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A NEW ACCELERATED OXIDATIVE STABILITY TEST FOR GLASS-FORMING ORGANIC COMPOUNDS *

JOHN P. ELDER

Merck & Co. Inc., P.O. Box 2000, Rahway, NJ 07065-0900 (U.S.A.)

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ABSTRACT

A new accelerated thermo-oxidative test has been developed for use with crystalline organic compounds which solidify to a stable vitreous solid from the melt. The principle of the method is based on the fact that the glass transition temperature of the solid is linearly dependent upon the prior oxidative history of the sample. The fixed time, isothermal DSC oxidative stress test is described, and examples of its use with a pharmaceutical compound are given. Results for material which has (a) been subjected to long-term, above-ambient thermo-oxidative ageing, and (b) undergone extended room temperature shelf-life testing, will be presented. The advantages of the procedure over conventional methods will be discussed.

INTRODUCTION

The traditional procedure whereby the shelf-life of solid pharmaceutical compounds is assessed is to stress them isothermally in air at a suitably chosen elevated temperature for different periods of time. The partially oxidized drug is then analyzed for the active component, usually by HPLC or TLC. Nash [1] has discussed these accelerated stability tests, and the various computerized statistical treatments of the results. Non-isothermal methods are not widely used. One can fit both isothermally or non-isothermally derived reaction kinetics data to an Arrhenius expression. However, caution must be exercised in employing the resulting kinetics parameters to predict behavior at room temperature. To minimize errors, the temperature interval over which the extrapolation is made should be kept as small as possible. Thus, one is limited in the selection of the isothermal stress temperature. If it is too high, one has the extrapolation problem. Moreover, the test sample may be over-oxidized, which may affect the active component analysis. The optimum temperature may be such that the test runs for a

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number of weeks, sometimes extending to several months. The term accelerated thereby becomes somewhat of a misnomer.

One of the widely used means for assessing the relative oxidative stability of such materials as lubricants and polymers is by employing the thermoanalytical techniques, DTA or DSC. The time to onset of the characteristic sample–reference temperature difference or differential heat flow signal excursion due to the exothermic reaction is monitored under isothermal conditions [2]. Such a procedure is potentially applicable to pharmaceuticals, or indeed any solid organic compound. It has the added benefit of limited sample oxidation, since the experimental program can be interrupted once the exotherm onset is recognized, either by the operator or by micro-processor controlled instrumentation. As an analytical technique, the onset time should not be too long; a 0–60 min range is acceptable. However, this does require that the isothermal stress temperature be carefully selected. This can be tedious in pharmaceutical development, since in optimizing the processing conditions for the production of a new compound having maximum oxidative stability, it may well be that the stress temperature will have to be raised several times.

If an easily measured physical characteristic of a material can be shown to be highly affected by oxidation, then the possibility of developing a new accelerated stress test for oxidative stability presents itself. Such a thermophysical characteristic has been found for an important pharmaceutical compound and an analog compound; namely, the glass transition temperature exhibited by the vitreous solidified melt, prior to its thermally stimulated recrystallization. Since many other compounds, pharmaceutical or otherwise, may exhibit similar behavior, it is considered important to describe the procedure in detail. It was initially developed to assess the oxidative stability of Lovastatin, the cholesterol biosynthesis-inhibiting drug. Salient features of the thermal and thermo-oxidative behavior of Lovastatin have been described [3]. In this paper, further details of the accelerated isothermal stress-glass transition temperature measurement procedure for assessing oxidative stability are presented.

PRINCIPLE OF THE METHOD

The test material is isothermally stressed in an open crucible in a flowing air environment in a DSC cell for a relatively short time, namely 45 min. The isothermal temperature is chosen such that the extent of oxidation in this time interval is small. A useful rule is to set the temperature at $\sim 10\,^{\circ}C$ below the onset of the exothermic oxidation at a dynamic heating rate of $10\,^{\circ}C\ min^{-1}$. Although not necessary, the DSC signal is usually monitored during the stress period. At the conclusion of the stress, the system is flushed with nitrogen to remove all traces of air from the furnace environment. The

sample is then held isothermally at or slightly above the melting point of the compound for ~ 2 min to ensure complete fusion. The melt is then rapidly cooled to a temperature ~ 20 °C below the glass transition temperature region. This region is then scanned dynamically in the usual manner. The sample temperature T_{g2} at the mid-point of the characteristic DSC record is used as an empirical measure of the relative oxidative stability of the sample.

EXPERIMENTAL

All heat flow measurements were made using the Mettler TA 3300 system, using the −160 °C to +600 °C furnace. For analytical purposes, 8–10 mg sized samples in the standard 40 µl aluminum crucibles were used. For preparative purposes, e.g. to provide sufficient material for HPLC or TLC active component analyses, 30–40 mg sized samples in the tall form, 150 µl crucibles were employed. Samples, randomly selected from the storage container, are loosely placed in the tared, open crucible, which is then weighed and transferred to the DSC cell at the selected isothermal temperature, and the experiment started. For Lovastatin, based upon its known thermal characteristics, the standard stress test is 130 °C/45 min. The start/end temperatures for the glass transition temperature measurement at 10 °C min^{-1} are −20 °C and 150 °C, respectively.

RESULTS AND DISCUSSION

As previously shown by non-isothermal TG measurements [3], generally there is a < 1% weight gain when Lovastatin is fully oxidized in air, followed by a sublimative loss of oxidation products at ~ 0.1% min^{-1}. Repeated measurements have shown that, during the 45 min isothermal stress and subsequent 2 min isothermal melt under nitrogen, significant weight changes do not occur.

Figure 1 shows typical oxidative stress data at 130 °C, a and b, and the resulting glass transition curves, c and d, for two samples, 1 and 2, of milled Lovastatin, prepared during the early stages of process development. The greater the extent of oxidation, as evidenced by the magnitude of the exothermic signal, the higher the glass transition temperature T_{g2}, as shown.

Figure 2 demonstrates how the character of the glass transition region changes with increase in extent of oxidation. In this case, sample 1 was oxidatively stressed at 130 °C for 5 min and 35 min, curves 1 and 2, respectively. Although ΔC_p is essentially the same in both cases, namely 0.38 J $g^{-1}K^{-1}$ and 0.37 J $g^{-1}K^{-1}$, which further confirms that weight changes do not occur, T_{g2} increases from 25.6 °C to 29.9 °C.

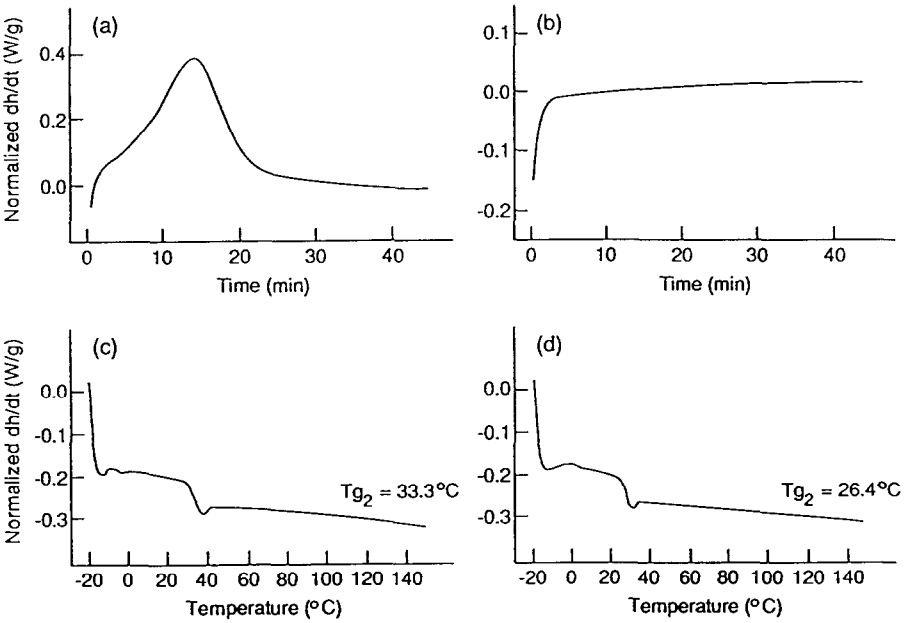

Fig. 1. Typical 130 °C oxidative stress and glass transition curves for milled Lovastatin samples: a and c, 1; b and d, 2.

A series of such variable time period stress tests at 130 °C were performed in triplicate. Two of the samples were used for duplicate HPLC assays of the residual Lovastatin content, and the third employed for the normal glass

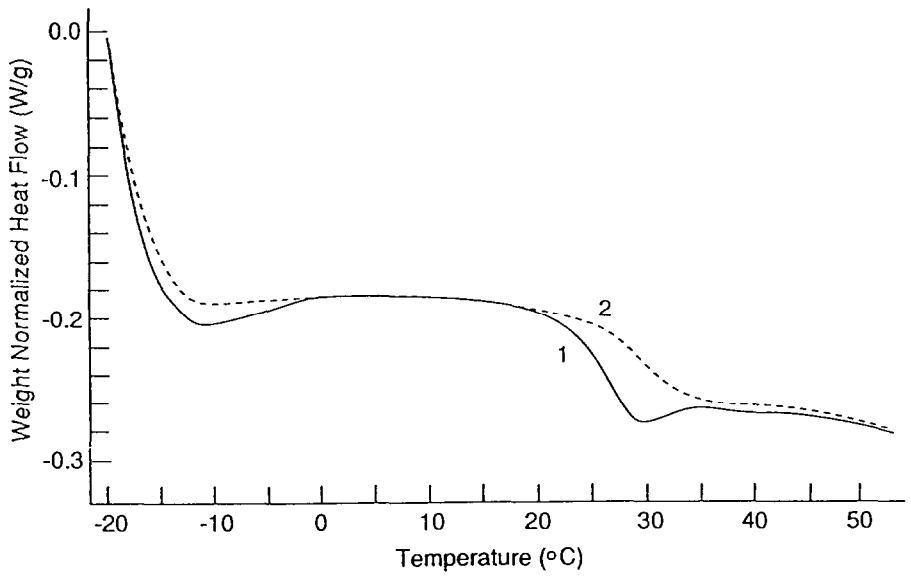

Fig. 2. Variation of the glass transition with the extent of oxidative stress in air at 130 °C of milled Lovastatin, sample 1: 1, 5 min; 2, 35 min.

TABLE 1

Thermo-oxidatively stressed Lovastatin glass transition temperature—HPLC assay data

Stress time at 130 °C (min)	T_{g2} (°C)	HPLC assay (%)
0	25.2	–
5	25.6	96.5, 96.6
10	26.1	93.6, 94.0
13	26.9	88.8, 90.1
15	27.6	81.0, 82.0
17	28.5	75.1, 78.7
20	29.5	67.7, 67.9
25	29.7	66.7, 67.6
30	29.9	66.6, 66.1
35	29.9	66.0, 66.1

transition temperature measurement. The results are given in Table 1. Both the T_{g2} and the HPLC assay data show a sigmoidal variation with stress time, and conform to eqn. (1) with a 0.997 correlation coefficient

$$T_{g2} = 38.7 - 0.14\text{HPLC}\ (\%) \quad (1)$$

Lovastatin samples with T_{g2} values of 25–26 °C, 27–29 °C and > 29 °C are referred to as of high, medium and low relative oxidative stability. As can be seen from Table 1, the greatest change in the extent of oxidative degradation and the T_{g2} values with time occurs in the second 10 min period of the isothermal stress. The greatest variation in T_{g2} is expected in the 27–29 °C range, and thus medium stability material is also expected to show the lowest measurement precision.

In order to assess the precision of the method, a milled pilot plant sample of Lovastatin was used as feed material for two different recrystallization procedures, producing more stable material. Ten consecutive standard stress tests were made on each of these products, designated to be of low, medium and high stability. The T_{g2} data are given in Table 2. As can be seen, even in the case of the medium stability samples, where the spread of the measured values is expected to be the largest, the precision is excellent.

This simple procedure has proved extremely useful in assessing the room temperature, long-term stability of Lovastatin. Table 3 lists T_{g2} values, following application of the standard stress for three different pilot plant, milled, recrystallized samples. Sample 65.6A initially appeared to be of high stability. However, over the 11 month period of the room temperature shelf-life test, its ability to withstand the accelerated stress decreased, as shown by the ever-increasing T_{g2} value. On the other hand, both the other recrystallized samples, although initially appearing to belong to the medium stable category, essentially retained their original ability to withstand the oxidative stress.

TABLE 2

Accelerated isothermal stress test precision study standard 130 °C/45 min stress: standard aluminum crucibles

High stability		Medium stability		Low stability	
Weight (mg)	T_{g2} (°C)	Weight (mg)	T_{g2} (°C)	Weight (mg)	T_{g2} (°C)
9.29	26.37	8.85	29.05	9.89	32.85
10.35	26.33	8.68	28.57	9.93	32.52
9.98	26.20	9.74	28.17	10.26	32.51
10.10	26.35	9.56	29.34	10.42	32.49
9.21	25.89	8.78	28.56	10.24	33.33
9.91	25.67	10.01	27.84	9.50	33.20
10.17	25.67	9.68	28.01	11.00	33.29
8.75	25.87	9.62	28.04	10.13	32.70
9.75	26.20	10.51	27.84	10.56	32.64
10.77	26.32	10.17	28.01	9.98	32.68
Mean value	26.09		28.34		32.82
Standard deviation	0.28		0.52		0.33

It should be emphasized that, like the time to exotherm onset isothermal DSC measurement, it is a relative and not an absolute oxidative stability assessment procedure. One cannot predict shelf-life from such measurements. However, for Lovastatin, a good correlation has been obtained between the results of this accelerated stress test and those obtained by 80 °C long term oven tests, which are, in many cases, used as predictors of shelf-life. As a relative oxidative stability test however, it has proved most useful in assisting in the optimization of processing conditions. This simple, rapid and reproducible method enables one to gauge the advantages, disadvantages and limitations of a wide range of material purification procedures. In this regard, it should be observed that well over 500 tests have been performed on samples of Lovastatin and an analog compound in the last two and a half years.

TABLE 3

Long-term stability assessment of pilot plant Lovastatin

Age (months)	T_{g2} values		
	65.6A	65.6B	66.6
0	26.2	27.4	28.4
0.75	27.1	26.3	28.3
2.0	28.4	27.0	29.1
3.5	28.7	26.5	28.1
7.0	29.1	27.1	28.9
11.00	30.0	27.8	28.4

As process optimization yields increasingly more stable material, often the T_{g2} values of samples, prepared by similar procedures, are too close. In such a case, raising the stress temperature by 1–5 °C usually enables a conclusive differentiation of the relative oxidative stability of the several samples. In this regard, it should be pointed out that the glass transition temperature measurement method has one important advantage over the time to exotherm onset method for the type of crystalline compound described here.

If, at a particular isothermal temperature, the oxidation rate is small, the heat flow signal may never depart markedly from the baseline, and one has, in effect, an infinite time to onset, indicating an oxidatively stable material, whereas this may not be the true state of affairs. As an example, a highly pure Lovastatin sample was oxidatively stressed at 135 °C. Using 1 mW as the exothermic limit for experiment termination, a time to onset of 16.7 min was recorded. The T_{g2} value of the solidified melt was 28.4 °C, indicating that the Lovastatin had been oxidatively stressed to a small extent under these conditions. Using the standard test temperature of 130 °C, even after 15 h stress in air, the heat flow signal did not deviate from the isothermal baseline. The T_{g2} value, 25.1 °C, was only slightly higher than that obtained

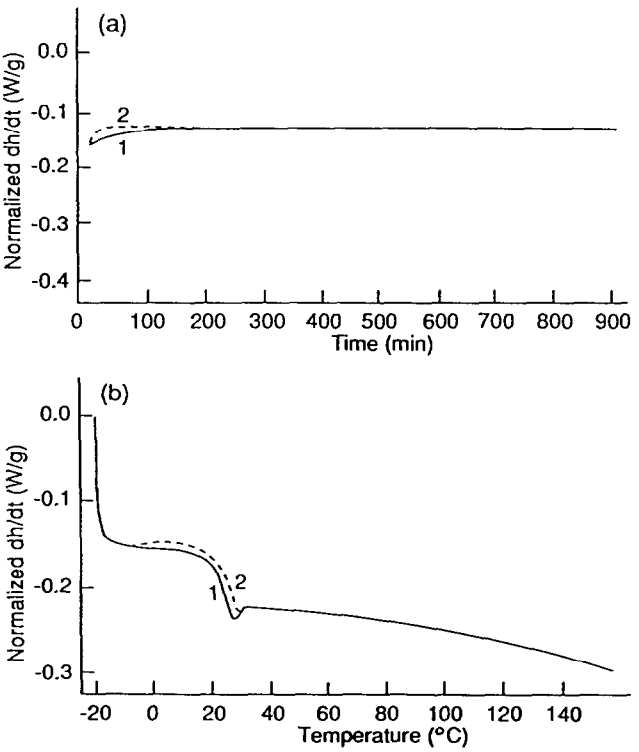

Fig. 3. 132 °C oxidative stress and glass transition curves for a Lovastatin standard (> 99.7%): 1, nitrogen; 2, air.

when the sample was stressed under the same conditions in a nitrogen atmosphere, namely, 24.8° C. However, sample discoloration indicated some oxidation had occurred. This state of affairs was maintained when the stress temperature was raised to 132° C, as shown in Fig. 3a. However, as indicated in Fig. 3b, there is a small but significant difference in the T_{g2} values; 24.4° C in nitrogen, 26.3° C in air.

CONCLUSIONS

The new accelerated isothermal DSC oxidative stress test has proved highly useful in assessing the relative oxidative stability of samples of a very important pharmaceutical compound, the cholesterol-inhibiting drug, Lovastatin. Furthermore, it is being employed effectively as an analytical support in the development and optimization of new purification procedures for an analogous drug.

The major advantage over the time to onset method is that it is a fixed time method. It appears not to be subject to differences in the kinetics of the oxidation process or processes, which control the slope of both the isothermal baseline and the initial part of the oxidative exotherm, from whose intersection the onset time is calculated. Measurement precision is excellent. Since, special sample preparative procedures are not required, and data analysis is essentially devoid of subjectivity, it is clearly a routine method which can be left in the hands of a technician with relatively little thermoanalytical experience.

There is every reason to believe that the method will prove effective with other organic compounds, which are susceptible to oxidation, and whose melt solidifies on cooling to a glass.

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