Ascorbic Acid: A Jaffé Interference

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The effects of ascorbate, the interference of the Jaffé reaction was individually studied by polarography, spectrophotometry, and thin-layer chromatography. Ascorbate was incubated in 28.4 mmol dm^{-3} picrate blank solution in 0.51 mol dm^{-3} NaOH at 25.00±0.02 °C and the reactivity of test solution containing ascorbate : picrate was investigated. Picramic acid formed in all ascorbate: 28.4 mmol dm^{-3} picrate test solutions which explained the observed positive interference of ascorbic acid. No fluorescent product was formed in ascorbate : picrate test solutions. The reactivity of ascorbate : picrate test solutions confirmed that ascorbate was a powerful reducing agent. 2-Furaldehyde which has been listed as one of the degradation products of ascorbic acid, is not identified under the experimental conditions.

In the Jaffé reaction, a red color formed at room temperature when creatinine was reacted with picric acid at an alkaline pH.[1] Even with the present automation and adapted kinetic Jaffé procedures, many authors have reported positive results due to interferences such as ascorbic acid.[2-4]

Momose et al.[5] classified ascorbic acid (vitamin C) under active methylene compounds along with pyruvic acid, whereas Bowers and Wong[6] considered ascorbate as another potential reducing substance. At therapeutic concentrations, vitamin C distinctly interfered with the determination of creatinine.[7] According to Taussky, ascorbic acid did not interfere in the serum procedure when iodine treatment preceded ether extraction.[8] Cook proposed two categories for creatinine interferences and classified ascorbic acid as a reductive interference.[9]

The purpose of the present study was to examine the interaction of ascorbic acid as an interference of the Jaffé reaction. The reactivity of ascorbic acid with 28.4 mmol dm^{-3} picrate in 0.51 mol dm^{-3} NaOH at 25 °C was investigated by polarography, spectrophotometry, and thin-layer chromatography (TLC) studies. The presence of 2-furaldehyde was studied by polarography as it has been listed as one of the degradation products of ascorbic acid,[10] and as an intereference of the Jaffé reaction.[11,12]

Materials and Methods

Materials. Picric acid was from BDH Chemicals, Toronto, Canada. L-Ascorbic acid (sodium salt) and 2-furaldehyde were purchased from Sigma Chemical Company, St. Louis, Missouri, USA. Model 4001 Pulse/DC Sargent-Welch Polarograph™ was from Sargent-Welch Scientific Co., Skokie, Illinois, USA. A Metrohm AG9100 Herisau reference electrode was from Brinkmann Instruments Canada Limited, Rexdale, Canada. A Model 8450A UV/vis spectrophotometer and a Model 7245A-plotter printer were from Hewlett-Packard, San Diego, California, USA. Type N-1 spectrophotometric cells (cuvets) with a light path of 2 mm were from Norell Inc., Landisville, New Jersey, USA. Whatman 20×20 cm K5 silica gel TLC plates (250 μm thickness) were purchased from Canlab, Winnipeg, Canada. All pH measurements were performed with a Fisher Accumet pH meter, Model 825 MP from Fisher Scientific Co., Fairlawn, New Jersey, USA.

Methods. Clear saturated picric acid solution (1.30 g/100 ml) was used for all studies at 25.00±0.02 °C. Sodium ascorbate was incubated with 28.4 mmol dm^{-3} picrate in 0.51 mol dm^{-3} NaOH. However, the concentration of picrate employed for all polarograms was maintained at 0.284 mmol dm^{-3} in 0.51 mol dm^{-3} NaOH.

A stock 2.04 mol dm^{-3} NaOH was prepared and the concentration of NaOH was maintained at 0.51 mol dm^{-3} for all studies by dilution with distilled water.

Polarography Studies: The reference, auxiliary (counter), and working electrodes were a saturated calomel electrode (S.C.E.), a wire platinum electrode, and a dropping mercury electrode (DME), respectively. The characteristics of the DME were m=17.68 mg s^{-1}; t=6.9 s; and a capillary constant of $m^{2/3}t^{1/6}$=9.38 mg$^{2/3}$ s$^{-1/2}$. The height of the mercury column was 70.5 cm. A mercury drop rate of 0.5 s/drop was maintained throughout. Direct current (DC) polarography was performed within a potential range of −0.40 to −1.80 V(volts) versus S.C.E., with a scanning time of 7 min.

Ascorbate Blank Solutions: A 50 ml volume of 0.51 mol dm^{-3} NaOH with a pH of 13.3 was purged with nitrogen for 20 minutes at 25.00±0.02 °C and a polarogram was recorded. 0.00281 g (0.284 mmol) sodium ascorbate, was added and polarograms of the ascorbate blank solution were recorded at 0, 1, and 3 h of incubation time. Similarly, polarographic analysis was performed on a 0.00843 g sodium ascorbate, the amount present in a 3 : 1 molar ratio ascorbate: 0.284 mmol dm^{-3} picrate test solution. All experiments were performed in duplicate.

28.4 mmol dm^{-3} Picrate Blank and Ascorbate : Picrate Test Solutions: All incubations were performed in saturated picric acid diluted with an equal volume of sodium hydroxide. A 5.0 ml volume of saturated picric acid was pipetted into a brown bottle immersed in a 25.00±0.02 °C water bath. A 5.0 ml volume of 1.02 mol dm^{-3} NaOH was added and the resulting 28.4 mmol dm^{-3} picrate blank solution was mixed and purged with nitrogen gas for 10 min.

A 0.50 ml aliquot of 28.4 mmol dm^{-3} picrate blank solution in 0.51 mmol dm^{-3} NaOH was diluted to 50 ml with nitrogen purged, 0.51 mmol dm^{-3} NaOH. A 50 ml volume of the 0.284 mmol dm^{-3} picrate blank solution was transferred to a 25.0±0.02 °C water-jacketed electrolysis vessel. The first aliquot of the blank solution, served as a point for comparison

purposes. Polarographic analysis of the diluted blank was performed. Similarly 0.50 ml aliquots of the 28.4 mmol dm^{-3} blank solution were withdrawn and diluted after 1, 2, and 3 h of incubation time for polarographic analyses. All experiments were conducted in duplicate.

0.0281 g sodium ascorbate was added to a fresh solution consisting of 5.0 ml of saturated picric acid, and 5.0 ml of 1.02 mol dm^{-3} NaOH, which was incubated for 10 min at 25.00±0.02 °C. This produced an approximately 0.5 : 1 ascorbate : 28.4 mmol dm^{-3} picrate test solution with a pH of 13.3. Polarographic analysis of a zero time blank was performed. Polarographic analyses were also performed for test solutions at 1/4, 1/2, 3/4, 1, 3/2, and 2 h of reaction times. Nitrogen gas was purged throughout the reaction time. The entire procedure was repeated for 1 : 1 and 2 : 1 ascorbate : 28.4 mmol dm^{-3} picrate test solutions. A 3 : 1 ascorbate : 28.4 mmol dm^{-3} picrate in 0.51 mol dm^{-3} NaOH was prepared and polarograms were recorded at 10-min intervals for a duration of one-hour. All experiments were performed in triplicate.

2-Furaldehyde Blank and 2-Furaldehyde: Picrate Test Solutions: An initial polarogram was recorded on a 50 ml volume of 0.51 mol dm^{-3} NaOH which was purged with nitrogen for 20 min at 25.00±0.02 °C. A 0.01 ml of 2-furaldehyde was added, mixed and a polarogram was recorded immediately.

A freshly prepared 50 ml of 0.284 mmol dm^{-3} picrate in 0.51 mol dm^{-3} NaOH solution was transferred into a water-jacketed cell and purged with nitrogen gas for 30 min. Polarographic analysis of the alkaline picrate blank was performed. A 0.01 ml of 2-furaldehyde was added, mixed, and a polarogram of the test solution was recorded. All experiments were performed in duplicate.

Acid/Alkali Nitro Group Regeneration Studies. 28.4 mmol dm^{-3} Picrate Blank and Ascorbate : Picrate Test Solutions: A 5.0 ml volume of saturated picric acid was mixed with 5.0 ml of 1.02 mol dm^{-3} NaOH and purged with nitrogen gas for 10 min. A 0.5 ml volume of the blank solution was diluted to 50 ml with 0.51 mol dm^{-3} NaOH which was already degased. Polarograms were recorded at 0 and 2 h of incubation time. At the end of 2 h, the blank solution was acidified to a pH of 1.4 with a 12.0 ml volume of 3 mol dm^{-3} HCl and a polarogram was recorded. The blank was returned to an alkaline pH of 12.8 by the addition of 3 ml of 4 mol dm^{-3} NaOH and a polarogram was recorded.

Polarographic analyses were performed for aliquots of 0.5 : 1, 1 : 1, 2 : 1, and 3 : 1 ascorbate: 28.4 mmol dm^{-3} picrate test solutions at 0 time and at 2 h, 1 h, 45 min, and 30 min of reaction times, respectively. Acid/alkali treatment and polarographic analyses of 2-h test solutions of 0.5 : 1 molar ratio, 1-h test solutions of 1 : 1 molar ratio, 45-min test solutions of 2 : 1 molar ratio and 30-min test solutions of 3 : 1 molar ratio ascorbate: 28.4 mmol dm^{-3} picrate were performed. All experiments were performed in duplicate.

Spectrophotometry Studies. Ascorbate Blank Solutions: 0.0562 g ascorbate, the amount present in a 1 : 1 ascorbate: 28.4 mmol dm^{-3} picrate test solution, was added to a nitrogen purged, 10 ml volume of 0.51 mol dm^{-3} NaOH solution. Nitrogen was purged for the entire experiment. Absorption spectra were recorded at 30-min intervals for a 3-h duration. The experiment was performed in duplicate.

28.4 mmol dm^{-3} Picrate Blank and Ascorbate: Picrate Test Solutions: A 0.10 ml aliquot of 28.4 mmol dm^{-3} picrate blank solution was transferred to a 10-ml volumetric flask containing 0.51 mol dm^{-3} NaOH and diluted to the mark with 0.51 mol dm^{-3} NaOH. This produced a 10 ml volume of diluted blank solution which was used for UV/vis spectrophotometry. A cuvet with a 0.2 mm light path was employed. Spectrophotometric analysis of the diluted zero time aliquot was performed at 25.00 °C between 200 nm and 650 nm versus a distilled water blank.

A 10 ml volume of 28.4 mmol dm^{-3} picrate blank solution was prepared fresh and a 0.0562 g quantity of ascorbate was added. This produced an approximately 1 : 1 ascorbate: 28.4 mmol dm^{-3} picrate test solution. Absorption spectra were recorded on the same chart paper at 30-min intervals for a 3-h duration. Absorption spectra were recorded for 2 : 1 and 3 : 1 ascorbate: picrate test solutions at 15-min intervals for a 2-h duration and at 10-min intervals for one-hour duration, respectively. All experiments were performed in duplicate.

Thin-Layer Chromatography Studies: Thin-layer chromatography plates were preheated to 110 °C for 30 min, and stored above silica gel in a desiccator at room temperature prior to use.

The solvent system consisted of ethyl acetate: 2-propanol: 28—30% aqueous ammonia: water (60 : 28 : 8 : 4, v/v/v/v).[13] The freshly prepared solvent system was mixed and allowed to stand at room temperature for 15 min prior to use. A 150 ml volume of the solvent mixture was transferred to the chromatography trough. The chromatography chamber was assembled and the solvent system was allowed to migrate a distance of 13 cm above the origin. The solvent migration time was approximately 80 min. The plate was air dried and visually examined. The chromatogram was also examined under 253.7 and 375 nm UV light.

A 0.5 : 1 molar ratio of ascorbate: 28.4 mmol dm^{-3} picrate test solution was prepared and a 10 µl of test aliquot was withdrawn at 0, 1/2, 1, 3/2, 2, and 3 h of incubation time and applied at 2 cm intervals onto a TLC plate. An aqueous picramic acid and a 28.4 mmol dm^{-3} picrate blank solution were used as reference materials. The TLC separation was performed using the solvent system. The chromatogram was air dried and visually examined under UV light. 1 : 1 and 2 : 1 molar ratio ascorbate: picrate test solutions were prepared and applied onto TLC plates. A TLC separation was performed on each plate. A test solution of 3 : 1 ascorbate: picrate was prepared and 10 µl of test solution was spotted onto a TLC plate at 10, 20, 30, 40, 50, 60, 90, and 120 min of incubation time and TLC separation was performed. All experiments were performed in duplicate.

Results

Polarography. Ascorbate Blank Solutions: A 0.284 mmol dm^{-3} ascorbate blank solution in 0.51 mol dm^{-3} NaOH did not produce any reduction waves during one hour of incubation time. However, the color of the solutions slowly changed from colorless to coral pink. A reduction wave with an average $E_{1/2}$ value of −1.43 V was observed after 3 h of reaction time. The ascorbate blank solution was purged with nitrogen during the 3 h of incubation time.

28.4 mmol dm^{-3} Picrate Blank and Ascorbate : Picrate Test Solutions: Polarographic analysis of 28.4 mmol dm^{-3} picrate blank solutions in 0.51 mol dm^{-3} NaOH showed three well-defined reduction waves with average $E_{1/2}$ values of −0.62, −0.78, and −0.92 V and a broad

fourth reduction wave of −1.30 V versus S.C.E. No significant changes either in I_d or in $E_{1/2}$ were observed during three hours of testing.

In the test procedure, a 28.4 mmol dm^{-3} picrate in 0.51 mol dm^{-3} NaOH solution was allowed to react with ascorbic acid. A general decrease in the I_d of the first

Table 1. Polarography Results of 0.5 : 1, 1 : 1, 2 : 1, and 3 : 1 Ascorbate : 28.4 mmol dm^{-3} Picrate Test Solutions[a] in 0.51 mol dm^{-3} NaOH, Incubated at 25 °C

Time	Picrate reduction waves[b–d] I		II		III		IV[e]	
	$E_{1/2}$	I_d	$E_{1/2}$	I_d	$E_{1/2}$	I_d	$E_{1/2}$	I_d
0.5 : 1 Ascorbate : 28.4 mmol dm^{-3} picrate test								
Blank[f]	−0.62	2.39	−0.78	1.48	−0.92	1.98	−1.30	1.16
1/4 h	−0.62	2.24	−0.78	1.42	−0.92	1.72	−1.29	1.13
% decrease		6.28%		4.05%		8.08%		
1/2 h	−0.62	2.10	−0.78	1.35	−0.92	1.60	−1.30	1.10
% decrease		12.1%		8.78%		19.2%		
3/4 h	−0.62	1.92	−0.78	1.23	−0.92	1.27	−1.30	1.05
% decrease		19.7%		16.9%		35.8%		
1 h	−0.62	1.81	−0.78	1.15	−0.92	1.16	−1.30	1.06
% decrease		24.3%		22.3%		41.4%		
3/2 h	−0.62	1.66	−0.78	1.13	−0.92	1.10	−1.29	1.09
% decrease		30.5%		23.6%		44.4%		
2 h	−0.62	1.53	−0.78	0.99	−0.91	1.00	−1.29	1.01
% decrease		36.0%		33.1%		49.5%		
1 : 1 Ascorbate : 28.4 mmol dm^{-3} picrate test								
1/4 h	−0.62	1.59	−0.78	0.95	−0.92	1.17	−1.29	0.98
% decrease		33.5%		35.8%		40.9%		
1/2 h	−0.62	1.09	−0.78	0.85	−0.92	0.73	−1.29	0.95
% decrease		54.4%		42.6%		63.1%		
3/4 h	−0.61	0.64	−0.78	0.61	−0.92	0.54	−1.28	1.03
% decrease		73.2%		58.8%		72.7%		
1 h	−0.60	0.54	−0.78	0.50	−0.92	0.44	−1.28	1.04
% decrease		77.4%		66.2%		77.7%		
3/2 h	−0.61	0.33	−0.77	0.35	−0.92	0.34	−1.28	1.04
% decrease		86.2%		76.4%		82.8%		
2 h	−0.60	0.16	−0.77	0.25	−0.92	0.22	−1.29	1.08
% decrease		93.3%		83.1%		88.9%		
2 : 1 Ascorbate : 28.4 mmol dm^{-3} picrate test								
1/4 h	−0.61	0.93	−0.78	0.69	−0.92	0.70	−1.27	1.14
% decrease		61.1%		53.4%		64.6%		
1/2 h	−0.61	0.37	−0.77	0.39	−0.92	0.35	−1.27	1.18
% decrease		84.5%		73.6%		82.3%		
3/4 h	−0.60	0.20	−0.76	0.22	−0.92	0.20	−1.26	1.19
% decrease		91.6%		85.1%		89.9%		
1 h	—	—	−0.75	0.18	−0.92	0.15	−1.26	1.18
% decrease		100%		87.8%		92.4%		
3/2 h	—	—	−0.75	0.18	−0.92	0.15	−1.26	1.25
% decrease		100%		87.8%		92.4%		
2 h	—	—	−0.75	0.16	−0.92	0.15	−1.26	1.20
% decrease		100%		89.1%		92.4%		
3 : 1 Ascorbate : 28.4 mmol dm^{-3} picrate test								
10 min	−0.61	1.03	−0.78	0.70	−0.92	0.68	−1.27	1.03
% decrease		56.9%		32.6%		65.7%		
20 min	−0.60	0.35	−0.76	0.39	−0.91	0.30	−1.25	1.18
% decrease		85.4%		73.6%		84.8%		
30 min	−0.60	0.18	−0.76	0.22	−0.92	0.19	−1.25	1.20
% decrease		92.4%		85.1%		90.4%		
40 min	—	—	−0.76	0.23	−0.91	0.19	−1.24	1.23
% decrease		100%		84.5%		90.4%		
60 min	—	—	−0.76	0.23	−0.92	0.16	−1.25	1.20
% decrease		100%		84.5%		91.9%		

a) 28.4 mmol dm^{-3} solutions were diluted to 0.284 mmol dm^{-3} for polarography testing. b) Each value reported represents an average of three analyses. c) The half-wave potential ($E_{1/2}$) is expressed in volts versus S.C.E. d) The diffusion current (I_d) is expressed in microamperes. e) Reduction wave IV was diffuse and not well-defined. Approximate $E_{1/2}$ and I_d measurements are presented. f) A zero time blank of 0.5 : 1 test solution served as blank for all test solutions.

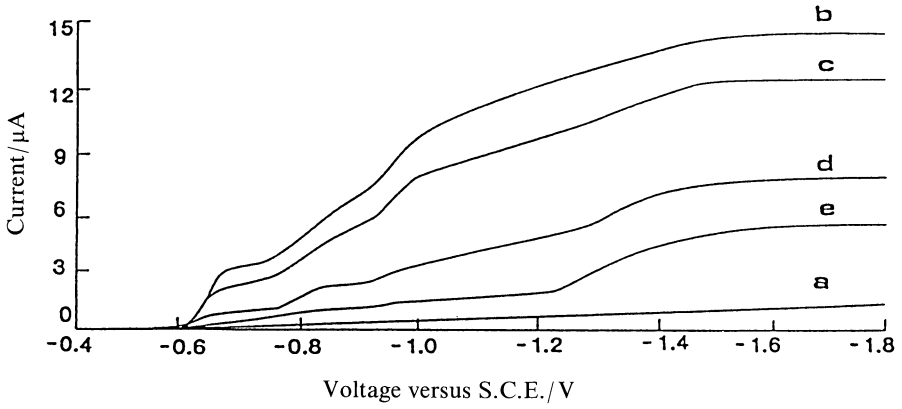

Fig. 1. Polarograms of an aqueous 0.51 mol dm^{-3} sodium hydroxide blank (a), a 28.4 mmol dm^{-3} picrate blank (b), and ascorbate: 28.4 mmol dm^{-3} picrate 0.5:1 (c), 1:1 (d), and 2:1 (e), test solutions reacted for 1 h at 25 °C, respectively.

three waves was registered with increasing ascorbate concentrations and prolonged incubation times (Table 1). Typical polarograms of 0.5:1, 1:1, and 2:1, ascorbate: 28.4 mmol dm^{-3} picrate test solutions incubated for one hour in 0.51 mol dm^{-3} NaOH are presented in Fig. 1. The decrease in the diffusion current for the first three reduction waves of ascorbate: 28.4 mmol dm^{-3} picrate test solutions was calculated versus a 28.4 mmol dm^{-3} picrate blank. For one hour reaction time, the I_d for the first reduction wave of 0.5:1, 1:1, and 2:1 ascorbate: 28.4 mmol dm^{-3} picrate test solutions were observed to decrease by 24.3%, 77.4%, and 100%, respectively (Table 1).

The 0.5:1 ascorbate: 28.4 mmol dm^{-3} picrate test solutions showed a greater decrease in I_d for reduction wave 3 than for waves 1 and 2 during two hours of reaction (Table 1). A similar response was observed for the 1:1 test solutions during the first 30 min of incubation time. A greater decrease in I_d was observed for reduction wave 1 during the remaining 90 min of reaction time. The 2:1 test solutions showed a greater decrease in I_d for reduction wave 1 than for waves 2 and 3 during two hours of incubation. However, for the 2:1 test solutions, some reactivity preference was shown for wave 1 with a 100% decrease in I_d observed after one hour of reaction time. With the addition of ascorbic acid, the broad diffuse wave 4 of picrate in 0.51 mol dm^{-3} NaOH became well-defined. The $E_{1/2}$ value of wave 4 shifted from −1.30 V to −1.25 V when ascorbic acid was increased from a 2:1 to a 3:1 molar ratio. However, the I_d of wave 4 remained almost constant. With the 3:1 test solutions, a similar reactivity preference was shown for wave 1 with 100% decreases in I_d observed after 40 min of reaction time.

2-Furaldehyde Blank and 2-Furaldehyde: Picrate Test Solutions: A polarogram of 2-furaldehyde in 0.51 mol dm^{-3} NaOH showed a well-defined reduction wave with an average $E_{1/2}$ value of −1.40 V.

2-Furaldehyde: 0.284 mmol dm^{-3} picrate test solution also showed four well-defined reduction waves with average $E_{1/2}$ values of −0.64, −0.80, −0.92, and −1.38 V, respectively.

Acid/Alkali Nitro Group Regeneration Studies: Results of the acid/alkali treatment procedure for 0.5:1, 1:1, and 2:1 ascorbate: 28.4 mmol dm^{-3} picrate test solutions are summarized in Table 2. The acid/alkali treated ascorbate: 28.4 mmol/dm^{-3} picrate test solutions were corrected for dilution. However, a decrease in the I_d of reduction waves 1—3 was observed for all test solutions. The I_d for reduction wave 4 also showed a decrease compared to the blank I_d. Taking into consideration the 28% dilution factor, regeneration to the blank level did not occur after the acid/alkali treatment.

Spectrophotometry. Ascorbate Blank Solutions: The absorption spectrum of an ascorbate blank solution in 0.51 mol dm^{-3} NaOH showed intense peaks near 211 and 299 nm versus water as a blank (Fig. 2). The absorbance near 211 nm remained almost constant whereas, the absorbance near 299 nm decreased slowly from 0.46 to 0.18 when measured between 15 and 180 min.

Picrate Blank and Ascorbate: 28.4 mmol dm^{-3} Picrate Test Solutions: A 28.4 mmol dm^{-3} picrate blank solution which was diluted for spectra showed absorption peaks near 210 and 356 nm (Fig. 3). Absorption spectra of 3:1 ascorbate: 28.4 mmol dm^{-3} picrate test solutions in 0.51 mol dm^{-3} NaOH, recorded at 10-min intervals, are also presented in Fig. 3. The absorbance near 356 nm decreased in intensity and shifted to 375 nm. The absorbance near 298 nm also decreased in intensity. The absorbance in the 450—650 nm region increased. The shoulder peak in the 400—450 nm region had a hypsochromic shift.

Thin-Layer Chromatography. For the TLC separated ascorbate: 28.4 mmol dm^{-3} picrate test solutions, picric acid with an R_f×100 value of 93.7, picramic acid with an R_f×100 value of 47.7, and off yellow band which was present in alkaline picrate blank with an R_f×100

Table 2. Acid/Alkali Nitro Group Regeneration Studies of Ascorbate : 28.4 mmol dm^{-3} Picrate 0.5 : 1, 1 : 1, 2 : 1, and 3 : 1 Molar Ratios Test Solutions[a] in 0.51 mol dm^{-3} NaOH

Time	Picrate reduction waves[b–d]							
	I		II		III		IV[e]	
	$E_{1/2}$	I_d	$E_{1/2}$	I_d	$E_{1/2}$	I_d	$E_{1/2}$	I_d
0.5 : 1 Ascorbate : 28.4 mmol dm^{-3} picrate test								
Blank[f]	−0.62	2.29	−0.78	1.45	−0.92	1.90	−1.30	1.12
2 h	−0.62	1.50	−0.78	0.94	−0.91	0.95	−1.30	1.02
Acid/alkali	−0.60	1.20	−0.78	0.84	−0.91	0.80	−1.22	0.49
Corrected		1.53		1.07		1.02		0.62
1 : 1 Ascorbate : 28.4 mmol dm^{-3} picrate test								
Blank[f]	−0.62	2.50	−0.78	1.53	−0.92	1.92	−1.29	1.18
1 h	−0.61	0.53	−0.78	0.57	−0.92	0.47	−1.28	0.98
Acid/alkali	−0.59	0.42	−0.76	0.38	−0.90	0.38	−1.23	0.60
Corrected		0.54		0.48		0.48		0.77
2 : 1 Ascorbate : 28.4 mmol dm^{-3} picrate test								
Blank[f]	−0.62	2.50	−0.78	1.53	−0.92	1.92	−1.29	1.18
3/4 h	−0.60	0.19	−0.77	0.21	−0.91	0.19	−1.26	1.20
Acid/alkali	−0.59	0.15	−0.76	0.17	−0.90	0.19	−1.23	0.51
Corrected		0.19		0.22		0.24		0.66
3 : 1 Ascorbate : 28.4 mmol dm^{-3} picrate test								
Blank[f]	−0.62	2.60	−0.78	1.55	−0.92	2.15	−1.28	1.25
30 min	−0.59	0.15	−0.76	0.19	−0.92	0.19	−1.25	1.20
Acid/alkali	−0.59	0.15	−0.76	0.15	−0.91	0.15	−1.22	0.60
Corrected		0.19		0.19		0.19		0.78

a) 28.4 mmol dm^{-3} solutions were diluted to 0.284 mmol dm^{-3} for polarography testing. b) Each value reported represents an average of two analyses except 3 : 1 test solution which is a single value. c) The half-wave potential ($E_{1/2}$) is expressed in volts versus S.C.E. d) The diffusion current (I_d) is expressed in microamperes. e) Reduction wave IV was diffuse and not well-defined. Approximate $E_{1/2}$ and I_d measurements are presented. f) A zero time blank of 0.5 : 1 test solution served as blank for all test solutions.

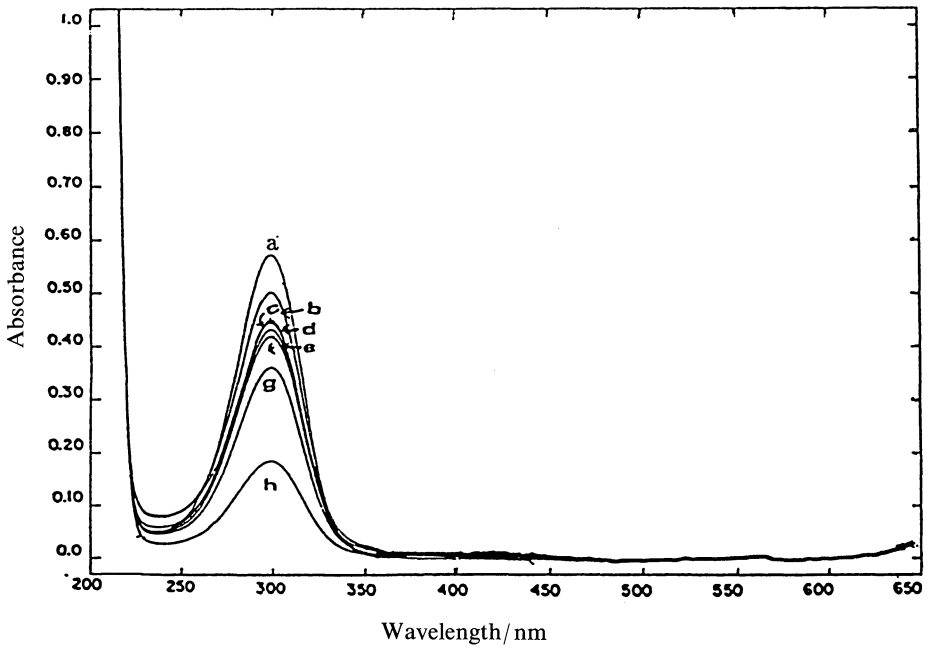

Fig. 2. Absorption spectra of 0.284 mmol dm^{-3} ascorbate blank solution in 0.51 mol dm^{-3} NaOH. Spectra were recorded at 15 (a), 30 (b), 45 (c), 60 (d), 75 (e), 90 (f), 120 (g), and 180 (h) min of incubation time, respectively versus water as blank at 25 °C.

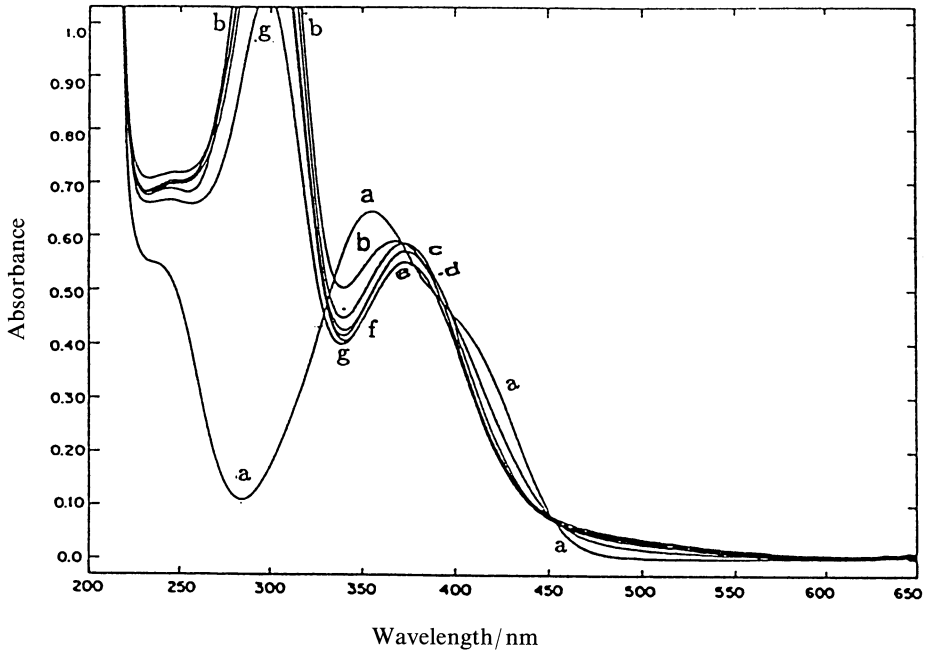

Fig. 3. Absorption spectra of a 28.4 mmol dm^{-3} picrate blank in 0.51 mol dm^{-3} NaOH at 25 °C (a), and of a 3 : 1 ascorbate : 28.4 mmol dm^{-3} picrate molar ratio tests solution, measured at 10 (b), 20 (c), 30 (d), 40 (e), 50 (f), and 60 (g) min of reaction times, respectively.

value of 41.2, were identified. No fluorescent material was observed for the ascorbate: 28.4 mmol dm^{-3} picrate test solutions. Picramic acid was present in the 30-min incubated 0.5 : 1 and 1 : 1 test solutions. Picramic acid was also observed in the 10-min reacted 3 : 1 ascorbate: 28.4 mmol dm^{-3} test solutions. Higher concentration of picramic acid was isolated by TLC for a 3 : 1 ascorbate: 28.4 mmol dm^{-3} picrate molar ratio test solution, incubated for 60 min at 25 °C.

Discussion

Ascorbate was incubated in 28.4 mmol dm^{-3} of picrate, but only the 0.284 mmol dm^{-3} picrate in 0.51 mol dm^{-3} NaOH solution was employed for polarography testing. A constant NaOH concentration of 0.51 mol dm^{-3} (pH 13.3) was maintained throughout all studies as the concentration of alkali had been shown to influence the diffusion current of picrate.[14]

Alkaline picrate blank solutions showed well-defined nitro reduction waves 1—3 and a broad, not well-defined fourth wave. The $E_{1/2}$ values for reduction waves 1—3 were in agreement with published values.[14] The $E_{1/2}$ value for wave 4 varied slightly due to the not well-defined nature of the wave. The I_d values for each of the nitro group reduction waves of picrate blank solutions were stable for three hours. It has already been shown in an earlier study that the first nitro reduction wave of alkaline picrate is due to the presence of an ortho nitro group and the reduction wave 4 has been attributed to the presence of nitro anion species.[14,15]

Each of the 0.5 : 1, 1 : 1, 2 : 1, and 3 : 1 ascorbate: 28.4 mmol dm^{-3} picrate test solutions showed a decrease in I_d for nitro reduction waves 1—3 (Table 1). Wave 3 decreased faster than waves 1 and 2 for the 0.5 : 1 test solution and the reduction wave 4 became well-defined. The I_d and $E_{1/2}$ measurements of wave 4 became more accurate but the increase in the I_d for wave 4 was very marginal. In general, for 1 : 1, 2 : 1, and 3 : 1 test solutions, the I_d of waves 1 and 3 decreased faster than for the wave 2. A greater decrease was observed for increased ascorbate concentrations and longer reaction time. For 3 : 1 test solutions, the $E_{1/2}$ value of wave 4 shifted to a slightly less negative potential with an average $E_{1/2}$ value of -1.26 V and the I_d showed a slight increase during 2 h of reaction time.

For 15 min of reaction time, the I_d for the reduction wave 1 of ascorbate : picrate 0.5 : 1, 1 : 1, and 2 : 1 test solutions decreased by 6.28%, 33.5%, and 61.1%, respectively (Table 1). Polarograms of 3 : 1 test solutions showed a 56.1% decrease in I_d for wave 1 within 10 min of reaction time. Greater reactivity was observed with increased concentration of ascorbate and lengthened reaction time at 25 °C.

For the acid/alkali treatment studies, quantitative recovery of waves 1, 2, and 3 for ascorbate : 28.4 mmol dm^{-3} picrate test solutions did not occur. A minor amount of regeneration of waves 1, 2, and 3 did result at the expense of wave 4 for 0.5 : 1 test solutions (Table 2). This was attributed to the interconversion of nitro anion groups to nitro groups.

The color of the 0.5 : 1 test solutions changed immediately from yellow to orange and then slowly to red. Red color formed at a much faster rate for 1 : 1 and 2 : 1 test solutions and the 3 : 1 ascorbate : 28.4 mmol dm^{-3} picrate test solution showed an intense red color within a few seconds. Picramic acid was identified by TLC, for 0.5 : 1, 1 : 1, and 2 : 1 ascorbate : 28.4 mmol dm^{-3} picrate test solutions incubated for 30 min at 25 °C. Picramic acid was also isolated on TLC from 3 : 1 test solutions incubated for 10 min at 25 °C.

If picramic acid were the only reduction product of ascorbate : 28.4 mmol dm^{-3} picrate test solution, the I_d values of waves 2 and 3 should remain constant (Table 1). However, there was a continuous decrease in I_d for waves 1—3. For 2 : 1 and 3 : 1 test solutions, 100% decreases in I_d for wave 1 were observed after 60 and 40 min of reaction time, respectively. But, almost a constant I_d of wave 4 was observed which indicates that ascorbic acid is not reactive with nitro anion groups of 1 : 1 and 2 : 1 hydroxide : picrate complexes under the present conditions. Picramic acid was isolated from all ascorbate : picrate test solutions. In this respect, glucose : picrate and ascorbate : picrate test solutions behaved similarly for the electrochemical studies.[15] Unlike glucose : 28.4 mmol dm^{-3} picrate test solutions, no fluorescent product was observed on TLC separated chromatograms of ascorbate : 28.4 mmol dm^{-3} picrate test solutions. However, picramic acid was formed for both ascorbate : picrate and glucose : picrate test solutions. This appears to indicate that, although the reduction product, picramic acid was the same for both, the intermediate chemical reduction products are different. Ascorbic acid has been reported as a positive Jaffé interference.[16–20] At therapeutic concentrations, ascorbic acid had distinctly been shown to interfere with the determination of creatinine.[7]

Ascorbic acid is a reducing agent and is subject to oxidative decomposition in solution.[21] In solution exposed to air or oxygen, ascorbic acid is subject to oxidation accelerated by dissolved trace minerals such as copper and light exposure. This proceeds first to dehydroascorbic acid, but continues to diketogulonic acid and various other breakdown products. Erdman and Klein suggested that the conversion of dehydroascorbic acid to diketogulonic acid occurs both aerobically and anaerobically and the total oxidation may result in the formation of 2-furaldehyde by decarboxylation and dehydration.[10]

Ono et al. investigated vitamin C by polarographic analyses and observed two oxidation waves under alkaline conditions.[22] They suggested that the electrooxidation of vitamin C produced dehydro L-ascorbic acid (DAA). DAA showed a reduction wave with values between −0.3 to −0.5 V in the pH range of 2—5 versus S.C.E. with the polarography reduction product identified as ascorbic acid. Kern reported the $E_{1/2}$ oxidation potentials of ascorbic acid at +0.1125 V and −0.0335 V for pH's of 3.58 and 6.67, respectively.[23] Dobos reported potentials of +0.22 V, 0.17 V, and −0.06 V for pH's of 1.8, 3.4, and 7.0, respectively.[24] The electrochemical reduction of ascorbic acid under alkaline conditions has not been reported. Under the present experimental conditions, ascorbic acid in 0.51 mol dm^{-3} NaOH did not record any reduction wave during two hours of incubation at 25 °C. However, after 3 h of incubation, 0.284 mmol dm^{-3} ascorbate in 0.51 mol dm^{-3} NaOH solution produced a pink color. The pink-colored ascorbate blank gave a reduction wave with an average $E_{1/2}$ value of −1.4 V which appeared to be a 2-furaldehyde reduction wave.

2-Furaldehyde has been indentified as one of the degradation products of ascorbic acid,[10] and has also been listed as an interference of the Jaffé reaction.[11,12] The reduction potential for furfural was listed as −1.43 V at a pH of 12.[24] Under the present experimental conditions, 2-furaldehyde blank in 0.51 mol dm^{-3} NaOH showed a single reduction wave with an average $E_{1/2}$ value of −1.40 V. During two hours of incubation time, ascorbate : picrate test solutions recorded four reduction waves which were similar to the reduction waves of alkaline picrate. 2-Furaldehyde did not form during two hours of reaction.

The relationship between the percent decrease in I_d for nitro reduction wave 1 of 0.5 : 1, 1 : 1, 2 : 1, and 3 : 1 molar ratios ascorbate : 28.4 mmol dm^{-3} picrate test solutions versus the reaction time did not appear to be linear. This may in part be attributed to the unstable nature of ascorbic acid under alkaline conditions. Similarities were observed for the acid/alkali treatment studies of ascorbate: picrate and glucose: picrate test solutions.[15] The I_d's of waves 1—4 could not quantitatively be recovered.

The absorption peak of ascorbic acid in water was reported to be 264 nm.[25] Under the present experimental conditions, ascorbic acid in 0.51 mol dm^{-3} NaOH solution recorded an absorption peak near 300 nm (Fig. 2). The decrease in absorbance near 300 nm was also seen for an ascorbate blank solution with extended time. This may in part be due to the unstable nature of ascorbic acid under alkaline conditions.

The absorption spectra of a 3 : 1 ascorbate : picrate test solution shifted slowly from 356 to 377 nm with a decrease in absorbance and showed a broad tailing absorption in the 450—600 nm region. An isobestic point was observed at 450 nm (Fig. 3). An absorbance peak near 300 nm which was due to the presence of ascorbic acid, was observed to decrease during the reaction period. This may be attributed to the unstable nature of ascorbic acid and the consumption of ascorbic acid in the reduction of picrate. The decrease in absorbance near 300 nm was also seen for an ascorbate blank solution. The decrease in absorbance near 356 nm was similar to that observed for glucose : picrate test solution.[15] This may be attributed to the effect of reduction of picric acid. Momose et al. classified ascorbic acid under the active methylene compounds.[5] How-

ever, the present experimental evidence indicates that ascorbic acid acts as a reducing agent in an alkaline picrate solution and forms picramic acid. This was in agreement with the observations of other investigators.[6,9]

Based on the polarography studies, ascorbate reacts with picrate of the Jaffé reaction, thus depleting the reagent and in turn results in an underestimation of creatinine. Thus it should be listed as a negative interference. But, ascorbate has been listed as a positive interference. The reports in the published literature have been explained by the present experimental data. Ascorbate reacts immediately with picrate to form picramic acid which provides a red color. The broad tailing shoulder absorbance between 475—600 nm was observed to increase gradually, which accounts for the observed positive interference of ascorbic acid.

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