temperature was employed in this experiment; the season of the year was different, summer *versus* spring; there was more intense illumination fortified by fluorescent lighting for 16 hr. daily in the previous experiment; younger plants (7-week *versus* 9-week at the final harvest) were used in this experiment; the two species were different. Any or all of these variables could account for the fact that an enhancement of the effects of the growth retardant was not noted when DMSO was combined with B995. Cathey (4) and other workers (10–12) have reported differences in the response of plants to growth retardants due to variations in the season of the year, temperature, and light intensity. The effect of the combined B995–DMSO treatment on plant habit and growth, chlorophyll formation, and fractions extracted by various selective solvents paralleled that noted in plants treated with B995 alone. In most cases similar trends were noted in the two groups (increases or decreases) in alkaloid concentration of the plant parts or total alkaloid content. The combination of DMSO with B995 appeared generally to have an additive effect.

The results of this experiment differed markedly from that of James and Sciuchetti (2), who employed concentrations of B995 one-twentieth or less to *D. innoxia* during a 4-week period. The pertinent differences between this experiment and the previous one based on data for the final harvest were: height reduction of 65% *versus* a 10% decrease, significantly delayed flowering and capsule formation *versus* no apparent effect, a 10% reduction in total weight and a 69% decrease in stem weight *versus* no loss in total weight and a 11% decrease in stem weight, a 90% increase in the concentration of stem alkaloids *versus* a 5% increase, a 21% decrease in the total alkaloid content per plant *versus* a 7% increase, a 14% decrease in the alcohol extract and a 33% increase in the water extract *versus* a 26% decrease in the petroleum ether extract, a similar decrease in the alcohol extract, and a 11% reduction in the water extract. These differences indicate that the strength of growth retardant employed can induce an entirely different response in sensitive plants and confirms our previous observation (2) that growth retardants in dilute concentrations can stimulate growth, whereas at high concentrations growth is inhibited. Recent work in our laboratory employing 2% aqueous sprays of B995 on *D. innoxia* and other species of *Datura* and 25 mg. of gibberellic acid either 1 week prior to or following treatment with retardant indicates that B995 has an anti-gibberellin effect.

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Use of 2-Thiobarbituric Acid–Malonaldehyde Reaction as a Measure of Antioxidant Effectiveness in Pharmaceutical Oils

By CHARLES A. BROWNLEY and LEON LACHMAN

A method for estimation of antioxidant efficiency in pharmaceutical oils is described. The procedure depends upon the measurement of malonaldehyde which is produced during the oxidative degradation of the oils. The malonaldehyde is condensed with 2-thiobarbituric acid (TBA) to obtain a pink chromogen which is measured spectrophotometrically at 530 mμ. The influence of 0.02 per cent butylated hydroxyanisole, butylated hydroxytoluene, nordihydroguaiaretic acid, propyl gallate, lauryl gallate, gentisic acid, ascorbyl palmitate, and acetone sodium bisulfite upon the formation of malonaldehyde in peanut, sesame, corn, cottonseed, and soya oils is reported.

OXIDATION IS ONE of the more prominent pathways by which pharmaceutical preparations undergo degradation. Fats and oils which can readily undergo oxidative deterioration are used commonly in ointments, creams, suppositories, and injectable solutions. Oxidative decomposition of pharmaceutical formulations containing these materials may occur during or after manufacture, manifesting itself by physical changes in the dosage form, such as texture, color, odor, flavor, or by change in potency of the active ingredients they contain.

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In fats and oils, oxidative changes are autoxidative in character, proceeding slowly under the influence of atmospheric oxygen. These reactions are of the free-radical type and proceed through the autoxidation of the unsaturated acids present in the oil or fat. The exact mechanism of this oxidation reaction is still largely a matter of conjecture, but the ultimate result of all these changes is the formation of products of lower molecular weights, *e.g.*, aldehydes, ketones, and fatty acids. These products are formed by cleavage of the hydroperoxide primary monomeric, nonvolatile oxidation products and are accelerated by light (ultraviolet being especially active), minute traces of metals and metallic salts, and by increased temperatures (1). This deterioration reaction can be retarded by antioxidants. Since all antioxidants do not have the same protective power and since their activity sometimes varies according to the nature of the substance to be protected and the concentration of the antioxidant, a considerable amount of effort has been expended to measure their degree of efficacy (2–4).

The activity of an antioxidant is usually evaluated by determining the stability of the oils with and without a definite quantity of antioxidant. The tests are hopefully designed to permit an estimation of the oxidative rancidity that would occur under actual shelf storage. The tests which have been most widely used are the oven test (5), which involves storing samples in a constant-temperature oven at 60° and supplementing periodic organoleptic examinations by peroxide determination; the active oxygen method (AOM) (6), which involves passing air at a controlled rate through samples maintained at 98.6° until the sample becomes rancid; the oxygen absorption tests (7), which involve the use of manometric respirometers; the Kreis test (8), which is based on the measurement of the epihydrin aldehyde derivatives formed as secondary products; the colorimetric tests for carbonyl compounds (9); and the ASTM bomb method (10), which is similar to the AOM method. A polarographic method (11) was recently reported which claims to provide a simple, rapid, precise measurement of lipid autoxidation and antioxidant value; but as the authors pointed out, the method measures only the antioxidant activity exerted during a brief portion of the total period of lipid peroxidation.

The above procedures are generally complicated, time-consuming, and are not sufficiently precise to give a clear understanding of the rate at which the oils rancidify. For the most part, they depend upon the determination of peroxide values which sometimes have no significance in relation to the keeping quality because of their simultaneous formation and decomposition. Hence, the development of a reliable and systematic measure of oxidative randicity would be extremely desirable.

A 2-thiobarbituric acid (TBA) test was devised by Kohn and Liversledge (12) for the qualitative examination of oxidation reactions occurring in tissues. It was stated that this test gives a reliable measure of the extent of oxidation in cases where substances other than fats are present and cause decomposition of the peroxides. Later, Wilbur (13) suggested that this acid could be employed to determine quantitatively the oxidation products of unsaturated fatty acids. Methods based on the use of TBA were developed by Patton (14) for use in the examination of the deterioration of milk fat and by Dunkley and Jennings (15) for use in the testing of milk. Following these developments, many investigators (16–20), including the authors (21), have turned to use of this reagent in the study of the stability of food products.

The TBA test involves the spectrophotometric determination of the pink color that results when rancid foods react with TBA in solution. This colorant has an absorption maximum at 530 to 535 mμ. The compound responsible has been established by Sinnhuber *et al.* (22) and confirmed by Schmidt (23) to be due to the condensation of two molecules of TBA with one molecule of the dicarbonyl compound, malonaldehyde (MA). This dicarbonyl compound originates from the autoxidation of certain unsaturated lipids and is a so-called secondary degradation product believed to be formed largely from primary hydroperoxide decomposition. According to Dahle *et al.* (24), MA comes from the oxidation of polyunsaturated fatty acids other than linoleic, and its presence in rancid foods and autoxidized fats and oils has been confirmed (22, 25). The high sensitivity of the TBA test compared with other methods in common use for oils and fats has been stressed frequently. Because of the sensitivity of this method, it has become widely used as an index for the stability of fats and oils and has been proved useful as an indicator of the effectiveness of antioxidants (26, 27).

Comprehensive data comparing the activities of various antioxidants for oils and fats are not available. In most published works, only a limited number of antioxidants have been tested at one time and often under conditions so different

that results cannot be compared. The information to be presented represents a preliminary report of our efforts in employing the TBA–MA reaction to evaluate several antioxidants as to their relative stabilizing effects on a number of oils commonly used in pharmaceutical preparations. Eight antioxidants were studied in five different oil substrates.

EXPERIMENTAL

Materials.—The oils selected for study were refined corn oil U.S.P.; prime winter yellow cottonseed oil U.S.P.; refined edible soyabean oil (obtained from Welch, Holme and Clark Co., Inc., New York); peanut oil U.S.P.; and sesame oil U.S.P. The antioxidants evaluated were butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Eastman Chemical Products, Inc., Tenn.); gentisic acid (GA) and lauryl gallate (LG) (K & K Laboratories, N. Y.); nordihydroguaiaretic acid (NDGA) (Aldrich Chemical Co., Inc., Wis.); propyl gallate (PG) (Nutritional Biochemical Corp., Ohio); ascorbyl palmitate (AP) (Chas. Pfizer and Co., Inc., New York); and acetone sodium bisulfite (ASB) (Ciba).

Analytical Method.—For this study, the single-phase system developed by Jacobson (20) was adopted. This single-phase solvent was a mixture of 50 parts of iso-octane, spectra grade (Phillip Petroleum Co., Okla.); 27 parts of *n*-propanol (b.p. 96–98°) (Matheson, Coleman and Bell, N. J.); and 3 parts of water for injection U.S.P. For the solvent to be acceptable for use in this analytical procedure, 10 ml. of solvent containing 10 mg. of purified TBA (see below) and heated at 60° for 30 min. should not exhibit any absorption from 400 to 600 mμ against a water blank. If any absorption is present, the solvent can be purified by the procedure employed by Jacobson or any reliable procedure for the removal of carbonyl compounds. The TBA was obtained from a number of suppliers (Eastman Chemical Products, Inc., K & K Laboratories; and Bios Laboratories, New York, N. Y.) and purified for use in this test. Figure 1 shows the absorption of TBA in the solvent as purchased *versus* material purified in our laboratories. If the TBA were pure, no absorbance would take place from 400 to 600 mμ against a water blank. To obtain pure TBA, the TBA is recrystallized twice from purified water by heating to 60° and cooling. The recrystallized TBA is next dissolved in purified water at 80°. The resulting slightly yellow solution is filtered while hot through a medium-porosity sintered-glass funnel directly onto a warmed 3 × 28-cm., or longer, column of Woelm acid aluminum oxide (activity grade 1 from Alupharm Chemicals, La.). The column is warmed by using 80° water as the final washing during its preparation. The column is discarded when the yellow band observed reaches within 2 cm. of the bottom. The solution of TBA is collected in shallow dishes and the water is removed by freeze-drying. The dried crystals were found to contain one molecule of water of hydration and were stable for several months when stored at low temperatures (6°). The yield of purified TBA was 25%. However, it is essential to employ purified TBA in order to obtain reproducible results and to decrease the involvement of the so-called troublesome interfering pigments that have been mentioned in the literature (28–30). It is interesting to note that no mention of the water of hydration has been made in previous studies dealing with oxidative evaluations.

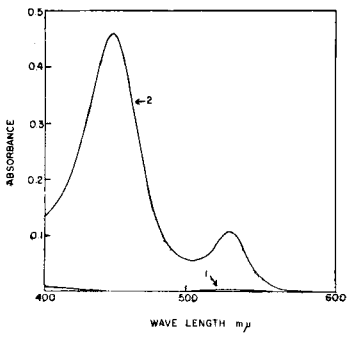

Fig. 1.—The absorption spectra for thiobarbituric acid before and after recrystallization (1 Gm./L.).

Equipment.—Absorbances of the TBA–MA complexes were read on a Beckman spectrophotometer model DB with a Sargent model SR recorder. Constant-temperature ovens (Precision Scientific Co.) were used at 80 ± 3° and 200 ± 3° for accelerated heating of the oil samples and at 60 ± 1° for development of the TBA–MA complex. A sand bath was used in the 60° oven to house the samples. A Wilmot-Castle steam sterilizer was used for heating the oil samples at 115°.

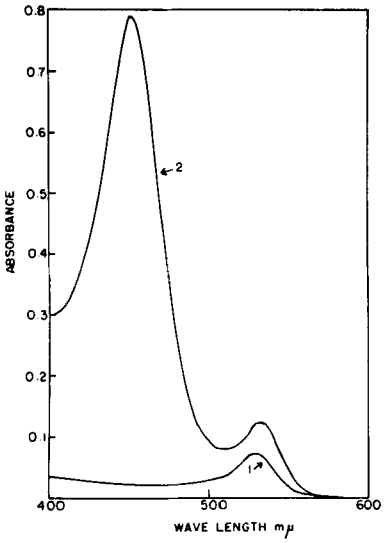

Fig. 2.—The absorption spectra for the TBA–MA colorant from a peanut oil sample heated at 60°. Key: 1, for 30 min.; 2, for 180 min.

Procedures.—Antioxidants at 0.02% were added to each oil sample. A 5-ml. sample of the protected oils was filled into glass ampuls, and the ampuls were pull-sealed under a gas-oxygen flame. The ampuls were heated at 200° or steam sterilized at 115°. Twenty grams of the protected oils was placed

into 100-ml. beakers. The height of these oils in the beakers did not exceed 2 cm. These beakers were placed into darkened cabinets at 80°. Samples of oils containing no antioxidant were placed along with each test series. At designated intervals, 100-mg. samples of the oil were removed and dissolved in 10 ml. of the solvent containing 10 mg. TBA in either 100-ml. volumetric flasks or 125-ml. iodine flasks. The flask was sealed with high vacuum silicone grease and placed into a sand bath at 60° for 30 min. The flask was removed and cooled under tap water before unstoppering. The color developed was read immediately at 530 mμ against a blank consisting of the solvent mixture and TBA treated in the same manner as the sample. No correction for oil color was necessary in the samples investigated. All analyses were run at least in triplicates. To facilitate running a large number of samples, the TBA can be added to a 90% propanol solution and aliquots removed for use in the TBA–MA reaction. This solution is stable for at least 2 hr. at room temperature and for a longer period of time if refrigerated. Slightly yellowed solutions can be used for qualitative comparisons but should not be used for quantitative analysis. The use of oil sample reservoirs in iso-octane will also facilitate triplicate determinations.

The relative effectiveness of the various antioxidants in the different oils was determined by comparing the absorbances of the TBA–MA complexes produced. The lower the absorbance the greater the apparent efficacy of the particular antioxidant in the particular oil system. This procedure was employed because it was felt that in the absence of standardized procedures for the TBA–MA complex, and in the absence of standardized reference samples, attempts to employ TBA numbers (31) or other quantitative numbers would be in error.

Discussion of TBA Test Procedures.—The TBA–MA color reaction ranges from light pink to deep pink to orange-yellow, depending upon MA concentration, as well as upon time allowed for the color development. Figure 2 shows the absorption spectra for the TBA–MA colorant from a peanut oil sample in the system studied at two different development periods at 60°. The lower curve shows the spectrum after 30 min. and absorption peak at 530 mμ. The upper curve represents the same sample, now orange-yellow, after 3 hr. at the same temperature. A second maximum is present at 450 mμ. Figure 3 shows the effect of time of heating on the TBA–MA pigment development at 530 mμ in an unprotected and an antioxidant-containing sample of peanut oil.

The rate of increase in color was greatest during the first hour. Thereafter the increase was slower, but continuous. Also, the difference in the absorbance in the unprotected sample *versus* the protected sample was far more pronounced after 3 hr.

The majority of TBA procedures reported employ a 20- to 40-min. pigment development time at 60–100°. The reasons usually given for the acceptance of these test time periods, although short of reaction completion, are instability of the MA, the development of the interfering pigment at 450 mμ, and the avoidance of additional heat-accelerated autoxidation of the test materials. Since this preliminary study was qualitative in nature, reaction times at 60° of 30, 120, and 180 min. were examined. The 30- and 60-min. heating times were found to give excellent replicate results. Two series of seven replicate determinations were made upon two separate autoxidized samples. The arithmetic means of the absorption maxima were found to be 0.371 and 0.365. The standard deviations from the means were ±0.023 and ±0.018, respectively. It was observed also that when highly autoxidized samples of oils were heated for the extended period of time with the TBA, the pink pigment would precipitate out, necessitating procedures for its resolution and measurement.

Figure 4 shows the development of the absorbance at 450 mμ in an unprotected sample of peanut oil at varying time periods during the color development at 60°. There was no development of

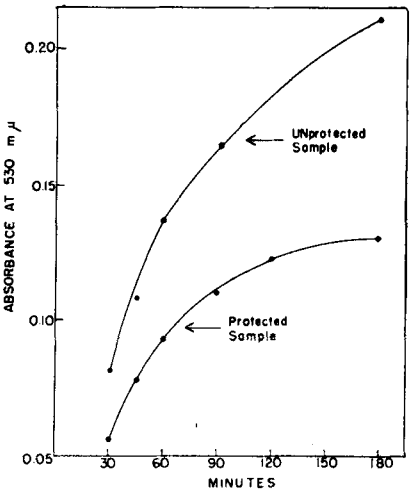

Fig. 3.—The effect of heating at 60° on the intensity of the TBA–MA colorant at 530 mμ from a sample of peanut oil with and without antioxidant.

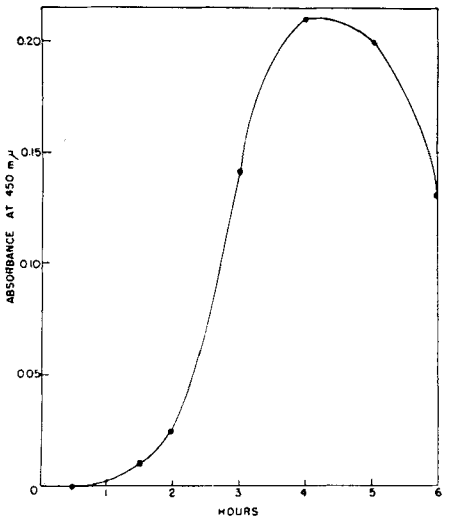

Fig. 4.—The influence of heating at 60° on the intensity of color development at 450 mμ from TBA with a sample of peanut oil.

color for the first hour, but thereafter the pigment developed rapidly, reaching a maximum intensity in 4 hr. and decreasing thereafter.

This possibly can be explained on the basis that the pigment forms as a result of a reaction between the TBA or its decomposition products with components of the oxidized oil since the determinations were made against blanks containing only the TBA. This absorption maximum at 450 mμ was reported by Tarladgis *et al.* (29) to interfere in the TBA test and to result in erroneously high values at 532 mμ because of the overlapping effects of the absorption spectra. Yu *et al.* (30) developed chromatographic procedures for separating and purifying the pigment having an absorption at 532 mμ. It was observed in this investigation that the two pigments could be separated completely by development of a two-phase system from our single-phase system by the simple addition of water. The pink pigment partitioned into the aqueous phase and the yellow pigment into the iso-octane phase. The spectra of each solution showed a complete absence of the other pigment. Yamada (32) in his studies to determine the nature of the 450-mμ absorption maximum concluded that, in heated oils, propionaldehyde, butylaldehyde, and caprylaldehyde were responsible along with TBA breakdown products.

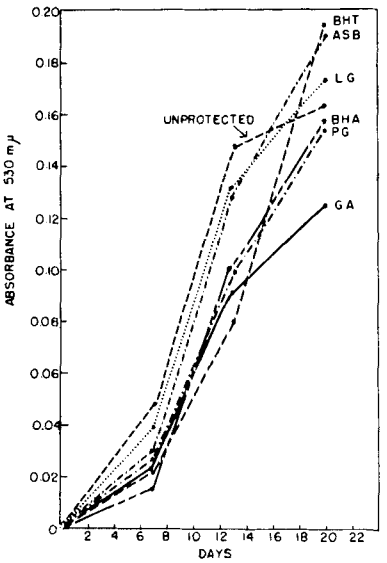

Fig. 6.—Curves showing the TBA–MA color development at 530 mμ from samples of peanut oil with and without antioxidant as a function of time at 80°.

RESULTS AND DISCUSSION

There have been references noting that more than 37 compounds (1, 33) react with TBA, some of which are not aldehydes. Therefore, an experiment was conducted to determine the reactivity of the antioxidants with TBA. Solutions of antioxidants at a concentration of 0.02% were prepared in propanol and water. The solutions were subjected, in closed ampuls, to 115° temperature for 8 hr. Samples from these solutions then were tested with TBA in the solvent system used for the oil solutions and in accordance with the heating procedures employed for color development of the oils. There was no absorption observed for either BHT, BHA, PG, LG, GA, ASB, AP, or NDGA at 530 mμ. In fact, no absorptions were observed at 530 mμ when the antioxidant–TBA solutions were heated for 3 hr. at 60°. Only with the antioxidant ASB was there any absorption at 450 mμ in the 3-hr. heating period when tested against a TBA blank. This possibly can be ascribed to the acetone portion of the molecule reacting with the TBA. However, it was observed that the yellow colorant developed (450 mμ) in the solutions containing the several antioxidants and the TBA held at 60° for 3 hr. was lower when water was used as the blank than that found for the TBA solution without antioxidant. The relative reduction of this TBA pigment development at 450 mμ in presence of the various antioxidants is shown in Fig. 5. The order of effectiveness is NDGA, AP, BHT, PG, LG, BHA, GA, ASB. This indicated the probability that a portion of the TBA degradation was due to oxidation. Since the antioxidants did not react with TBA to produce a colorant which absorbs at 530 mμ, several experiments were performed to evaluate the antioxidant effectiveness in oils subjected to various exaggerated conditions of storage favoring autoxidation. In addition, one experiment was performed in which the influence of the antioxidants on the yellow chromogen absorbing at 450 mμ was evaluated.

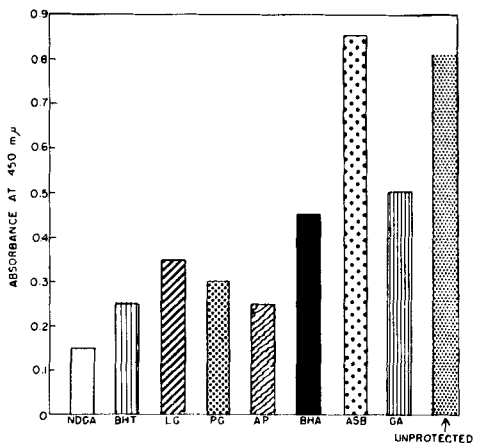

Fig. 5.—The relative effect of several antioxidants on the color development of TBA in solvent at 450 mμ.

Experiment 1.—This experiment involved the examination of the TBA–MA complex development at 530 mμ in samples of peanut oil containing 0.02% of BHA, BHT, PG, LG, GA, and ASB. The samples were stored in open glass beakers at room temperature in a darkened, but vented, cabinet.

The initial absorbance at 530 mμ of the TBA–MA complex developed in the peanut oil used was 0.000 to 0.015. The rate of increase was extremely slow at room temperature. After 60 days, the absorbance was only 0.115. In the samples containing the antioxidants, the absorbances were 0.050 (ASB), 0.062 (GA), 0.065 (PG), 0.068 (LG), 0.080 (BHT), and 0.094 (BHA). This indicated that at these

conditions, ASB was the more effective antioxidant in inhibiting the development of MA, and BHA and BHT were the least effective.

Experiment 2.—This experiment involved the examination of the TBA–MA complex development at 530 mμ in samples of peanut oil containing 0.02% of the same antioxidants used in *Experiment 1.* However, the samples were stored in the open glass beakers at 80°.

The initial absorbance at 530 mμ of the TBA–MA complex developed in the peanut oil used was also 0.000 to 0.015. The TBA test was conducted on these samples daily for 7 days and then on the 13th and 20th day. The first discernible absorbances appeared on the seventh day. The increase in absorbance of the complexes in the presence of the antioxidants is shown in Fig. 6. Following the lag or very slow initial period, there is a period of rapid increase, followed by an apparent decrease in MA being produced. After 7 days, the sample containing BHA showed the least formation of TBA–MA complex. After 20 days, the sample containing GA showed the least formation, followed by PG, BHA, LG, ASB, and BHT. The importance of the time of testing is highlighted in this experiment.

As can be seen from Fig. 6, the TBA–MA colorant is more intense at 20 days in the samples containing LG, ASB, and BHT than in the sample of oil not protected by an antioxidant. One can possibly deduce from these results that the antioxidants are accelerating the development of MA. According to Jaminet (34), an antioxidant can exert a "pro-oxidant" effect and promotes degradation of the principles requiring protection against autoxidation. If this had taken place only in the samples which were stored in open beakers and heated at 80°, it could also be reasoned that the MA was being lost to the atmosphere. However, this same effect was also observed in sealed ampuls containing each of the antioxidants in the several oil systems when stored at 115 and 200°. Consequently, it seems more reasonable to assume that the MA in the antioxidant-containing samples (LG, ASB, and BHT) is being protected from subsequent heat degradation which occurs in the unprotected sample.

Experiment 3.—Samples of corn, cottonseed, peanut, sesame, and soyabean oils containing 0.02% of BHA, BHT, PG, LG, GA, ASB, and AP were filled into glass ampuls, sealed, and heated at 200° from 2 to 24 hr. This evaluation was performed to determine the influence of temperatures approximating that used in the sterilization of oils for injectable use on the effectiveness of the antioxidants.

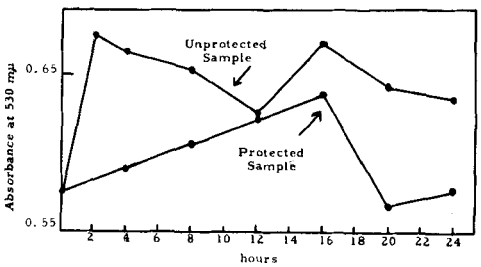

Fig. 7.—Changes in the MA concentration of peanut oil at 200° for 24 hr. as measured by the TBA–MA color reaction at 530 mμ.

The initial absorbance of the oils at 530 mμ was corn oil, 0.030; cottonseed oil, 0.050; peanut oil, 0.058; sesame oil, 0.074; and soyabean oil, 0.035. The order of effectiveness of the antioxidants in samples exposed to 200° for 2 hr. is shown in Table I. In general, it appeared that the gallic acid esters were the most effective antioxidants, the butylated hydroxy esters next, and GA and ASB least effective.

It was interesting to note the AP which was least effective in protecting peanut and corn oils was most effective in protecting soyabean oil at this temperature. Consequently, in choosing the most appropriate antioxidant for a particular system, it is unwise to rely on data obtained from other systems. Instead, each system should be evaluated independently to obtain the antioxidant of maximum effectiveness.

Figure 7 shows the TBA absorbance for peanut oil over the 24-hr. heating period at 200°. It is readily seen that the TBA–MA absorbance values at 530 mμ are not directly related with the time of heating. Similar results were obtained with the other oils. Yamada (35) reports a similar curve, which shape he explained on the basis that his soyabean oil was heated in opened dishes and loss of the MA reactant had occurred. He obtained a smoother rising curve when he employed a reflux condenser, but a dip of some magnitude was still seen after 10 hr. of heating.

A more appropriate explanation of the curve possibly lies in the dynamics of autoxidation occurring in the oils at these relatively high temperatures. Initially, there is development of MA which is unstable and destroyed rapidly by the accelerated heat conditions as can be seen by the drop in absorbance for the TBA–MA complex after 2 hr.

TABLE I.—RELATIVE EFFECTIVENESS OF ANTIOXIDANTS IN PHARMACEUTICAL OILS STORED AT 200° FOR 2 HR. AS MEASURED AT 530 mμ

Oils	Antioxidants
Corn oil	PG > LG > BHT > ASB > GA > BHA > AP
Cottonseed oil	LG > PG > GA > BHT > AP > BHA > ASB
Peanut oil	PG > LG > BHT > BHA > GA = ASB > AP
Sesame oil	LG > BHA = ASB > AP > PG > BHT > GA
Soyabean oil	AP > PG = LG > BHA > GA > ASB > BHT

TABLE II.—RELATIVE EFFECTIVENESS OF ANTIOXIDANTS IN PHARMACEUTICAL OILS STORED AT 115° FOR 8 HR. AS MEASURED AT 530 mμ

Oils	Antioxidants
Corn oil	ASB > BHT > BHA > PG = AP > LG = NDGA > GA
Cottonseed oil	BHT = ASB = NDGA > LG > BHA = GA > AP > PG
Peanut oil	AP > BHT > NDGA = ASB > BHA = PG > LG > GA
Sesame oil	GA > AP > PG > NDGA > ASB > BHA = BHT = LG
Soyabean oil	AP > BHT > PG = ASB > BHA = GA > LG > NDGA

Also plotted on Fig. 7 is the curve for a sample of oil protected with BHA. There is an increasing amount of MA being produced over the first 16 hr. At that time, there is the sudden drop as is seen in the unprotected samples after 2 hr. Consequently, the active period of MA production appears to have been increased in the presence of the antioxidant.

Experiment 4.—The five oils, as used in *Experiment 3*, containing 0.02% of BHA, BHT, PG, LG, GA, ASB, AP, and NDGA were filled into ampuls, pull-sealed, and heated at 115° for 8 hr. Table II indicates the relative effectiveness of the antioxidants as shown by their absorbances at 530 mμ. From the general pattern, AP and ASB appeared to be the most effective, and LG and GA the least effective. Here again, the antioxidant exhibiting maximum effectiveness was dependent on the particular oil tested and the degree of heat accelerated autoxidation.

Experiment 5.—It has been observed that the yellow pigment (450 mμ) formed after heating a solution of TBA in solvent at 60° is reduced in presence of the antioxidants, except in the case of ASB. It was felt that there was a possibility that the absorption maximum at 450 mμ could also serve as a test for the effectiveness of the antioxidants in the oils. Therefore, samples of peanut oil and several antioxidants were stored at 80° and reacted with TBA. The pigment was allowed to develop for 3 hr. and measured at 450 mμ. Figure 8 shows the influence of the antioxidants on the absorbance at this wavelength, indicating NDGA as the most effective of the antioxidants. Table III shows the influence of the antioxidants on the absorbances at 450 mμ produced in the samples of oil from *Experiment 4* which were stored at 115°. As can be seen from Tables II and III, the relative effectiveness of the antioxidants measured at 450 mμ and 530 mμ differed. Although information has been

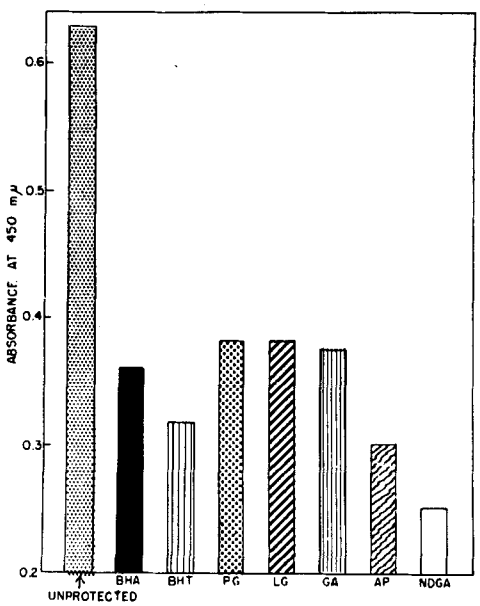

Fig. 8.—The relative effect of antioxidants on the color produced between oxidized peanut oil and TBA as measured at 450 mμ.

TABLE III.—RELATIVE EFFECTIVENESS OF ANTIOXIDANTS ON PHARMACEUTICAL OILS STORED AT 115° FOR 8 hr. AS MEASURED AT 450 mμ

Oils	Antioxidants
Corn oil	ASB > PG > AP > NDGA > LG > BHA > GA > BHT
Cottonseed oil	ASB > BHT > NDGA > GA > BHA > LG > PG > AP
Peanut oil	AP > LG > PG > GA = ASB > NDGA = BHT > BHA
Sesame oil	GA > NDGA > PG > AP > BHA > ASB > LG > BHT
Soyabean oil	AP > BHT > PG > BHA > ASB > LG > NDGA > GA

presented on the influence of several antioxidants in the color development at 450 mμ, considerably more work will be required before it can be stated whether the absorbance at 530 mμ or 450 mμ gives a truer indication of the antioxidant effectiveness.

SUMMARY

The antioxidant effect of butylated hydroxytoluene, butylated hydroxyanisole, propyl gallate, lauryl gallate, acetone sodium bisulfite, nordihydroguaiaretic acid, gentisic acid, and ascorbyl palmitate on peanut, cottonseed, corn, sesame, and soyabean oils was studied for varying periods of storage time at room temperature, 80, 115, and 200°. A thiobarbituric acid test (TBA) procedure for following the antioxidant effectiveness was described. The procedure employs the reaction of TBA with malonaldehyde, a degradation product obtained from oxidation of the oil, which develops a pink colorant, measured spectrophotometrically at 530 mμ. The effectiveness of the various antioxidants was found to vary (*a*) with the temperature used to accelerate the oxidation of the oils, (*b*) with the length of time at these accelerated temperatures, and (*c*) with the individual oils. The TBA test procedure described closely reflects the oxidative condition of the oil and is a sensitive and simple method for studying the effectiveness of antioxidants in pharmaceutical oils and possibly in complex pharmaceutical systems.

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Interaction of Parachlorometaxylenol with Macromolecules

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A quantitative evaluation of the interaction between parachlorometaxylenol and various macromolecules was obtained by solubility and dialytic procedures. The macromolecules studied were polyvinylpyrrolidone, polyethylene glycol 6000, polysorbate 80, methylcellulose, and poly(methyl vinyl ether/maleic anhydride). The binding affinity of polyvinylpyrrolidone and poly(methyl vinyl ether/maleic anhydride) for parachlorometaxylenol exhibited only a minor temperature dependency. The parachlorometaxylenol–polyethylene glycol interaction was found to be temperature independent. Polysorbate 80 interacted with parachlorometaxylenol to a greater degree than did the other macromolecules studied. Interpretation of the data relative to the possible mechanisms of these interactions is considered.

In recent years, nonionic polymeric substances have been used extensively in the formulation of pharmaceutical dosage forms. Although these polymers are usually considered to be chemically inert, many of them undergo interaction with drug molecules in aqueous solution. These interactions may result in physical incompatibilities as well as the inactivation of preservatives and antimicrobial agents. The inhibitory effect of nonionic macromolecules on phenolic preservatives has been reported (1–12).

Higuchi and Lach (13) and Guttman and Higuchi (14) have investigated the complex formation between phenols and polyethers such as polyethylene glycols. More recently, Patel and Foss (15) have studied the interaction of parabens and phenols with polysorbate 80 and polyethylene glycol 4000. They demonstrated that *p*-chlorophenol exhibited a greater tendency to interact with polysorbate 80 than did phenol.

Parachlorometaxylenol (PCMX) has been used as an antimicrobial agent for a number of years in Great Britain. The biological activity and clinical usefulness of the compound have been reported (16–18). In a study conducted by Mulley and Metcalf (19), it was demonstrated that the solubility of PCMX in aqueous solution was augmented by the presence of polyethylene glycol 1000 monocetyl ether. They attributed the increased solubility of PCMX to its incorporation into micelles.

The present investigation was undertaken to obtain a quantitative evaluation of the interaction between PCMX[1] and various macromolecules. An equilibrium dialysis technique was utilized to study the binding of polyvinylpyrrolidone[2] (PVP), polysorbate 80,[3] and methylcellulose[4] with PCMX. The solubility method was used to study the interaction of PVP, poly-

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[1] Ottasept Extra. Supplied through the courtesy of the Ottawa Chemical Co., Toledo, Ohio.
[2] Plasdone, type K 29–32. Supplied through the courtesy of Antara Chemicals, a division of General Aniline and Film Corp., New York, N. Y.
[3] Marketed as Tween 80 by Atlas Chemical Industries, Inc., Wilmington, Del.
[4] Methocel 15 cps. Supplied through the courtesy of Dow Chemical Co., Midland, Mich.