. This is in agreement with the findings of Dekker and Dickenson (20) who stated that the copper-catalyzed oxidation of ascorbic acid in water is a first-order kinetic process. The straight lines shown were obtained by the method of least squares (21), but the points shown represent the experimental values. The slopes obtained from the least-squares calculations were used to calculate values (k) for the first-order rate constants (22). First order-rate constants were calculated for each experimental situation and were plotted as a function of polysorbate 80 concentration at all temperatures. These graphs appear as Fig. 5.

[6] Analytical reagent grade, Fisher Scientific Co., Pittsburgh, Pa.

[7] Deionized water was used throughout the study and was prepared by passing distilled water through two glass percolation columns containing a mixture of cationic- and anionic-exchange resins (Rexyn AG 501, Fisher Scientific Co.). Conductivity was below 0.1 p.p.m. (as sodium chloride) as determined with a Barnstead purity meter (Barnstead Still and Sterilizer Co., Boston, Mass.)

[8] Percentage values indicate percent by weight.

[9] Fisher Scientific Co., Pittsburgh, Pa.

[10] Analytical reagent grade, Merck and Co., Rahway, N. J.

[11] E. H. Sargent and Co., Chicago, Ill.

[12] Blue M Stabil-Therm Mechanical Convection Oven and Labline Radiant Heat Oven, Aloe Scientific, St. Louis, Mo.

[13] Beckman Instruments, Inc., Fullerton, Calif.

[14] These plots were prepared by determining absorbance values of solutions containing 2.00, 1.75, 1.50, 1.00, 0.50, and 0.25 mg.% ascorbic acid against a suitable blank. This was done for each concentration of polysorbate 80 used in the study.

TABLE II—PERCENTAGE CONCENTRATION OF ASCORBIC ACID REMAINING AS A FUNCTION OF TIME IN VARIOUS CONCENTRATIONS OF POLYSORBATE 80 AT 30° IN THE PRESENCE OF 1×10^{-4} *M* CUPRIC ION

Time, days	Water	Concn. of Polysorbate 80, % w/w							
		0.015	0.05	10	30	40	50	60	70
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1	95.00	95.90	95.04	92.86	—	—	—	—	—
2	89.20	91.02	90.87	94.00	98.71	98.35	92.46	96.15	94.91
3	92.93	91.02	90.08	91.26	—	—	—	—	—
4	90.86	89.40	90.87	90.43	—	—	—	—	—
7	86.70	86.15	85.52	85.87	—	—	—	—	—
8	—	—	—	—	96.62	95.75	—	83.65	93.62
11	83.33	81.27	81.82	82.47	96.08	94.93	92.46	87.56	93.62
18	78.73	75.22	78.51	79.54	94.53	93.22	83.73	84.95	93.62
25	73.73	69.11	72.70	72.91	92.03	88.09	79.13	81.52	91.52
32	71.66	64.17	62.78	71.70	90.34	88.09	79.13	81.52	91.52
39	70.96	60.92	55.38	69.34	88.65	86.79	79.13	74.72	91.52
46	67.06	55.26	49.17	68.13					

TABLE III—PERCENTAGE CONCENTRATION OF ASCORBIC ACID REMAINING AS A FUNCTION OF TIME IN VARIOUS CONCENTRATIONS OF POLYSORBATE 80 AT 40° IN THE PRESENCE OF 1×10^{-4} *M* CUPRIC ION

Time, days	Water	Concn. of Polysorbate 80, % w/w							
		0.015	0.05	10	30	40	50	60	70
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1	95.00	95.08	95.83	96.13	97.52	100.00	100.00	93.53	89.01
5	—	—	—	—	—	96.64	91.41	87.53	88.13
6	86.70	85.24	86.78	91.80	92.52	—	—	—	—
8	83.33	80.78	83.01	88.52	91.02	94.04	91.41	93.33	89.01
12	76.67	75.80	78.51	86.88	89.79	91.51	84.13	90.86	89.01
19	68.33	65.57	66.49	81.83	84.13	85.49	84.13	90.00	89.01
26	58.33	54.75	56.17	77.44	81.27	80.36	76.44	85.00	88.54
33	50.40	46.29	49.57	70.09	74.38	78.45	76.44	83.33	88.13

TABLE IV—PERCENTAGE CONCENTRATION OF ASCORBIC ACID REMAINING AS A FUNCTION OF TIME IN VARIOUS CONCENTRATIONS OF POLYSORBATE 80 AT 50° IN THE PRESENCE OF 1×10^{-4} *M* CUPRIC ION

Time, days	Water	Concn. of Polysorbate 80, % w/w							
		0.015	0.05	10	30	40	50	60	70
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1	92.93	95.00	96.56	84.85	98.71	99.17	98.71	100.00	100.00
4	80.00	84.52	86.78	91.80	96.08	94.04	97.42	98.33	92.40
7	73.73	75.16	75.21	85.24	92.03	88.50	90.23	92.06	91.52
11	68.73	60.14	61.92	77.44	84.40	81.66	83.86	83.73	84.74
14	63.33	54.48	52.87	72.13	81.02	76.94	75.38	81.66	81.35
18	52.06	46.74	42.96	67.60	72.18	70.51	71.59	75.00	77.55
21	45.00	40.63	38.00	61.44	65.83	66.68	67.79	71.73	71.18
25	36.66	32.89	31.59	56.52	59.55	61.55	63.11	66.67	69.49
28	32.06	27.63	27.23	54.09	55.70	58.13	57.22	61.66	66.91

TABLE V—PERCENTAGE CONCENTRATION OF ASCORBIC ACID REMAINING AS A FUNCTION OF TIME IN VARIOUS CONCENTRATIONS OF POLYSORBATE 80 AT 60° IN THE PRESENCE OF 1×10^{-4} *M* CUPRIC ION

Time, days	Water	Concn. of Polysorbate 80, % w/w							
		0.05	5	10	30	40	50	60	70
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1	91.67	95.00	96.67	99.67	100.00	96.67	91.33	90.87	99.67
5	71.67	78.33	80.80	90.00	92.53	86.67	85.00	88.33	92.53
8	58.80	65.00	69.10	83.40	88.45	83.40	80.80	85.00	85.00
12	45.00	50.75	58.80	75.80	80.80	75.00	76.70	80.00	76.70
15	38.33	45.87	55.00	70.00	75.00	70.86	75.00	76.70	75.00
19	30.87	35.87	50.87	64.13	68.33	65.00	68.33	70.00	71.67
22	25.87	33.33	48.33	61.67	66.67	60.87	64.13	66.67	66.67
26	20.00	26.67	44.13	57.53	60.00	54.20	60.67	62.47	62.47
29	17.53	25.87	41.67	54.20	56.67	51.67	55.00	56.67	60.87

As can be seen from the plots in Fig. 5, the rate of oxidation in 0.015% (slightly below the CMC of 0.03%)[15] and 0.05% (slightly above the CMC) is slightly higher than that of water at all temperatures except at 60° where a slightly lower value for 0.05% polysorbate 80 was observed. These increases are followed by a lowering of the rate at a 10% polysorbate 80 concentration at all temperatures. At the lower temperatures, this is followed by a further decrease at 30% polysorbate 80, somewhat of a leveling effect at 40%, an increase at 50%, and subsequent decreases at 60 and 70%. At the higher temperatures, the general trend seems to be a leveling off between 10 and 40% polysorbate 80 followed by a gradual decrease up to 70%, the limiting concentration to which the study was performed.

DISCUSSION

Weissberger *et al.* (23) have reported that the copper-catalyzed oxidation of ascorbic acid is a

[15] This value for the CMC was determined at 30° with a Fisher Surface Tensiomat, Fisher Scientific Co., Pittsburgh Pa.

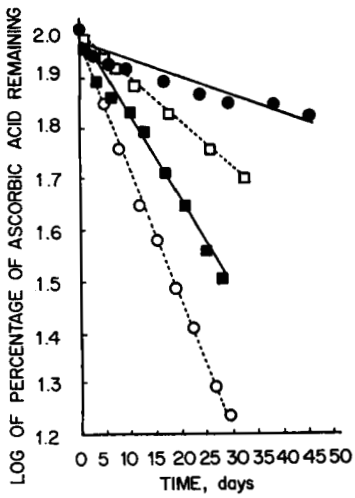

Fig. 1—Log of percentage of ascorbic acid remaining as a function of time in water. Key: ●, 30°; □, 40°; ■, 50°; ○, 60°.

first-order kinetic process and that the rate is proportional to the oxygen concentration in neutral and acid solutions. (While not carefully controlled through the use of buffers, pH values for the systems employed in this study fell within a range of 2.5 to 4.0.) They indicated that the rate-determining step is the formation of an intermediate semiquinone-like structure from the ascorbate ion, and that the formation of this intermediate is catalyzed by the presence of the cupric ion. Subsequent oxidation of this intermediate to dehydroascorbic acid is therefore dependent upon the concentration of oxygen present. Dekker and Dickenson (20) have reported much the same findings and are in agreement with Weissberger *et al.* as to the proposed mechanism of oxidation. As was mentioned previously, the decomposition kinetics of ascorbic acid for the systems investigated in the present study appears to be first-order, which is in agreement with the literature references indicated here.

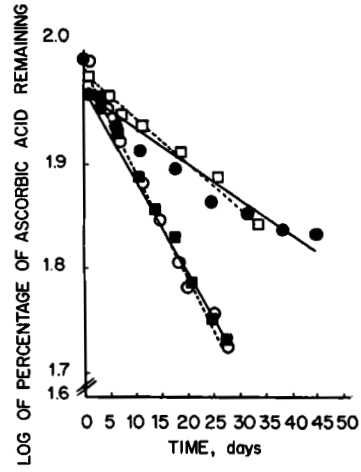

Fig. 2—Log of percentage of ascorbic acid remaining as a function of time in 10% polysorbate 80. Key: ●, 30°; □, 40°; ■, 50°; ○, 60°.

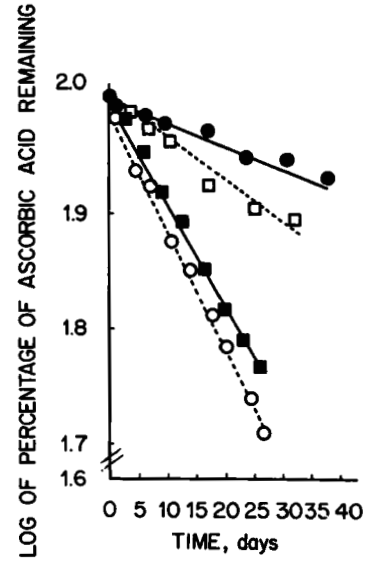

Fig. 3—Log of percentage of ascorbic acid remaining as a function of time in 40% polysorbate 80. Key: ●, 30°; □, 40°; ■, 50°; ○, 60°.

As illustrated in Fig. 5, the general effect observed upon the addition of polysorbate 80 to aqueous solutions of ascorbic acid is the occurrence of a reduction in rate constant. However, it should be noted that this reduction seems to be significantly influenced by surfactant concentration only up to a polysorbate 80 concentration of approximately 30% at 30° and 10% at the other temperatures. Then there was a general leveling off of the rate constants at higher surfactant concentrations. In all studies except that carried out at 60°, an initial increase in rate constants was observed in the systems containing 0.015 and 0.05% polysorbate 80. This may be due to an association complex formed

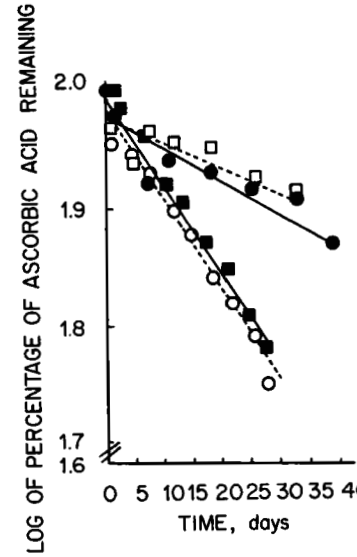

Fig. 4—Log of percentage of ascorbic acid remaining as a function of time in 60% polysorbate 80. Key: ●, 30°; □, 40°; ■, 50°; ○, 60°.

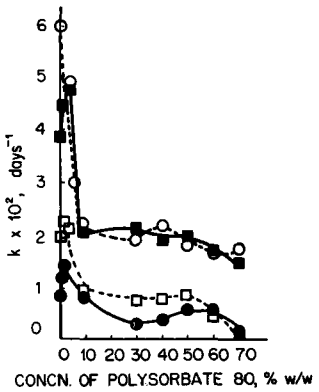

Fig. 5—First-order rate constants as a function of polysorbate 80 concentration. Key: ●, 30°; □, 40°; ■, 50°; ○, 60°.

between the ascorbic acid and polysorbate 80 molecules in which the ascorbic acid is made more readily susceptible to oxidative degradation. Another possibility might be that the ascorbic acid molecules are adsorbed onto the surface of the surfactant molecules, again making them more susceptible to oxidative attack due to a surface-type catalysis. These findings are in agreement with the observation of Nixon and Chawla (15) that low surfactant concentrations caused a rise in the rate of oxidation of ascorbic acid. Whatever the reasons for this phenomenon, these effects are apparently overcome at higher polysorbate 80 concentrations where a decrease in the rate of oxidation (as compared with the rate in systems containing no surfactant) occurred at all concentrations of polysorbate 80 studied. This decrease in the rate, or protection from oxidation, is most probably related in some way to micelle formation.

As was mentioned previously, Nixon and Chawla (15) carried out a stability study with ascorbic acid which in some respects resembles the present study. However, there are significant differences in the results of the two studies which could be accounted for in terms of differences in experimental conditions. The results in the present study showed that the greatest decrease in the copper-catalyzed oxidation rate occurred between 0.05 and 10% surfactant except at 60° where there was no initial increase in the rate with the very low surfactant concentration. At polysorbate 80 concentrations higher than 10%, a general leveling off of the rate constants occurred with an additional slight decrease occurring between 50 and 70%. However, in their study, Nixon and Chawla found an increase in the copper-catalyzed oxidation rate at the lower concentrations up to about 40%, where a sharp decrease in the rate occurred which continued up to a concentration of about 75% polysorbate 20, followed by a leveling off. It should be pointed out that different surfactants were utilized in each of the studies—polysorbate 80 in the present study and polysorbate 20 in the other. Also, with polysorbate 20, no gel formation, such as that which occurred in the present study, was observed. Perhaps the most significant difference was the method of assay employed by Nixon and Chawla in which a Warburg apparatus was used to measure the oxygen uptake for the total system, thus introducing the possibility that substances other than ascorbic acid may utilize oxygen and alter the rate of oxygen uptake. However, in the present study, the assay method was specifically designed to determine only the concentration of ascorbic acid remaining in the system thus eliminating any interference introduced by other components of the system. The initial concentration of ascorbic acid could also be responsible for differences in the results. Nixon and Chawla employed saturated solutions of ascorbic acid in polysorbate 20 whereas 3% (w/w) ascorbic systems were employed in the present study. The use of buffers, such as the dibasic sodium phosphate–citric acid buffer utilized by Nixon and Chawla, may have caused some difference in the results since no buffers were used in the present study. Finally, the stability studies carried out by Nixon and Chawla were at 25° exclusively, while four different temperatures—30, 40, 50, and 60°—were employed in the present study.

If the stabilizing effects of polysorbate 80 are, in fact, related to the presence of micelles, then those factors which are known to alter the micellar properties of a system should also influence stabilization profiles such as those observed in this study. Becher (24), in a discussion of micelle formation has stated that the aggregation number (number of monomer units per micelle) of a micelle appears to increase with increasing surfactant concentration up to those concentrations that are between 100 and 1,000 times the CMC. Thus, based on this idea, micelles in the surfactant systems of this study would increase in aggregation number and hence in micelle size between concentrations of 3 and 30% polysorbate 80.[16] At higher surfactant concentrations, the aggregation number levels off and thus the size of the micelles is somewhat the same at the various higher concentrations of polysorbate 80 employed in this study. Micellar shape can also vary with surfactant concentration as has been indicated by Nakagawa (25) who stated that micelles in dilute solutions are spherical or globular, but in more concentrated solutions the micelles tend to assume a lamellar structure. It has been found (24) that increased temperatures cause increases in the micellar weight suggesting that larger micelles would occur at higher temperatures. The addition of electrolytes has been found to cause a drop in the CMC, but causes very little, if any, change in the size of the micelle. Thus, the addition of ascorbic acid to these systems will have no appreciable effect on the size and shape of the micelle.

Based on the ideas in the preceding discussion and the plot of rate constants presented in Fig. 5, it appears possible that increases in the number of micelles and increases in the micellar aggregation number, *i.e.*, increases in the size of the micelles, which occur up to about 30% polysorbate 80, may cause the sharp decrease in the rate constants which is observed in this concentration range. The more extensive formation of lamellar-type micelles may also come about in this concentration range, and this might be another factor responsible for the decrease in rate constants. This decrease in oxidation rate may be due to a decrease in the collision

[16] These figures are based on the previously mentioned CMC of 0.03% for polysorbate 80 at 30°.

frequencies caused by the increasing size of the micelles. The larger micelles may slow down the rate of oxygen diffusion and thus slow down the rate of oxygen attack of the semiquinone-like ascorbic acid intermediate. As was indicated previously, the micellar size remains essentially constant at polysorbate 80 concentrations above 30% as does the rate of oxidation as can be seen by the general leveling off in Fig. 5 at higher surfactant concentrations. The increase in micellar size would be even larger at the higher temperatures possibly causing the sharper drop in the rate constants which was observed. In fact, different micellar properties at the higher temperatures might be responsible for the interesting grouping and similarity of slope observed with the results at 30 and 40° as compared to the results at 50 and 60° as illustrated in Figs. 2–4.

Based on these observations, it would seem that the increased number of micelles and changes in micellar shape that may occur with increasing polysorbate 80 concentrations (above 30%) have no appreciable effect on the rate of oxidation. It was noted that a slight decrease in the rate of oxidation occurred between 50 and 70% polysorbate 80, a region where gels and the liquid of maximum viscosity occurred. Nixon and Chawla (15) stated that at very high polysorbate 20 concentrations water was enclosed as a discontinuous pseudophase inside the micelle and that the ascorbic acid dissolved in it was protected from the oxygen by the surfactant. This might account for the slight additional decrease in oxidation rate at these higher surfactant concentrations. Nixon and Chawla have also proposed that the stabilizing effect of polysorbate 20 is closely related to the increasing viscosity of the systems which decreases the rate of oxygen diffusion. The work of Bartilucci and Foss (14) indicates that it would be possible that retardation of oxidation of ascorbic acid may be due primarily to the decrease in water content as surfactant concentrations increase. The results of this study, however, would seem to indicate that increased viscosity or simple reduction in water content are not the primary factors responsible for surfactant stabilization of ascorbic acid in the case of polysorbate 80. The viscosity of the systems employed in this study increased markedly between 40 and 50% polysorbate 80 (where gel formation occurred), whereas no significant change in the rate constants occurred in this region. The 60% polysorbate 80 gel and the 70% liquid also have viscosity values which are much higher than the 40% surfactant system, but no corresponding decrease in the rate constants was observed at these concentrations as would be expected if viscosity were the primary factor responsible for stabilization of ascorbic acid. Atlas Chemical Industries (16) has reported that only a gradual increase in viscosity of polysorbate 80–water systems occurs between 20 and 40% surfactant concentrations. Such a gradual increase in viscosity does not account for the large decrease in rate constants which was observed around 10% polysorbate 80. Similarly, a more gradual decrease in rate constants would probably have been observed if simple reduction in water content had been the stabilizing factor. Thus, it would seem that changes in micellar structure provide a more suitable explanation for the results obtained in this study.

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Keyphrases

Ascorbic acid stability—polysorbate 80 solutions, gels
Polysorbate 80 effect—ascorbic acid oxidation
Copper-catalyzed oxidation—ascorbic acid
UV spectrophotometry—analysis