Ball's Test.—Ball (5) proposed the use of potassium ferrocyanide as a reagent for cinchonine.

The test is described as follows: "Potassium ferrocyanide if added to the solution of a salt of this alkaloid, produces a yellowish white curdy precipitate, which is dissolved upon the application of a gentle heat, but is again deposited, when the liquid cools, as an abundant crop of golden yellow crystals; a slight excess of ferrocyanide should be used."

This test, with some modifications was used on quinicine and cinchonicine. The test as used was: to an aqueous solution of the toxin, add an equal volume of dilute sulfuric acid (6*N*) and then an excess of a solution of potassium ferrocyanide.

Quinicine yields a brilliant yellow precipitate not dissolved by boiling.

Cinchonicine yields a salmon pink precipitate not dissolved by boiling.

Sensitivity for both toxins 1:60.

The phenylhydrazine and nitrous acid tests of Ganassini (6) were tried and discarded as unsatisfactory.

SUMMARY

1. Diazobenzene sulfonic acid reagent gives reliable tests for quinicine and cinchonicine in aqueous or alcoholic solutions and in the presence of the parent alkaloid or alkaloidal salts.
2. Dinitro thiophene reagent gives reliable tests for quinicine and cinchonicine in alcoholic or ethereal solution and in the presence of the parent alkaloid, but not in the presence of the alkaloidal salts.
3. The modified Lipkin test may be used to differentiate between quinine and quinicine and between cinchonine and cinchonicine.
4. The modified Ball test may also be used to differentiate between quinicine and cinchonicine.
5. The phenylhydrazine and nitrous acid tests are less satisfactory than others described.

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"Clearness is the ornament of profound thought"—Vauvenargues

The Stabilizing Effects of Antioxidants upon Solution of Tannic Acid*

By K. P. DuBois† *and C. O. Lee*‡

The decomposition of tannic acid solutions is believed to be due, in part at least, to oxidation. This belief suggested the possibility of using antioxidants as protective agents for such solutions. As a result of our study we believe that very satisfactory stabilizing agents have been found for tannic acid solutions.

In so far as we were able, we applied the information which had been obtained in various other antioxidant studies to the problem of stabilizing tannin solutions. It was realized that an antioxidant must be very active in order to be effective in stabilizing tannin solutions because they themselves act as very active antioxidants under certain conditions.

EXPERIMENTAL

Assay Method for Tannin Solutions.—The effectiveness of the various antioxidants is dependent upon their ability to prevent the loss of tannin in tannic acid solutions. This makes it necessary to determine the tannin content of such solutions at frequent intervals. A modification of the Lowenthal-Proctor method was found to be satisfactory, especially so with simple solutions of tannic acid (1).

The following tannic acid solution has been recommended for therapeutic use (2). This solution without the salicylic acid was made the subject of our stabilization study.

Tannic acid	100.00 Gm.
Sodium chloride	10.50 Gm.
Potassium chloride	0.42 Gm.
Calcium chloride	0.84 Gm.
Salicyclic acid	1.00 Gm.
Distilled water to make	1000.00 cc.

One hundred cubic centimeter portions of this solution were placed in 120-cc. prescription bottles. To each of 17 of them a different antioxidant was added. In addition one sample was stored under carbon dioxide, another under nitrogen to exclude the air, and a third served as the control.

Effectiveness of Common Antioxidants.—The antioxidants used are listed in Table I. Most of these

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substances have been known to behave as antioxidants or are thought to possess such property because of their ease of oxidation. The antioxidants used in this series were representative compounds selected from each of the various classes of chemical antioxidants.

Table I.—Effectiveness of the More Common Antioxidants

		Per Cent Tannin			Per Cent Loss of Tannin	
Sample No.	Antioxidant	Immediately after Preparation	After 60 Days	After 120 Days	After 60 Days	After 120 Days
16	Sodium sulfite	9.35	9.35	9.35	0	0
18	Sodium thiosulfate	9.35	9.37	9.35	0	0
15	Sodium sulfite (1%)	9.35	9.28	9.26	0.7	0.96
19	Carbon dioxide	9.35	9.18	9.02	1.81	3.53
17	Phosphoric acid	9.35	9.00	8.90	3.94	4.81
5	Tartaric acid	9.35	9.08	8.90	2.78	4.81
8	Resorcinol	9.35	8.95	8.75	4.81	6.41
7	Hydroquinone	9.35	8.95	8.75	5.33	6.41
12	Sodium citrate	9.35	9.04	8.69	3.30	7.16
2	Salicylic acid	9.35	8.85	8.69	5.33	7.16
3	Benzoic acid	9.35	8.85	8.69	5.33	7.16
20	Nitrogen	9.35	8.85	8.61	5.33	7.91
10	Allyl alcohol	9.35	8.85	8.52	5.33	8.87
4	Citric acid	9.35	8.61	8.52	7.91	8.87
1	No antioxidant	9.35	8.64	8.44	7.59	9.73
11	Benzyl benzoate	9.35	8.52	8.38	8.87	10.38
14	Zinc chloride	9.35	8.84	7.95	5.36	15.90
13	Methenamine	9.35	8.64	7.81	7.59	16.47
9	Glycine	9.35	8.78	7.70	6.01	17.64
6	Pyrogallic acid	9.35	8.28	5.02	11.33	46.31

Table II.—Effectiveness of Less Common Oxidants

			Per Cent Tannin		Per Cent Loss of Tannin
Sample No.	Antioxidant	Concentration of Antioxidant	Immediately after Preparation	After 60 Days	After 60 Days
35	Maleic acid	0.2	8.58	8.20	4.43
32	Anisol	0.2	8.58	8.20	4.43
33	Chloroform	0.2	8.58	8.20	4.43
34	Parahydroxybenzaldehyde	0.2	8.58	8.20	4.43
38	Malonic acid	0.2	8.58	8.20	4.43
39	Gum guaiac	0.2	8.58	8.20	4.43
40	Soy bean oil	0.2	8.58	8.20	4.43
42	Tincture of Merthiolate	0.2	8.58	8.20	4.43
43	Salicylic acid	0.2	8.58	8.20	4.43
41	Glycerin	1.0	8.58	8.12	5.36
22	Control	...	9.32	8.80	5.58
36	Cholesterol	0.2	8.58	8.03	6.40
37	Aniline	0.2	8.58	8.03	6.40
24	Fructose	0.2	9.32	8.72	6.44
27	Sodium hypophosphite	0.2	9.32	8.46	9.22
26	*l*-Arabinose	0.2	9.32	8.37	10.19
23	Rhamnose	0.2	9.32	7.21	22.64
25	*l*-Xylose	0.2	9.32	6.94	25.53

The concentration of the antioxidants was 0.2 per cent in all samples except those stored under the gases and sample 15 to which 1.0 per cent of sodium sulfite was added 24 hours after being prepared.

All samples were assayed for tannin content immediately after being prepared. The bottles were then sealed with paraffin and stored in a dark place. Assays were again made after 60 days and 120 days. The results of the assays are shown in the table arranged in the order of the increasing loss of tannin after 120 days.

Observations and Comments.—The addition of 0.2% sodium sulfite prevented the characteristic loss of tannin and the usual discoloration of the preparation. The addition of the same concentration of sodium thiosulfate produced a similar effect, except that a small amount of free sulfur was deposited in the container. Storing under carbon dioxide gas partially prevented tannin loss and discoloration. Nitrogen was also partially effective. It is believed that absolute exclusion and displacement of oxygen from these samples was not obtained. The addition of sodium sulfite 24 hours after preparation of the sample 15 did not give full protection against decomposition. This seems to indicate that the deterioration begins immediately after preparation of the samples and that the antioxidants should be added during preparation of the solution.

It was noted that in all samples decrease in tannin content was accompanied by increase in discoloration. Hydroquinone, although it has been used as a very effective antioxidant in other stabilization studies, was of no particular value in this experiment. This bears out the fact that the effectiveness of an antioxidant is dependent upon the nature of the compound which is to be stabilized. Other substances used in this series showed no appreciable stabilizing effect, some of them even hastening decomposition. These substances were eliminated from further consideration of this problem.

Addition of Less Common Antioxidants to Tannic Acid Solutions.—Caldwell and Bibbins (3) in their work on antioxidants concluded that the "trial and error" method must be used to determine the possible effectiveness of an antioxidant and the amount necessary to stabilize the substance under study. Sugars have unexpectedly shown antioxidant properties. Amines also are known to have antioxygenic properties (4).

For a second study some less commonly used antioxidants such as aniline, maleic acid, anisol and some sugars were added to a series of tannic acid solutions. These were stored in prescription bottles and protected from light. Tannic acid determinations were made when prepared and at the end of sixty days. The results are given in Table II.

Observations and Comments.—None of the antioxygenic substances used in this series prevented tannin loss. The sugars apparently hastened decomposition and provided a media for mold growth. Tincture of Merthiolate, although not an antioxidant, hindered mold growth but did not prevent discoloration of the solutions or loss of tannin. Anisol, chloroform, *p*-hydroxybenzaldehyde, aniline, soy bean oil, malonic acid and gum guaiac were not effective stabilizing agents and were eliminated from further study.

Comparison of Sulfite Compounds as Stabilizing Agents.—Since sodium sulfite and sodium thiosulfate had previously appeared to be the only good antioxidants for the preservation of tannic acid solutions a number of more detailed experiments were carried out with sulfite compounds. It would seem logical to expect all sulfite salts to act in a manner similar to sodium sulfite. However, in order to obtain experimental evidence regarding the action of these compounds, comparisons between sodium sulfite, sodium thiosulfate, potassium sulfite and sodium bisulfite were made. The substances were added in concentrations of 0.2% to the tannic acid solution previously described. After standing protected from light for 60 days tannin determinations were made, the results of which are given in Table III.

Observations and Comments.—Each of these antioxidants prevented tannin loss and discoloration of the solutions. There was light decomposition of the sample containing sodium thiosulfate, as well as liberation of free sulfur.

Minimum Effective Concentration of Sodium Sulfite.—In order to determine the minimum concentration of sodium sulfite which would completely prevent tannin loss various concentrations of it were added to a number of tannic acid solutions. Concentrations of sodium sulfite ranging from 0.01 to 2.0% were used in this experiment. The results obtained on tannin determinations appear in Table IV.

Table IV.—Minimum Effective Concentration of Sodium Sulfite

Sample No.	Concentration of Sodium Sulfite	Per Cent Tannin Immediately after Preparation	Per Cent Tannin After 60 Days	Per Cent Loss of Tannin After 60 Days
58	Control	8.58	8.03	6.40
45	0.01%	8.58	8.03	6.40
46	0.03	8.58	8.12	5.36
47	0.05	8.58	8.33	2.91
48	0.07	8.58	8.49	1.04
49	0.10	8.58	8.56	0
50	0.20	8.58	8.59	0
51	0.30	8.58	8.59	0
52	0.40	8.58	8.59	0
53	0.50	8.58	8.59	0
54	0.70	8.58	8.64	0
55	1.00	8.58	8.59	0
56	1.50	8.58	8.64	0
57	2.00	8.58	8.59	0

Observations and Comments.—The minimum concentration of sodium sulfite required to prevent tannin loss was 0.1%. Concentrations of the antioxidant below 0.1% were only partially effective, the 0.01% being ineffective. All concentrations above 0.1% were effective. The solutions containing 1.5 and 2.0% contained a portion of undissolved sodium sulfite.

The prevention of tannin loss and discoloration of tannic acid solutions by sodium sulfite suggests its use in the preparations of tannic acid solutions to be used medicinally. Sollmann (5) states that sulfites are not active therapeutically in low concentrations, thus they may be used without fear of producing untoward physiological effects.

The use of antioxidants as protective agents for tannin solutions presents the probability that atmospheric oxygen plays a part in the decomposition. This was substantiated by Lefevre and Lee

Table III.—Comparison of Effectiveness of Sulfite Compounds

Sample No.	Antioxidant	Concentration of Antioxidant	Per Cent Tannin Immediately after Preparation	Per Cent Tannin After 60 Days	Per Cent Loss of Tannin After 60 Days
21	Control	...	9.32	8.80	5.58
28	Sodium sulfite	0.2	9.32	9.35	0
29	Sodium bisulfite	0.2	9.32	9.35	0
30	Sodium thiosulfate	0.2	9.32	9.26	0.6
31	Potassium sulfite	0.2	9.32	9.35	0

(6) who stored Fluidextracts of Krameria for 12 months in air-tight containers and compared the loss of tannin with the loss observed in the same samples which had not been sealed. It was found that the tannin loss in unsealed samples was much greater than that in sealed samples. This further indicates that oxidation plays a part in the decomposition of tannic acid solutions.

SUMMARY AND CONCLUSIONS

1. Results obtained from this study seem to indicate that most of the well-known chemical antioxidants are ineffective in the stabilization of tannic acid solutions.

2. Sodium sulfite and other sulfite salts are effective in preventing discoloration and loss of tannin in aqueous solutions of tannic acid, at least for a period of four months.

3. In aqueous tannic acid solutions decrease in the tannic acid content is not only immediate but accompanied by marked discoloration of the solutions.

4. There is evidence that the presence of atmospheric oxygen contributes to the decomposition of tannic acid solutions.

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Homogeneity of Tablet Mixtures Before Granulating*

By E. C. Beeler and E. N. Gathercoal†

Tablet forms have been recognized for many years as one of the most convenient methods of administering medicines with uniform and exact dosage. There have been many phases of tablet making reported in the literature, but few of them have considered the homogeneity of tablet mixtures. The fabrication of compressed tablets consisting of multi-component substances can be divided into four factors wherein uniformity is either consummated or modified; named in the order of processing tablets, they are (1) mixing before granulating, (2) granulating, (3) mixing after granulating prior to compression and (4) compression. The first three factors involve the mixing of relatively small solid particles to uniformity, while the fourth, compression, involves the effect of mechanical vibrations of the tablet machine; any mechanical disturbance in the latter could break the homogeneity established in the granulation.

Any type of mixture, *i. e.*, liquid solution, solid solution, gases, mechanical mixtures, etc., is homogeneous when each component substance possesses a constant proportional relationship to every other component substance in any fractional part of the mixture. An ideal mixture is one in which intermingling of particles of the component substances down to molecular magnitude can be accomplished. A solid substance reduced to powder form by mechanical means could never be made to reach such a minute size; therefore, medical powder mixtures are classified as mechanical mixtures in which homogeneity can never be completely realized. Snow and Fantus (1) advanced the theory on tablet making that the material to be compressed must be heterogeneous with respect to the electrical potential set up by mechanical friction of the particles being compressed.

Homogeneity will be used in this paper to indicate the uniformity of composition to which a tablet mixture can be prepared under specific physical conditions of the component substances. Experimental evidence of homogeneity is obtained from the results of several analyses on at least one of the component substances. Consequently, the term homogeneity as applied to a powder mixture is a general description of the uniformity of composition in a fractional part of the mixture convenient for ordinary analysis.

Conditions entering into the problem of mixing solids with solids are of a mechanical or physical nature. Physical, or inherent, characteristics of the component substances comprise the factors which cannot be controlled while mechanical factors can be

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