Studies on the Effect of Gallate on the Oxidation of Aldehydes Dispersed in Cetomacrogol Solutions

LUCY S. C. WAN and C. L. HWANG*

Abstract □ A series of gallates of varying alkyl chain length was used to study the effect of antioxidants on the oxidation of benzaldehyde and *p*-methylbenzaldehyde dispersed in aqueous solutions of cetomacrogol at 25° in the presence of cupric sulfate. Prooxidation increased with gallate concentration until a maximum was reached and then decreased with further addition of the antioxidant until antioxidation appeared. Only antioxidation was observed in the presence of butylated hydroxyanisole and butylated hydroxytoluene. The prooxidation could be attributed to the action of a complex formed between gallate and cupric sulfate.

Keyphrases □ Aldehyde dispersions—cetomacrogol solutions □ Gallate effect—aldehyde oxidation □ Antioxidants—prooxidant activity □ Oxygen uptake, aldehydes—surfactant effect

Generally it is not common for the antioxidant to act as a prooxidant. In the studies of the effect of histamine and trace metals on the oxidation of linoleate ester in emulsions, Saunders *et al.* (1) reported that at low concentrations, histamine acted as an antioxidant while at high concentrations it acted as a prooxidant. These observations are contrary to those obtained with the gallates in this study. The action of histamine was thought to be due to the formation of prooxidative complexes with trace quantities of ionic iron. Investigations of the effect of prooxidants in preparations of aqueous surfactant solutions have been made by Bergström *et al.* (2) who found that the oxidation rates of solutions of sodium linoleate were dependent on the content of copper chloride, while Loncin and Jacqmain (3) observed a reduction rate in emulsions in the presence of copper when complexing agents were added to the system. They suggested that the addition of such compounds transferred the prooxidant from the oil to the aqueous phase.

Using the systems adopted by Mitchell and Wan (4) it seemed useful to study the effect of gallates of varying alkyl chain length on the oxidation of aldehyde dispersed in aqueous solutions of cetomacrogol. These gallates are used in pharmaceutical practice to prevent or reduce oxidation.

EXPERIMENTAL

Materials—Benzaldehyde[1] and *p*-methylbenzaldehyde[2] were purified and stored as stated previously (5). Methyl gallate, m.p. 195–196°; ethyl gallate, m.p. 147–148°; propyl gallate, m.p. 150–151°; octyl gallate, m.p. 90–91.5°; decyl gallate, m.p. 94–94.5°; dodecyl gallate, m.p. 96–96.5°; butylated hydroxyanisole, m.p. 50–51°; and butylated hydroxytoluene,[1] m.p. 70–70.5° were used as supplied. Cetomacrogol 1000 B.P.C.[3] is the same as that mentioned in a previous paper (6). Cupric sulfate[1] was included as a catalyst.

Apparatus—Warburg apparatus.[4]

Measurement of Solubilities of Aldehydes in Cetomacrogol Solutions at 25°—The method described by Mitchell and Wan (5) for determining the solubilities of aldehydes in cetomacrogol was used.

Measurement of Oxygen Uptake—This was determined using a Warburg apparatus and adopting the procedure of Mitchell and Wan (6), who found that when the ratio of the amount of aldehyde present to its solubility in the surfactant solution was unity, the oxidation rate constants were the same at and above 0.04 *M* surfactant solution concentration. Hence the system selected for this investigation was one which contained benzaldehyde or *p*-methylbenzaldehyde solubilized in 0.04 *M* cetomacrogol solution such that the above-mentioned ratio = 1.0. The amount of aldehyde to be added to the surfactant solution could be obtained from Fig. 1. Cupric sulfate, 1×10^{-4} *M* was included in addition to the antioxidant. A control without the antioxidant was set up with every oxidation determination.

RESULTS AND DISCUSSION

Figure 1 shows the solubilities of benzaldehyde and *p*-methylbenzaldehyde in cetomacrogol solutions at 25°. Aldehyde solubility

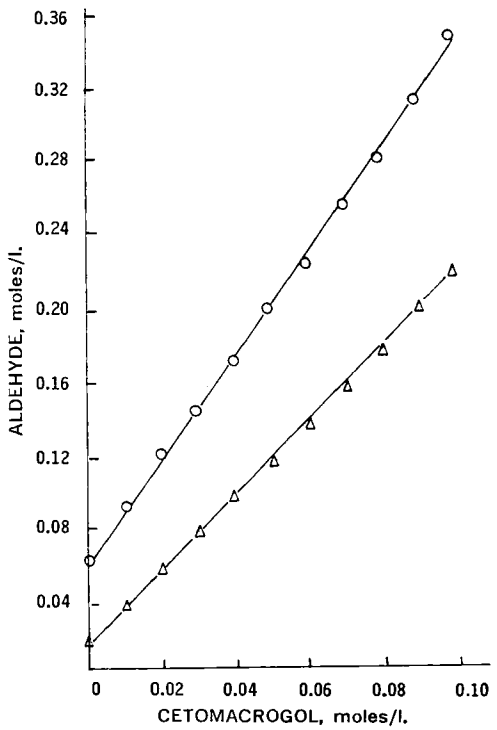

Figure 1—*Solubilities of aldehydes in aqueous solutions of cetomacrogol at 25°. Key:* ○, *benzaldehyde;* △, *p-methylbenzaldehyde.*

[1] A. R., British Drug House, Ltd.
[2] Pract. Grade, Eastman Organic Chemicals, Rochester, N. Y.
[3] Texofor A1P, Glovers Chemicals Ltd.
[4] Braun model VL-85.

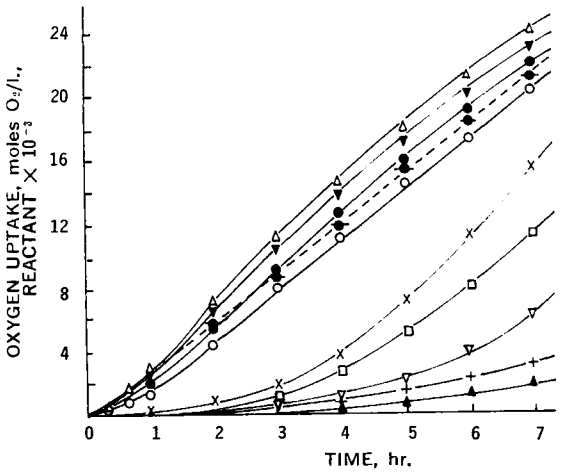

Figure 2—*Effect of ethyl gallate on the oxidation of benzaldehyde (0.1729 M) solubilized in cetomacrogol solution (0.04 M) at 25° in the presence of cupric sulfate (1 × 10⁻⁴ M). Key: ▼—, 0.15 × 10⁻⁴ M; △—, 0.3 × 10⁻⁴ M; ●—, 0.45 × 10⁻⁴ M; ○—, 0.6 × 10⁻⁴ M; ×—, 1.2 × 10⁻⁴ M; □—, 1.35 × 10⁻⁴ M; ▽—, 1.5 × 10⁻⁴ M; +—, 1.8 × 10⁻⁴ M; ▲—, 3.6 × 10⁻⁴ M; ●- - -, control, without ethyl gallate.*

increases with surfactant concentration, benzaldehyde being more soluble than *p*-methylbenzaldehyde. In dispersions containing aldehydes and ethyl gallate, prooxidation and antioxidation are produced (Figs. 2 and 3). This behavior is also observed with methyl, propyl, octyl, decyl, and dodecyl gallates. The prooxidant effect is very marked in dispersions containing *p*-methylbenzaldehyde (Fig. 3). Generally prooxidation occurs at low gallate concentration and antioxidation at high gallate concentration. The induction period is much less obvious in the prooxidation-rate curves obtained from the oxidation of benzaldehyde than from those obtained from *p*-methylbenzaldehyde. The prooxidant effect increases with gallate concentration up to a maximum, then decreases with further addition of the antioxidant until eventually antioxidation appears.

When varying amounts of cupric sulfate are added to surfactant solutions containing benzaldehyde the oxygen uptake increases with catalyst concentration (Table I). At a given concentration of ethyl gallate and using different quantities of cupric sulfate, prooxidation occurs when the catalyst concentration is high and antioxidation when the concentration is low. Similar results are obtained with other members of the gallate series.

Table II shows that oxidation does not occur in the aqueous phase in the presence of cupric sulfate (A) or cupric sulfate and ethyl gallate (B) and that cetomacrogol is not likely to be responsible for the prooxidation observed since no oxygen is consumed

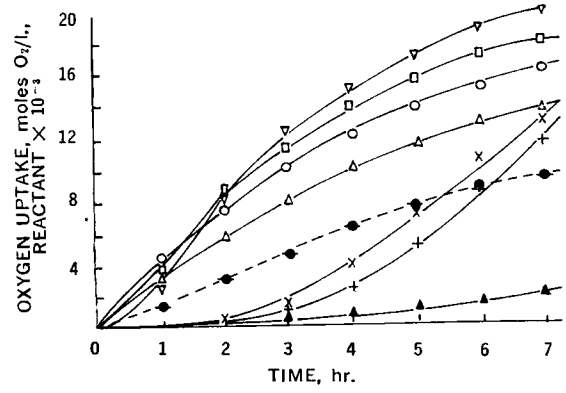

Figure 3—*Effect of ethyl gallate on the oxidation of p-methylbenzaldehyde (0.099 M) solubilized in cetomacrogol solution (0.04 M) at 25° in the presence of cupric sulfate (1 × 10⁻⁴ M). Key: △—, 0.3 × 10⁻⁴ M; ○—, 0.6 × 10⁻⁴ M; □—, 1.2 × 10⁻⁴ M; ▽—, 1.8 × 10⁻⁴ M; ×—, 2.1 × 10⁻⁴ M; +—, 2.4 × 10⁻⁴ M; ▲—, 2.7 × 10⁻⁴ M; ●- - -, control without ethyl gallate.*

Table I—Effect of Varying Concentrations of Cupric Sulfate on the Oxidation of Benzaldehyde (0.1729 *M*) Solubilized in Cetomacrogol Solution (0.04 *M*) Containing a Fixed Concentration of Ethyl Gallate at 25°

	Cupric sulfate, moles/l.			
	1×10^{-3}	5×10^{-4}	1×10^{-4}	1×10^{-5}
	Oxygen Uptake at 6th hr. (moles O_2/l. $\times 10^{-3}$)			
In the absence of ethyl gallate	19.4	18.6	18.0	13.0
In the presence of ethyl gallate 3×10^{-5} *M*	25.0[a]	22.8[a]	21.0[a]	4.1[b]
In the presence of ethyl gallate 6×10^{-5} *M*	27.6[a]	25.5[a]	17.2[b]	0.52[b]

[a] Prooxidant. [b] Antioxidant.

[(C), (D), and (E)]. The oxygen uptake of a solution of cetomacrogol and benzaldehyde (F) is much less than that of the same system with cupric sulfate included (H). In the presence of ethyl gallate (G), only antioxidation has been found with surfactant solutions containing benzaldehyde. However when cupric sulfate is added to the same system, prooxidation and antioxidation are produced (H). Butylated hydroxyanisole and butylated hydroxytoluene have been substituted for the gallates but no prooxidation is observed even when the antioxidant concentration is 10 times more dilute than that used with the gallates; only antioxidation is produced. This would indicate that prooxidation is not merely the result of the presence of a low concentration of an antioxidant and cupric sulfate but that it is likely to occur if there is an interaction between the antioxidant and cupric sulfate. Thus the presence of cupric sulfate and gallate is essential for prooxidation to occur. Since the rate of prooxidation observed does not increase markedly with time, the possibility that the prooxidation is being catalyzed by an oxidation product of benzaldehyde or by a complex formed between this product and the gallate is unlikely. In fact, benzoic acid has been reported as inhibiting the process of aldehyde oxidation (7). Interaction of gallate and cupric sulfate resulting in a cupric-gallate complex is more likely to be responsible for the observed results. Complexes between metals and gallic acid have been reported by various workers (8–10).

Table II—Oxygen Uptake of Various Systems at 25°

System	Oxygen Uptake at 6th hr. (moles O_2/l. $\times 10^{-3}$)
(A) Benzaldehyde in water[a] + cupric sulfate[b]	nil
(B) Benzaldehyde in water[a] + cupric sulfate[b] + ethyl gallate, as for (G)	nil
(C) Cetomacrogol solution[c] + ethyl gallate, as for (G)	nil
(D) Cetomacrogol solution[c] + cupric sulfate[b]	nil
(E) Cetomacrogol solution[c] + cupric sulfate[b] + ethyl gallate, as for (G)	nil
(F) Benzaldehyde in cetomacrogol solution[d]	6.64
(G) Benzaldehyde in cetomacrogol solution[d] + ethyl gallate, *M*	
0.00	7.23
0.30×10^{-6}	7.21
0.45×10^{-6}	7.06
0.60×10^{-6}	6.98
0.90×10^{-6}	6.55
(H) Benzaldehyde in cetomacrogol solution[d] + cupric sulfate[b] + ethyl gallate, *M*	
0.00	18.0
0.30×10^{-6}	21.42
0.45×10^{-6}	20.62
0.60×10^{-6}	17.23
0.90×10^{-6}	14.45

[a] Benzaldehyde in water 0.0617*M* which is the water solubility value at 25°. [b] Cupric sulfate concn. 1×10^{-4} *M*. [c] Cetomacrogol 0.04 *M* [d] Benzaldehyde in cetomacrogol solution 0.1729 *M* which is the aldehyde solubility in the surfactant solution.

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Simultaneous Quantitative Gas Chromatographic Determination of Atropine and Scopolamine

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Abstract □ Atropine and scopolamine as free bases were simultaneously quantitated by GLC. Atropine and its sulfate salt produced peaks as apoatropine and atropine base. Scopolamine base produced peaks of scopolamine and a product of lower molecular weight. Linear standard curves were obtained between 0.5 and 200 mcg. for each alkaloid. Pure compounds and plant extracts were compared with the USP XVII assay for total alkaloids of belladonna.

Keyphrases □ Atropine, scopolamine—simultaneous determination □ TLC—identity □ GLC—analysis

The work of Quin (1) on tobacco smoke and Lloyd (2) on the gas chromatography of high molecular weight alkaloids led Brochmann-Hanssen and Svendsen (3) to attempt separation of a large number of alkaloid mixtures from various plant sources, including atropine and scopolamine. No attempt was made to quantitate these alkaloids, but some of the difficulties to be anticipated in a quantitative GLC procedure were enumerated. The formation of apoatropine catalyzed by glass wool in the column was discussed. The use of GLC in the simultaneous determination of methapyrilene fumarate, ephedrine hydrochloride, and codeine phosphate in syrup was recently demonstrated by Wesselman and Koch (4). In an intensive study Rader and Aranda (5) extended the applicability of GLC quantitative procedures to various drug mixtures. Wesselman (6) assayed terpin hydrate and codeine elixir by GLC procedures.

This paper reports the quantitation of atropine and scopolamine simultaneously as pure compounds and from plant extracts.

EXPERIMENTAL

Equipment—A linear programmed temperature gas chromatograph[1] equipped with a flame-ionization detector was used. The detector signal was printed on a 1-mv. recorder[2] with a chart speed of 1.27 cm. (0.5 in.)/min. and 1-sec. full-scale response. The 2-μl. samples were injected with a 10-μl. syringe.[3] Continuous extraction apparatus was used for all extractions of plant powders according to the method of USP XVII (7). No attempt to estimate the combined precision of the extraction procedure with the GLC procedure was made.

Materials—The carrier gas was helium. Hydrogen and air were used in the flame-ionization detector. The stationary phase was diatomite aggregate,[4] DMCS 80/100 with methyl silicone gum rubber[5] liquid phase at a concentration of 2.5%. Dual borosilicate glass columns 182.88 cm. (6 ft.) × 0.19 cm. (0.075 in.) inside diameter were filled with prepared packing material[6] under reduced pressure with uniform vibration. Packing material was held in place in the column by the smallest pledgets of Pyrex glass wool practicable. Chromatographically pure chloroform was used throughout as the solvent for the free bases of atropine and scopolamine. Atropine sulfate was dissolved in reagent grade anhydrous methyl alcohol. The powders of *Atropa belladonna* and *Datura stramonium* used in this work were 60-mesh powders. Thin-layer chromatograms of each compound indicated one spot for each standard.[7]

Operating Conditions—The column temperature was programmed from 150–275° at the rate of 6°/min. At the end of each run the column was cooled for 10 min. and then equilibrated for 3 min. at 150° before injecting the next sample. The injection port temperature was maintained at 315°. The mean helium flow rate was 99.5 ± 0.5 ml./min. Air and hydrogen were maintained at 48 and 24

[1] Perkin-Elmer model 881.
[2] Sargent model SR (S-72180-20).
[3] Hamilton No. 701.
[4] Chromosorb G, acid-washed.
[5] SE30/S.
[6] Perkin-Elmer Co.
[7] Atropine and scopolamine used as standards were obtained from the Aldrich Chemical Co.