Drug Development and Industrial Pharmacy, 33:683–689, 2007

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040601012890

informa
healthcare

A Rapid Technique to Evaluate the Oxidative Stability of a Model Drug

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ABSTRACT The objective of the current study was to investigate the oxidative induction time (OIT) as a measurement of the stability of an oxygen-sensitive model drug. The OIT was determined by differential scanning calorimetry and represents the time required for oxidative decomposition to occur at a given temperature. Samples were heated to a specific temperature under a nitrogen blanket then held isothermal while exposed to oxygen. The experiment proceeded until oxidative degradation of the sample was apparent from the real-time heat flow graphs. Variables investigated in this study included different lots and suppliers of a model drug as well as the addition of antioxidants. Results demonstrated that the stability of the drug was dependent on the supplier. All antioxidants investigated in this study improved oxygen stability of the model compound, as evidenced by a longer OIT. Butylated hydroxyanisole (BHA) was found to better stabilize the drug than butylated hydroxytoluene at equivalent concentrations. The combination of ascorbic acid and BHA provided the greatest protection against oxidation of the model compound. The results of this study demonstrate the usefulness of OIT to investigate the oxygen stability of pharmaceutical compounds.

KEYWORDS Oxidation, Degradation, Stability, Antioxidants, Differential scanning calorimetry (DSC), Oxidative induction time (OIT)

INTRODUCTION

Oxidative degradation of active pharmaceutical ingredients (APIs) in solid and liquid formulations has been widely reported in the literature (Townsend et al., 1990; Khossravi & Borchardt, 1998; Farmer et al., 2002). Decreased potency of the drug and a reduction in the shelf-life of the product can occur as a result of this degradation process (Monkhouse, 1984). Oxidative decomposition can also produce changes in the dissolution rate and cause discoloration of the dosage form (Templeton et al., 2002).

Oxidation involves the removal of electrons from a substance, involving the loss of hydrogen or addition of oxygen. The most common type of oxidative degradation is considered autooxidation, a reaction that involves the API reacting with molecular oxygen through a 3-step, free radical-mediated process:

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1. Initiation (free radical generation): $RH \rightarrow R\bullet$
2. Propagation (reaction with oxygen): $R\bullet + O_2 \rightarrow ROO\bullet$
   $ROO\bullet + RH \rightarrow ROOH + R\bullet$
3. Termination (reaction between 2 radicals): $2\,R\bullet \rightarrow$ molecular products

These processes can be quite complex and are often catalyzed by light, temperature, hydrogen ion concentration and trace metal ions (Skiba et al., 2000; Hong et al., 2004). These catalysts can reduce the time for initiation and increase the rate of oxidation (Kumar et al., 1992; Khossravi & Borchardt, 1998). Impurities in pharmaceutical excipients, such as peroxides, can act as free radical initiators and also accelerate the oxidative degradation process (Hartauer et al., 2000).

A number of approaches have been taken during development of pharmaceutical products to improve chemical stability and prolong product shelf-life of drugs susceptible to oxidation. The obvious approach during processing is to reduce oxygen levels and minimize contact of the API with oxygen. For example, processing under nitrogen and minimizing container headspace may improve the stability of oxygen sensitive drugs, although removal of all oxygen present in a container is quite difficult and trace amounts may be sufficient to initiate oxidation (Hovorka & Schoneich, 2001). Processing at low temperatures may also help to enhance chemical stability. Polymeric film coatings can be used to reduce the rate of oxygen permeation into the dosage form (List & Kassis, 1982; Felton & Timmins, 2006). In addition, special packaging has been developed to minimize oxidative degradation (Waterman & Roy, 2002). Since contact with aqueous environments may catalyze oxidative processes, switching coating or granulating systems to organic solvents may improve stability. The use of organic solvents, however, presents its own set of environmental and safety issues (Obara et al., 1999) and may not be able to be implemented in all pharmaceutical firms. A very practical method to prevent or retard oxidation is by the inclusion of antioxidants in the formulation (Waterman et al., 2002). Antioxidants can decrease localized O_2 concentrations, scavenge free radicals, quench singlet O_2, bind metal ions, and remove peroxide. Antioxidants can be classified as primary (free radical terminators), secondary (oxygen scavengers) or tertiary (chelating agents). Caution must be used when selecting the amount and type of antioxidant as, in excess, these molecules may catalyze or directly initiate oxidation of the API through complex mechanisms. Regardless of the approach taken, a rapid screening tool to evaluate the effectiveness of the treatment to stabilize an oxygen-sensitive compound would significantly aid the pharmaceutical scientist in the development of these types of compounds.

The current study investigated the potential use of differential scanning calorimetry with oxygen as the purge gas to quantify the oxidative induction time (OIT) as an analytical screening tool to rapidly predict the stability of an oxygen sensitive API. With this technique, a solid sample is placed in an open pan and heated to a specific temperature, generally several degrees below the melting temperature of the sample, under a nonreactive purge gas. The temperature is held isothermal and oxygen is pumped into the environment. As the sample undergoes degradation, the instrument measures a characteristic change in heat flow. The OIT is then calculated using appropriate analytical software. This technique has been used in other industries to study oxidative processes (Phease et al., 2000; Rosa et al., 2000) and was shown to have a good correlation with conventional oxidative stability index (OSI) measurements for edible oils (Tan et al., 2002). The objective of the current study was to investigate the oxidative degradation of a model oxygen-sensitive drug alone and in the presence of antioxidants using differential scanning calorimetry.

MATERIALS AND METHODS

An oxygen-sensitive model drug containing conjugated alkene moieties was purchased from two different suppliers, A and B. The antioxidants used in this study included butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and ascorbic Acid. These materials were purchased from Spectrum Chemicals (Gardena, CA) and were used as received.

Oxidative Induction Time

The optimal temperature for testing the oxidative degradation of the model API was first determined. Approximately 5–10 mg of the API was placed in open aluminum pans and the pans set in the differential

scanning calorimeter cell (TA Instruments Model 2920, New Castle, DE). Nitrogen was used as the purge gas. The samples were equilibrated to 10°C and then heated at 10°C/min to 110, 120, or 125°C. After 5 min at isothermal conditions, the purge gas was switched to oxygen and the heat flow was recorded. The experiment proceeded until oxidative degradation of the sample was apparent from the real-time heat flow graph. The procedures were repeated without switching the purge gas to oxygen to ensure that the transitions noted in the thermograms were due to oxidation and not thermal degradation. The OIT of the API was determined using TA Instruments Universal Analysis software. Based on the overlay plot of the thermograms showing the OIT at the different hold temperatures (Fig. 1), 125°C was selected as the optimal isothermal hold temperature for subsequent experimentation. This temperature provided a relatively fast OIT and would likely allow differences in oxidative degradation to be determined in a timely manner. Three samples were tested for each formulation.

Statistical Analysis

Statistical analysis was carried out using SigmaStat 3.0 software (SPSS, Inc., Chicago, IL). For statistical comparison, a student *t*-test was used to compare two groups and one-way analysis of variance test was applied to compare more than two groups with a pair wise multiple comparison Holm-Sidak post-test was used to determine differences between groups. A $p < 0.05$ was considered statistically significant.

RESULTS

Comparison of Sources of API

The OIT of three API samples (two lots from source A and one lot from B) was determined and the data are presented in Fig. 2. Statistical evaluation of the data demonstrated no difference in OIT for the two lots of API from supplier A ($p=0.288$). However, the lot from supplier B exhibited a significantly longer OIT than the API from the A lots ($p < 0.001$). According to the Certificate of Analysis, the A source contained not more than 0.1% BHT whereas 0.008–0.012% (80–120 ppm) BHA was included in the B product. These data suggest that BHA, even at lower concentrations, is more effective than BHT in stabilizing the API.

Influence of BHA Concentration on Oxidative Protection

To quantify the oxidative protection provided by BHA, the OIT of the model API from mixed samples was determined. The BHA was ground in a mortar and pestle, passed through a 40 mesh screen, and manually mixed with the API in a scintillation vial. To reduce error due to nonuniform blending, a 5 wt. % BHA mixture was prepared and aliquots of this powder were further diluted with the API. Three samples from each blend were tested and the data are presented in Table 1. A small addition of BHA significantly prolonged the OIT of the drug. Moreover, at concentrations between 0.2 and 2 wt. %, a linear

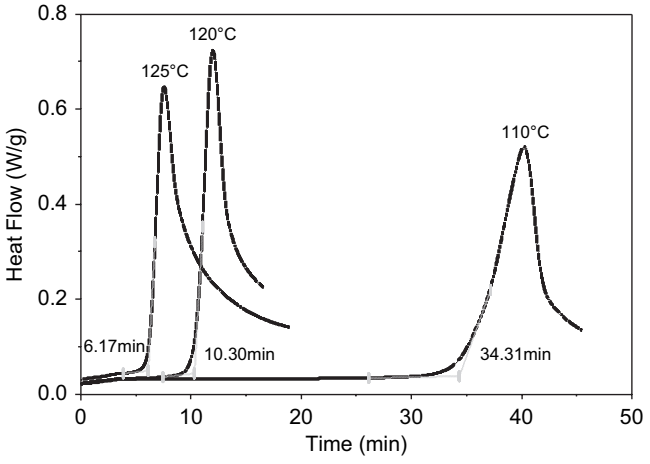

FIGURE 1 **Overlay Plot of the Thermograms Showing OIT of the Model Drug as a Function of Hold Temperature.**

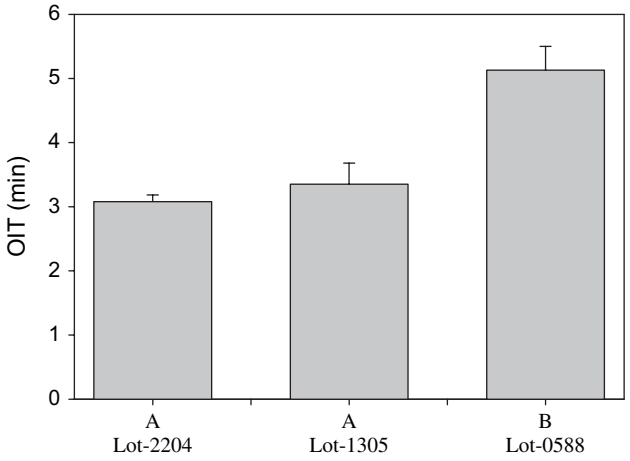

FIGURE 2 **OIT of the Model Drug from Various Lots and Suppliers.**

TABLE 1 **Influence of BHA Concentration on the Oxidative Protection of the Model Drug. API Raw Material from Source A, Lot-1305, and Containing < 0.1% BHT**

BHA added to API (wt. %)	Average OIT(min)	Standard deviation
0	3.35	0.33
0.2	11.65	0.58
0.5	16.07	1.57
1	23.73	1.50
2	36.38	2.38
5	> 180	0

relationship (R^2=0.998) between BHA concentration and the OIT was evident, as seen in Fig. 3. At 5 wt. % BHA, no oxidative degradation occurred within the 3 hr test period. The linear regression model had predicted an OIT time of around 80 min and thus suggests that the linearity between stabilizer concentration and OIT is limited.

Influence of BHT on Oxidative Protection

BHT was ground using a mortar and pestle and manually mixed in a scintillation vial with the drug at a concentration of 2 wt. %. The average OIT was 10.14 min, with a standard deviation of 3.23. The high deviation was attributed to error introduced by mixing very small quantities of powders, likely producing a nonuniform blend. Comparison of the OIT for the BHT and the BHA mixtures revealed a statistically significant difference ($p < 0.001$), indicating that BHA is a better stabilizer for the API than BHT, as seen in Fig. 4. These data are in agreement with the data comparing supplier, where the more stable product contained BHA.

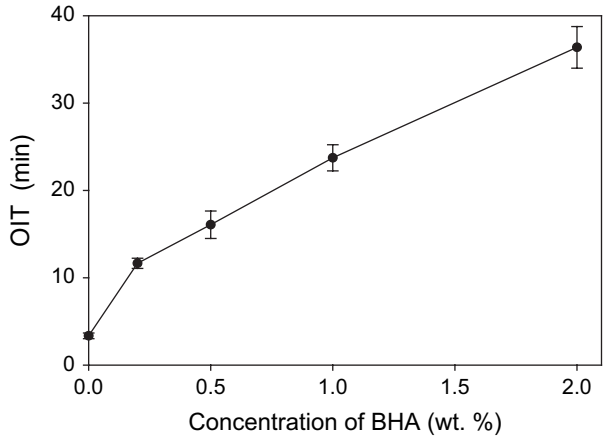

FIGURE 3 **Relationship Between BHA Concentration and the OIT of the Model Drug. API Raw Material from Source A, Lot-1305, and Containing < 0.1% BHT.**

Effect of Combined BHA and BHT on Oxidative Protection

BHT was added to the well-blended BHA/API mixtures and analyzed for OIT. The concentrations investigated were 1 wt. % BHA/1 wt. % BHT and 2 wt. % BHA/2 wt. % BHT. The results of these experiments are shown in Fig. 4. The addition of the BHA/BHT combination significantly prolonged the OIT in comparison to the API raw material ($p < 0.001$) and higher concentrations of the stabilizers further improved the stability of the drug against oxidation. However, statistical comparison of the OIT from the BHA/BHT (2 wt. % each) to the 2 wt. % BHA only blend found no significant difference between the two groups ($p > 0.05$).

Influence of Ascorbic Acid on Oxidative Protection

The influence of ascorbic acid alone and in combination with BHA on the OIT of the API was determined and these data are presented in Fig. 5. Both the ascorbic acid and BHA were ground in a mortar and pestle and then passed through a 40-mesh sieve. The

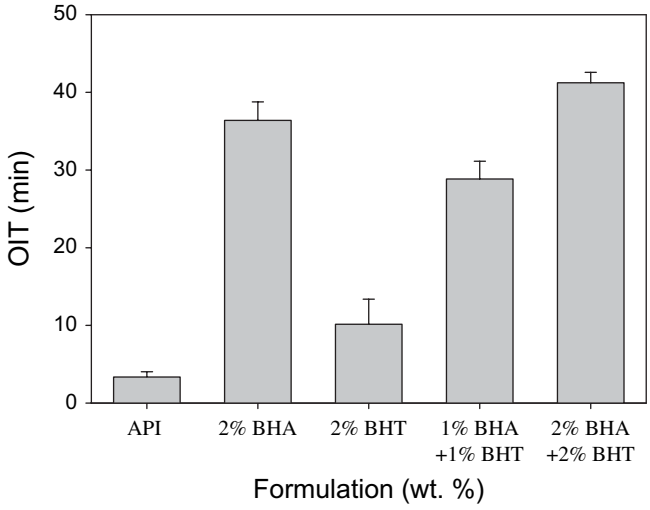

FIGURE 4 **Influence of BHA and BHT on OIT of the Model Drug. All Reported Concentrations of Antioxidants are in Addition to that in the API Raw Material (Source A, Lot-1305, < 0.1% BHT).**

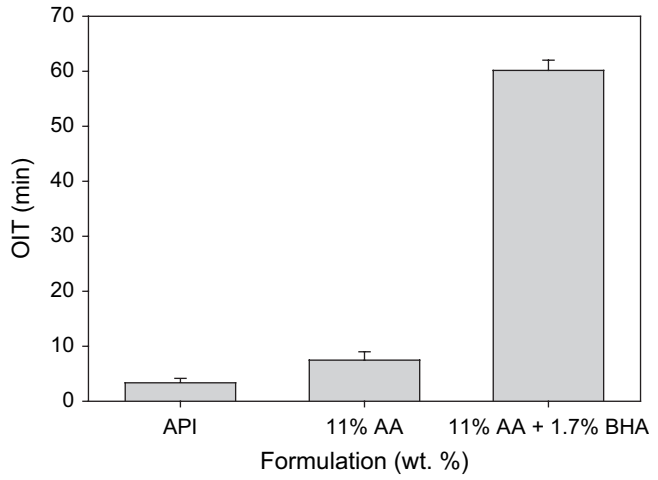

FIGURE 5 **Influence of Ascorbic Acid on the OIT of the Model Drug. API Raw Material From Source A, Lot-1305, and Containing < 0.1% BHT.**

API was also passed through a 40-mesh screen. The powders were then weighed, placed in a scintillation vial and manually mixed. The concentration of the ascorbic acid was 11 wt. % and this value was based on a desired level of ascorbic acid in the final drug product. The addition of ascorbic acid was found to provide some protection against oxidation (3.35 min drug versus 7.47 min when ascorbic acid was added, $p=0.01$). However, BHA exhibited superior oxidative protection. Using the data in Fig. 3, linear regression ($R^2=0.998$) predicted an OIT of 32.58 min for a mixture of drug plus 1.7% BHA while measured data for the 2% BHA mixture exhibited an OIT of 36.38 min. Interestingly, the combination of ascorbic acid and BHA demonstrated significantly enhanced protection against oxidation in comparison to the 2 wt. % BHA mixture (60.15 min versus 36.38 min).

DSC Analysis of the Antioxidants

BHA, BHT, and ascorbic acid were subjected to DSC testing to evaluate their susceptibility to oxidation and to ensure that the thermal transitions determined in these experiments were due solely to oxidation of the API, BHA and BHT were evaluated for 3 hr at 110, 120, and 125°C under both nitrogen and oxygen purge; ascorbic acid was tested for 3 hr at 125°C under oxygen purge. No peaks were noted during these test periods at all conditions investigated, supporting our assumption that the OIT observed during DSC experimentation was due to the oxidative degradation of the API.

DISCUSSION

Many drugs have been reported to be susceptible to oxygen degradation, with conjugated alkenes, aromatics, ethers, thioethers, and amines being the likely functional groups to react with oxygen (Hovorka & Schoneich, 2001). One simple and straightforward method to improve stability of oxygen sensitive materials is the addition of antioxidants. As mentioned previously, antioxidants can be classified as free radical terminators, oxygen scavengers and chelating agents. The chelating agents, such as citric acid and EDTA, complex with metal ions present to essentially sequester these catalysts. Oxygen scavengers consume oxygen and can be effective without direct contact with the drug. Free radical terminators possess higher oxidative potentials than the drug and disrupt chain propagation by reacting with the free radical to terminate the reaction. The effectiveness of free radical terminators can be predicted based on the difference in redox potential (the thermodynamic tendency to lose an electron) between the drug and the antioxidant. In complex pharmaceutical systems, however, electrometric measurements rarely accurately predict antioxidant efficacy (Waterman et al., 2002). Real time or accelerated stability testing of the drug product is often undertaken to screen antioxidants, a process that can add months to the development timeline (Waterman & Adami, 2005). The current study demonstrated the usefulness of the OIT as a rapid predictor of antioxidant effectiveness of a model oxygen sensitive drug. This DSC technique may also be suitable to evaluate the oxygen sensitivity of new chemical entities.

Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are common antioxidants used in the pharmaceutical, cosmetic, and food industries (Rowe et al., 2006). In the current study, simple binary mixtures of the API and antioxidant were used. Results from this study demonstrate that BHA is a more effective stabilizer for the API than BHT. These results are likely due to the differences in the chemical structures of the two compounds, with the two tert-butyl functional groups of the BHT providing steric hindrance of the hydroxyl and reducing its ability to interact with free radicals. These results are in agreement with

Nash, who suggested that the more sterically hindered antioxidant would be less susceptible to oxygen attack (Nash, 1958).

Ascorbic acid is also considered a free radical scavenger. Our results, however, demonstrate that this antioxidant was not as efficient as either BHA or BHT in API stabilization. Moreover, significantly higher levels of ascorbic acid (11%) were used in comparison to the other antioxidants. It should be noted that ascorbic acid is a relatively polar compound and likely separated from the model API, creating polar microenvironments; hence ascorbic acid was not as readily available to react with free radicals. These results suggest that free radical scavenging antioxidants must be in intimate contact with the active in order to be effective. Interestingly, the combination of ascorbic acid and BHA provided a synergistic effect, producing the greatest level of stabilization of the API for all antioxidants investigated in the current study. These results are in agreement with previously published studies that showed synergistic antioxidant activity when ascorbic acid was combined with other more hydrophobic antioxidants (Packer et al., 1979; Szymula, 2004). The proposed mechanism of action for this synergistic antioxidant activity is the ability of ascorbic acid to interact with and regenerate other antioxidants. In the current study, it is possible that ascorbic acid was able to react with the BHA radical to essentially regenerate BHA.

The current study investigated simple mixtures of a model API and several antioxidants to demonstrate the usefulness of OIT as a rapid screening tool to evaluate oxygen stability of pharmaceutical compounds. However, as with most analyses, testing parameters can significantly impact results. For example, the ratio of surface to volume of the sample has been shown to influence measured OIT (Tan et al., 2002). In another study, Rosa and coworkers found that particle size significantly affected the OIT of high density polypropylene (Rosa et al., 2000). The particle size of the API was not characterized nor controlled in the current study. The physical state of the mixtures was also not a focus of this study yet could impact OIT and oxygen stability. Both BHA and BHT were in the melt state at the testing temperature of 125°C, presumably in much more intimate contact with the API than solid-state ascorbic acid. It should be noted that the stability of the API in the final dosage form is of primary concern and thus OIT experiments should be considered only as a screening tool. Excipients, processing conditions, dosage form, and packaging may significantly influence the API stability of a drug product (Hartauer et al., 2000; Waterman et al., 2002).

CONCLUSIONS

The current study demonstrated that conventional DSC with gas switching capabilities provides a rapid method to predict stability of oxygen sensitive APIs. Stability of the API was dependent on the supplier, with the B product being more stable than the A. The addition of all antioxidants investigated improved the stability of the model drug against oxidation, as demonstrated by an increase in the OIT. BHA was found to better stabilize the drug than BHT at equivalent weight concentrations. The addition of increasing amounts of BHA provided increased protection against oxidation, and this relationship was linear between 0.2 and 2 wt. % BHA. The combination of BHA and BHT also improved the stability of the drug compared to the control (API raw material) and higher concentrations of the two stabilizers further prolonged the OIT. Of the formulations investigated, the combination of ascorbic acid and BHA provided the greatest protection against oxidation.

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