The Preparation and Antioxidant Activity of BHA Bonded to Porous Glass

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ABSTRACT

Butylated hydroxyanisole (BHA) has been bonded to porous glass beads, thus utilizing its functional characteristics while preventing it from entering the final food products. The glass-bonded BHA has been tested in lard and found to have only one percent of the free BHA activity, which can be attributed to stereochemical shielding effects. Evidence is presented strongly indicating that the observed activity resulted from BHA bonded to glass, although activity resulting from BHA leached from the glass could not be conclusively ruled out.

INTRODUCTION

Ideally, preservatives and antioxidants used to protect and stabilize food systems should be functional without necessitating their ingestion. One recently reported approach (1,2) deals with oil-soluble, polymeric antioxidants which have good activity and remain largely unabsorbed in the digestive tract of test animals. Potential heterogeneous polymeric antioxidants, an ethyleneimine polymer, and an ion exchange resin have also been reported (3); however, the ethyleneimine polymer dissolved in the oxidized liquid.

Over the past few years, enzymes (4) and chromatographic phases (5) have been bonded to porous glass beads. In addition, colors have been bonded to fiberglass (6) and algicides to aquarium plate glass and filters (7). In keeping with these concepts the present paper describes the preparation and effectiveness of an antioxidant, butylated hydroxyanisole (BHA), which has been covalently bonded to inert porous glass beads. While the material has not been evaluated in depth, several uses for this bonded antioxidant can be visualized. The BHA-glass powder may be added to edible liquids which are prone to oxidation during storage and subsequently removed by filtration prior to use. Alternatively, the antioxidant could be placed in "tea bag" type porous containers or fritted glass tubes which are immersed in the liquid or through which the liquid is allowed to circulate. If only a small amount of the antioxidant is required, BHA could be bonded to the inside of the glass container itself. In all of these potential applications, the amount of antioxidant ingested would be limited to very small amounts which may leach from the glass. Finally, the powder might be useful in polymer formulations as a nonvolatile antioxidant. The present work describes the synthesis of BHA-glass and the determination of its antioxidant activity.

EXPERIMENTAL PROCEDURES

Materials

Food grade BHA was provided as a gift by Eastman Chemical Products, Kingsport, TN. Pure lard with no preservatives nor hardening agent was provided as a gift by Sunnyland Foods, Inc., Thomasville, GA. CPG-550 porous glass (particle diameter 74-125μ, surface area 70 m^2/g) is a product of Corning Biological Products Department, Medfield, MA. Microanalyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, MI, 49951 and Midwest Microlab, Ltd., Indianapolis, IN 46250.

Arylnitro Glass (II)

II was prepared by a modification of the method of Venter (8). Triethylamine (0.6 ml, distilled and dried over KOH) was added to p-nitrobenzoylchloride (1.2 g) previously dissolved in chloroform (100 ml). The mixture was added to 10 g of the amine glass (I) (8) in a 500 ml one-necked flask and deaerated for 5 min with shaking under aspirator vacuum. The stoppered flask was shaken on a flatbed stirrer for 1.5 hr, then equipped with a condenser and drying tube and gently refluxed in an oil bath with periodic shaking. Some bumping occurs if refluxing becomes too vigorous. After heating 14 hr, a small aliquot of glass was checked for primary amine with picrylsulfonic acid (8). The test was positive indicating the reaction had not gone to completion. Consequently, a mixture of p-nitrobenzoyl chloride (5 g), triethylamine (5 ml) and chloroform (50 ml) was added to the glass and heated just below reflux for 18 hr followed by 4 hr at reflux. The glass was filtered, washed with a series of solvents and dried at 50 C in a heated vacuum desiccator under aspirator vacuum. The negative primary amine test showed the reaction had essentially gone to completion.

Arylamine Glass (III)

III was prepared from II (8). Analysis: C, 2.57; H, 0.45; N, 0.59. 0.214 meq of organic ligand/g of III based on C; 0.210 meq/g based on N.

BHA Glass (IV)

BHA solution. BHA (0.3 g) was dissolved in IN NaOH (8 ml) and placed under N_2. The pH of the solution was adjusted to ca. 12.8 with 1N HCL. The solution was then diluted to 50 ml with H_2O and cooled in an ice bath.

Diazotized glass. Diazotized glass was prepared by the addition of 2N HCl (50 ml) to 5 g of the arylamine-glass (III). The solution was cooled in an ice bath, and sodium nitrite (1.25 g) was then added which resulted in the immediate evolution of a yellow gas. The mixture was evacuated with aspirator vacuum while rotating in an ice

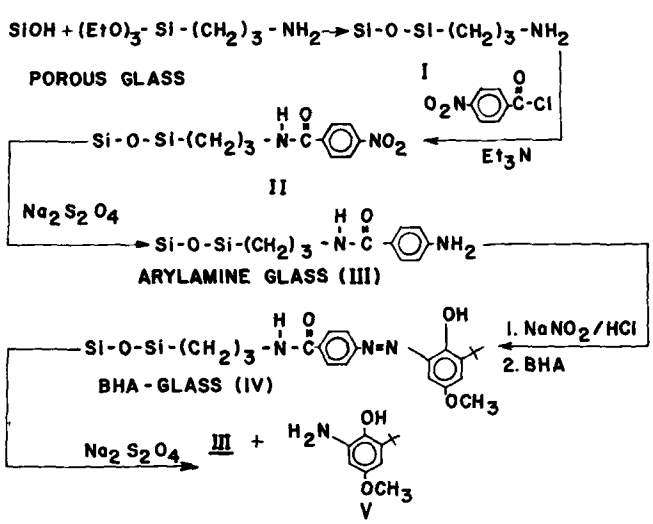


FIG. 1. Preparation of BHA-glass (IV) and cleavage.

bath for 30 min. The diazotized glass was isolated by filtration through a fritted glass funnel and washed well with H_2O (10 C) and 1% aqueous sulfamic acid (10 C).

Coupling. The diazotized glass was added to the cold BHA solution and the glass immediately turned dark red. The mixture was shaken periodically in an ice bath for 30 min, following which the glass was removed by filtration and washed successively with water, benzene, and methanol. Finally, 0.5 g of glass was placed in a small diameter glass column and washed with 150 ml of N_2 saturated CH_3OH over a period of one day. The methanol, following the removal of the first 15 ml, did not absorb in the UV, nor was there any more color removed. Exposure to air during the washing was minimal. Analysis: C, 4.14; H, 0.56; N, 0.70. Meq organic ligand/g of IV: calculated from C, 0.17; from N, 0.16.

Cleavage of BHA Glass to Yield III + V

IV (1.5 g), previously washed for several days in a column with methanol, was washed with water, added to 50 ml of 0.05 M sodium dithionite and deaerated as previously described. The red color very quickly faded to light yellow. The mixture was shaken overnight, filtered, and the glass washed with 10% HCl to give a filtrate V having a pH of 1. The glass residue (III) gave a positive primary amine test with picrylsulfonic acid. Analysis: C, 3.27; H, 0.47; N, 0.56; percent cleavage based on micropanalysis is at least 55%. V; λ_{max} 280 nm (pH = 1 in HCl - H_2O) and after workup 300 nm (pH = 7 in CH_3OH). Calculated from UV data 0.082 meq of V/g of BHA glass (IV) is obtained assuming an ϵ value of 2934. Percent cleavage based on UV is ca. 50%. The ϵ value of 2934 was determined for the model compound *o*-aminophenol. For *o*-aminophenol; λ_{max} = 269 nm at pH = 1 (H_2O - HCl) and 283.5 nm at pH = 7 (95% EtOH).

Antioxidant Activity

Oxidations of lard were performed using the Active Oxygen Method of Wheeler (9) as modified by Eastman Chemical Products, Inc. (10). Basically, a typical test included exposing 0.95 g of BHA-glass (IV) and 20 g of lard at 95 C ± 1 to a constant stream of air in a standard aeration tube.

Analysis of Lard

The molecular weight of the fresh lard employed was 840 by osmotic pressure. Analysis: C, 72.02; H, 12.22; N, 0.11. Lard oxidized to a peroxide value > 500 gave a molecular weight of 895.

Oxidized BHA Glass

BHA-glass which had been used in the above test was washed with a series of solvents followed by Soxhlet extraction for several days first with $CHCl_3$:CH_3OH (95:5) and then with CH_3OH:$CHCl_3$ (10:1). Analysis: C, 5.9; H, 0.82; N, 0.65.

RESULTS AND DISCUSSION

The BHA-glass (IV) was prepared by the sequence outlined in Figure 1. The proposed structure for IV is based on the following evidence: (a) the reactions yielded a brightly colored (red) material typical of a diazo compound; (b) the C,N microanalyses were consistent with compounds I-IV with a coating of 0.16 meq of organic ligand per gram of IV; and (c) treatment of IV with sodium dithionite, known to cleave diazo linkages (8), yielded a glass residue which is light yellow in color. Further, the residue has a C,N analysis, indicating at least 55% of the bonded V had been cleaved and gave a positive primary amine color test. UV data for the cleaved material gave a $\Delta\lambda_{max}$ of 20 nm over a pH range of 1-7, which was consistent with compound V and in addition indicated 50% of the bonded V had been cleaved.

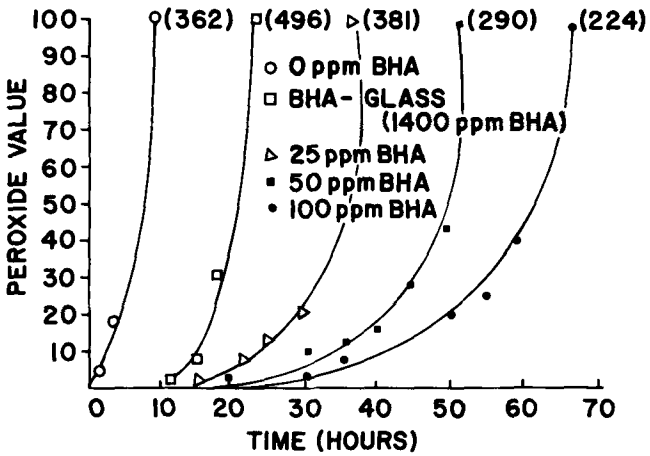


FIG. 2. Peroxide value vs. time:oxidation of BHA-glass and controls in lard.

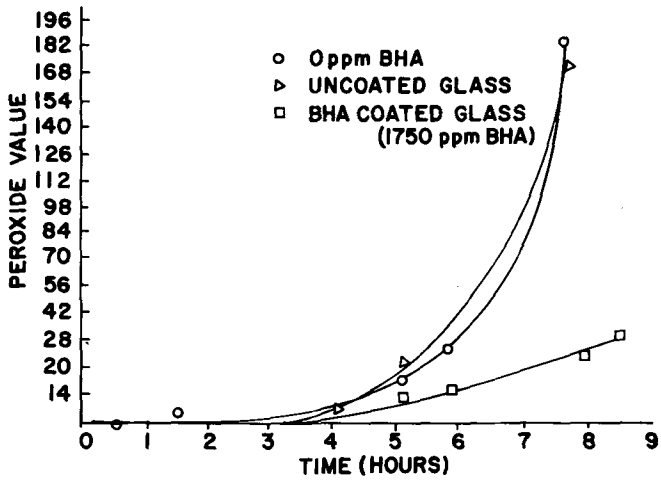
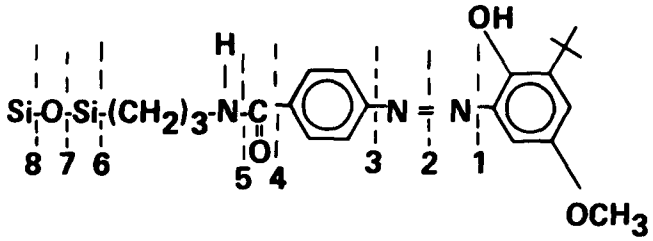


FIG. 3. Peroxide value vs. time:oxidation of selected samples in lard.

Sherwin et al. (11) have shown that lard is readily stabilized by BHA. Therefore, the BHA glass was tested for antioxidant activity in lard using the AOM method. Good agitation was maintained in the heterogeneous glass-lard system, since the air stream was directed to the bottom of the standard aeration tube (12). It can be seen in Figure 2 that bonded BHA is much less effective than free BHA on an equal BHA concentration basis. Thus, a sample of BHA glass (IV) added to lard to yield a BHA concentration of 1400 ppm (4.8×10^4 ppm of the coated glass) was less effective than 25 ppm of free BHA but had considerably greater activity than no BHA. It should be noted that even though the activity is low relative to free BHA, the test conditions are stringent and in many cases a relatively low level of antioxidant is required to preserve a liquid under normal storage conditions.

Figure 3 shows the comparative antioxidative effect in lard of BHA-glass (IV), uncoated porous glass and a control with no BHA. It can be seen that lard containing uncoated porous glass gave the same effect as lard without BHA, in contrast to the BHA-glass which afforded some protection. In addition, the oxidized BHA-glass used in the initial experiment was filtered from the lard, washed with chloroform and shown to have very little activity when compared to fresh BHA-glass in lard. Similarly, a sample of arylnitro



FORMULA A.

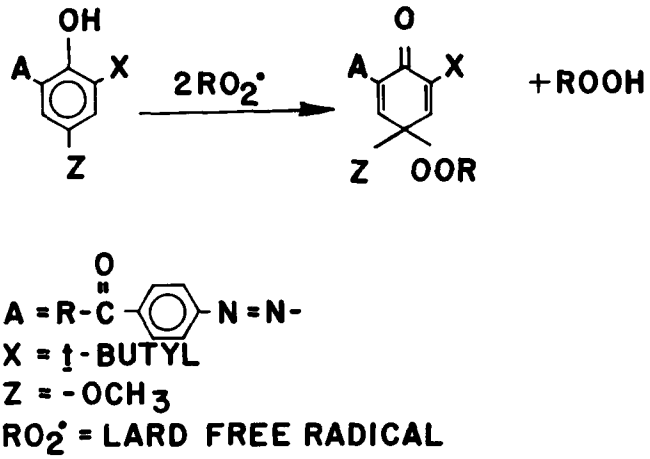


FIG. 4. A mechanism for BHA reaction with lard.

glass (II) showed very little activity.

It was necessary to determine if enough BHA was leached from the glass during testing to account for all or some of the observed activity. This hypothesis was tested by analysis of BHA-glass. Conceivably, the organic side chain could cleave from the bonded BHA-glass (IV) at various points as shown in A, and the cleavage fragments containing BHA would be expected to react similarly to free BHA.

In model experiments it was found that BHA did not survive the stringent test conditions. It was thus felt that analysis of the lard for BHA or fragments from A would be useless. It was therefore decided to analyze the glass itself.

Boozer et al. (13) and Pospisil (14) proposed an antioxidant mechanism for a phenolic antioxidant which involves the covalent trapping of alkyl radicals. It has also been proposed by Stuckey (16) that the same mechanism operates with fatty acid radicals (lard triglyceride peroxide radicals in this case) as shown in Figure 4. The BHA-glass that had undergone two oxidations in lard was cleaned with solvent prior to cleavage with dithionite. No cleavage occurred, either because the glass contained a lard residue, or more likely, because it was covalently bonded to the lard triglyceride as noted above.

Since the oxidized beads did not associate with water, they must be associated with a very hydrophobic residue. However, because derivatives I-IV associate with water, they behave like free-flowing sand in water settling to the bottom, whereas oxidized IV clumped and partially floated indicating very little water association. All the materials I-IV and oxidized IV are readily dispersible in polar organic solvents. Analysis of the trace organic residue from the attempted dithionite cleavage showed no nitrogen indicating no BHA-NH_2 (V) cleavage. The C analysis of the glass was high as expected. The N analysis, after correction for increased C, was very close to starting material indicating that if measurable cleavage had occurred during oxidation it was at the unlikely position 1 (see A).

It was conclusively shown that measurable cleavage did not occur at positions 2-8 since the C, N analysis remained unchanged following an exhaustive Soxhlet extraction with various ratios of chloroform-methanol solutions. This indicated that some of the triglyceride peroxy radicals are bonded to the BHA-glass. It is also doubtful that significant bond cleavage occurred at the 1-8 positions (A) because BHA-glass retained its red color after two oxidation tests in lard. If it is assumed that no BHA was cleaved during testing, that all the increase in carbon in the BHA-lard derivative is due to lard and that only one triglyceride peroxide radical is attached to one BHA molecule, then calculations from microanalytical and molecular weight data show ca. 20% of the attached BHA is substituted by lard triglyceride peroxide. This value is based on the partial lard analysis. A fatty acid analysis of the lard was not performed since variation in the fatty acid composition within the limits found in lard (15) or a selective substitution of a particular fatty acid does not significantly change the 20% substitution. The substitution indicates that BHA functioned at least partially in its normal manner and remained substantially bonded to the glass. This finding is important since the activity of the BHA-glass is so low that the method of analysis (microcombustion) would not detect the loss of small amounts of BHA which would be necessary to effect the observed activity. It should be noted, however, that the carbon increase in the oxidized glass could be explained by other mechanisms. The present data do not allow a choice between the fact that BHA-glass is a poor antioxidant under these test conditions or that BHA-glass is inactive and the activity is due to BHA fragments generated in the testing.

The low activity of the BHA-glass can be at least partly explained. It has been reported (16) that in very sterically hindered phenols, such as the BHA-glass, antioxidant activity is lowered. Finally, much of the bonded BHA may be unavailable to the very viscous lard. That is, the lard may not freely traverse the pores of the glass powder where a very large fraction of the BHA resides and therefore, this material may find greater utility in liquids of lower viscosity.

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