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Report

Applications of Oxygen Polarography to Drug Stability Testing and Formulation Development: Solution-Phase Oxidation of Hydroxymethylglutaryl Coenzyme A (HMG-CoA) Reductase Inhibitors

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The kinetics of oxidation of the HMG-CoA reductase inhibitors lovastatin, simvastatin, L-157,012, and L-647,318 were studied in an aqueous surfactant solution. A thermally labile free radical initiator was used to attain measurable reaction rates at 40°C and rate constants were determined by measuring oxygen consumption using an oxygen electrode. The stability of the drugs was found to increase in the order lovastatin = simvastatin < L-157,012 < L-647,318. The addition of butylated hydroxyanisole (BHA) was found to stabilize the drugs. For the oxidation of lovastatin, the effectiveness of antioxidants increased in the order propyl gallate < BHA < alpha-tocopherol. It is concluded that the stability of oxidizable drugs can be rapidly and conveniently assessed by the techniques described herein.

KEY WORDS: oxidation; antioxidants; stability testing; lovastatin; simvastatin.

INTRODUCTION

The shelf life of many pharmaceutical products is limited by the susceptibility of the active drug to undergo oxidative degradation. From a developmental viewpoint, it is highly desirable to assess the oxidative stability of a new drug substance at an early stage of research so that stabilization strategies can be pursued. Nonetheless, several factors complicate the study of oxidation reactions. For example, many oxidation reactions exhibit an induction period during which little or no drug decomposition occurs; consequently, experimental data obtained during the induction period will tend to overestimate the oxidative stability of a drug. Moreover, oxidation reactions display complex kinetic behavior due to the accumulation of reaction products which may catalyze further reactions. Further complicating the experimental assessment of oxidative stability is that the oxidative process is frequently initiated by trace impurities with levels that are difficult to control and/or quantitate (1). Since the overall rate of oxidation depends in part on the rate of initiation, it is difficult to obtain reproducible reaction rate data without some prior knowledge of the initiation step. Finally, it is worth noting that the mechanistic complexity of oxidation reactions often results in nonlinear Arrhenius behavior and that the extrapolation of elevated temperature data to room temperature must be interpreted with caution.

From the above discussion, it is clear that an assessment of the intrinsic oxidative stability of a drug requires that the rate of initiation be carefully controlled and that the extent of drug degradation be kept small to prevent product autocatalysis. It is also preferable for the experiment to be performed as close to room temperature as possible. In this report, an experimental system is described which meets these requirements. The key feature of this system is the use of an oxygen electrode to monitor the uptake of molecular oxygen by a drug in aqueous solution. By monitoring the loss of free oxygen from solution as a function of time, it is possible to construct oxygen uptake curves which reflect the oxidative stability of a drug. In addition, it is possible to obtain the necessary data at very low extents of drug degradation (typically 0.5–1% loss of drug) so that product effects on reaction rates are negligible. Finally, we have made use of a thermally labile free radical initiator in these studies. The concentration of the initiator is kept high enough so that conveniently measured reaction rates are attained at a relatively low temperature (40°C), and initiation due to adventitous impurities is insignificant. Consequently, it is possible to make meaningful comparisons of oxidation rates since the initiation rate is kept constant and the overall rate is determined by the intrinsic susceptibility of the drug to free radical induced oxidation.

In the present study, the oxidation of the hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors lovastatin, simvastatin, L-157,012, and L-647,318 was investigated in an aqueous surfactant solution. The structures of these compounds, which are known to be highly effective cholesterol-lowering agents (2), are shown in Fig. 1. In addition, the utility of the electrode test system in formulation

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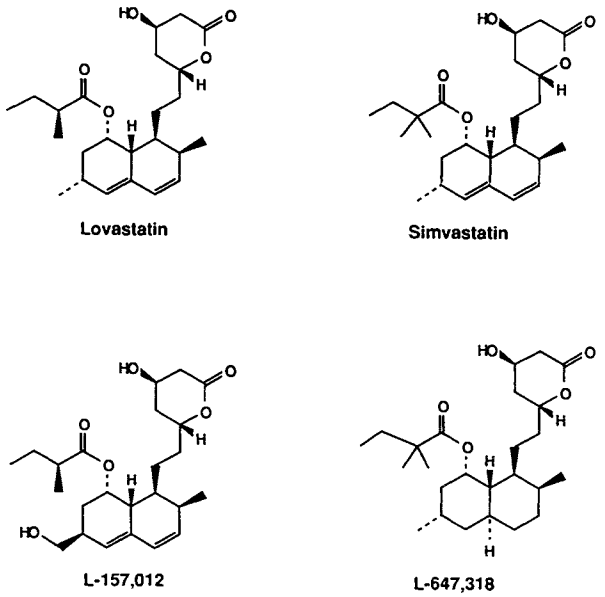

Fig. 1. Chemical structures of HMG-CoA reductase inhibitors.

development was investigated by monitoring oxidation rates in the presence of various antioxidants.

MATERIALS AND METHODS

Oxygen concentrations were measured using a YSI Model 5300 Biological Oxygen Monitor. Prior to each kinetic run, the instrument was calibrated using a value of 220 μM as the solubility of oxygen in distilled water at 40.0°C (3). The instrument was also checked for noise and drift during the calibration procedure. The drug substances used in this study were obtained from the Merck Sharp and Dohme Research Laboratories and were all at least 98% pure. The initiator, 2,2′-azobis(4-methoxy-2,4-dimethylvaleronitrile), was obtained from Wako Chemicals, Inc., and was used as received. Antioxidants were obtained from commercial sources and used without further purification.

Stock solutions of each drug were prepared in 2% aqueous sodium dodecyl sulfate (SDS) buffered to pH 7.0 with 0.01 *M* sodium phosphate; these solutions were freshly prepared just prior to use. The drug concentration was 7.2 m*M* (ca. 3 mg/ml) in all experiments. Stock solutions of the initiator, 16 mg/ml in methanol, were prepared on a daily basis.

In a typical experiment, 6.0 ml of the stock drug solution was added to a reaction vessel thermostated to 40°C. The oxygen electrode, which is designed to form a reasonably air-tight seal with the reaction vessel, was inserted into the solution and the electrode output was monitored for several minutes to confirm that no oxygen was being consumed at this point. A 100-μl aliquot of the stock initiator solution was then added via syringe through a small port in the assembly, and the resulting decrease in oxygen concentration was monitored as a function of time. Generally, the reactions were followed for 30-40 min, corresponding to about one half-life in oxygen concentration. In practice, it was most convenient to sample the electrode output at recorded time intervals so that the digitized data could be transferred directly to a computer for further manipulation.

RESULTS AND DISCUSSION

General Kinetic Features

In the absence of an added initiator, no oxygen uptake is observed for solutions of the drugs at 40°C. This indicates that the spontaneous oxidation rate of these drugs at 40°C is too slow to be detected by the oxygen electrode. In contrast, the addition of the free radical initiator to these solutions results in the rapid depletion of free oxygen from solution; this is attributed to the oxidation of the drug, which consumes dissolved oxygen as it reacts. It is notable that oxygen consumption commences immediately upon introduction of the initiator with no observable induction period.

A typical oxygen consumption curve determined for simvastatin is shown in Fig. 2. In this run, the dissolved oxygen concentration decreased from its initial value of 200 μ*M* (slightly below saturation) to about 100 μ*M* over the course of 32 min. Exponential oxygen decay was observed in all the experiments described in this report, indicating that the overall oxidation kinetics of these drugs follows a first-order rate law in oxygen. This is somewhat unusual in that solution-phase oxidation reactions are often found to exhibit zero-order kinetics in oxygen (4); i.e., the reaction rate is independent of oxygen concentration. The departure from zero-order kinetics may indicate that the mechanism of these reactions involves the utilization of more than one equivalent of molecular oxygen per equivalent of substrate.

To quantitate the data further, the integrated form of the general first-order rate law [Eq. (1)] was used:

$$\log [O_2]_t = -kt + \log [O_2]_i \qquad (1)$$

where $[O_2]_t$ = concentration of dissolved oxygen at time t, $[O_2]_i$ = initial concentration of dissolved oxygen, and k = pseudo first-order rate constant.

Equation (1) predicts that a plot of log $[O_2]$ vs time should be linear with a slope equal to the rate constant k for oxidation. Such a plot is shown in Fig. 3, and the excellent linearity confirms the first-order nature of these reactions. Rate constants were evaluated by least-squares line fitting. It should be noted that these rate constants are pseudo first

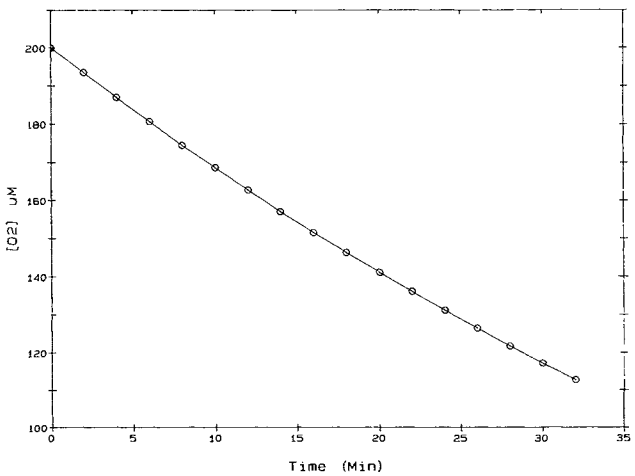

Fig. 2. Plot of oxygen concentration in solution vs time for the initiated oxidation of simvastatin in 2% aqueous SDS at 40°C.

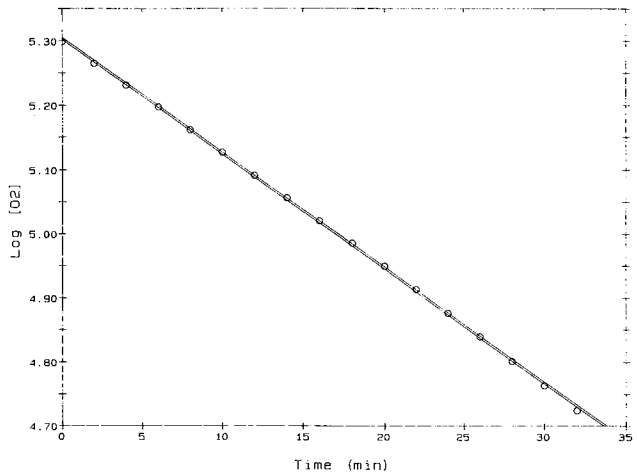

Fig. 3. Plot of log oxygen concentration vs time. The data points (circles) are from Fig. 2 and the regression equation is log $[O_2]$ = −0.0179 time (min) + 5.304; r^2 = 0.9997.

order in that they incorporate the kinetic terms for the constant substrate and initiator concentrations.

Drug Oxidation Kinetics

The oxygen uptake curves obtained from a series of experiments with the four drugs are shown in Fig. 4. Despite the close structural similarities among these compounds, the oxygen uptake curves reveal pronounced differences in stability. The apparent order of oxidative stability is L-647,318 > L-157,012 > lovastatin ≈simvastatin. Rate constants for these compounds, calculated as average values of duplicate runs, are shown in Table I. Table I also shows the rate constant for oxygen uptake when no substrate is present. This reflects the consumption of oxygen due to the decomposition of the initiator and serves as a control for evaluating the other rate constants.

The comparison of oxidation rates shown in Table I provides several mechanistic insights regarding the oxidation of this class of compounds. Consider first the oxidation

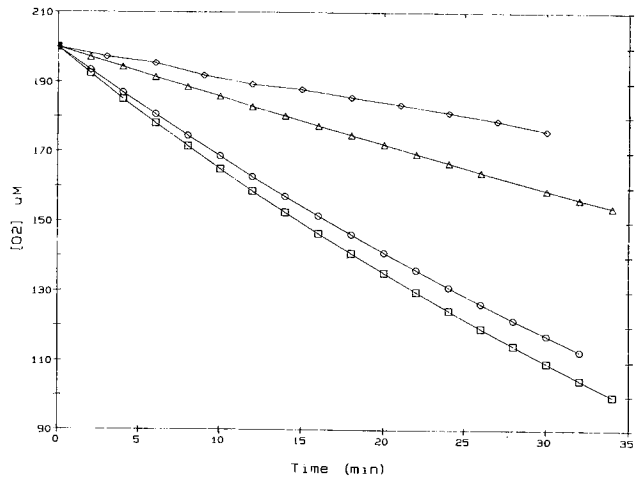

Fig. 4. Oxygen consumption curves of HMG-CoA reductase inhibitors. Diamonds, L-647,318; triangles, L-157,012; circles, simvastatin; squares, lovastatin.

Table I. Pseudo First-Order Rate Constants[a] for Uninhibited (k) and Inhibited[b] (k_i) Oxidation of HMG-CoA Reductase Inhibitor

Substrate	1000 k	1000 k_i
None	4.1 ± 0.5	—
L-647,318	4.1 ± 0.3	—
L-157,012	6.9 ± 0.8	4.9 ± 0.4
Simvastatin	18 ± 2	13 ± 2
Lovastatin	18 ± 2	10 ± 1

[a] In units of min^{-1}.
[b] Inhibition by BHA at 0.1% (w/w) relative to the substrate.

rates of simvastatin and L-647,318. Structurally, these compounds are identical except for the diene system present in simvastatin. However, the oxidation kinetics clearly reveal that saturation of the diene system as in L-647,318 completely deactivates the compound toward oxidative degradation, whereas simvastatin is among the most reactive of the compounds examined in this study. This result demonstrates that the reactive site of simvastatin, and by analogy the other unsaturated compounds, is located about the conjugated double bonds. Consistent with this assignment, it is found that lovastatin and simvastatin have identical oxidation rates; the structural difference between these compounds occurs in an ester side chain which is too far removed from the diene system to have a significant effect on reactivity. In contrast, the low reactivity of L-157,012 compared to lovastatin and simvastatin indicates that structural modifications near the diene system can have a major effect on overall reactivity. The stabilizing effect of the hydroxymethyl group in L-157,012 is probably due to an inductive effect since free radical formation is generally inhibited by electron withdrawing substituents (5).

Antioxidant Effects

Phenolic compounds such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are effective antioxidants in solution. The protective effect of these compounds is generally attributed to their ability to scavenge substrate-derived free radicals and thereby decrease the overall rate of oxygen consumption (6). Since this effect should be directly measurable with the oxygen electrode, it was decided to investigate the effect of BHA on drug oxidation rates.

Figure 5 shows the oxygen uptake curves for L-157,012 without an antioxidant, and in the presence of BHA (drug: antioxidant = 1000, w/w). It is apparent that even at the 0.1% level, BHA is highly effective at inhibiting the oxidation of L-157,012. Similarly, the oxidation rates of simvastatin and lovastatin are also decreased in the presence of 0.1% BHA, and these data are given in Table I.

During formulation development it is often necessary to determine the most effective antioxidant for use with a particular drug. (Using conventional stability testing protocols, this can be a very time-consuming task if several antioxidants at varying concentrations are to be screened.) In light of the results described above for the BHA solutions, it was of interest to determine the effect of other antioxidants and, in particular, whether the effectiveness of these antioxidants

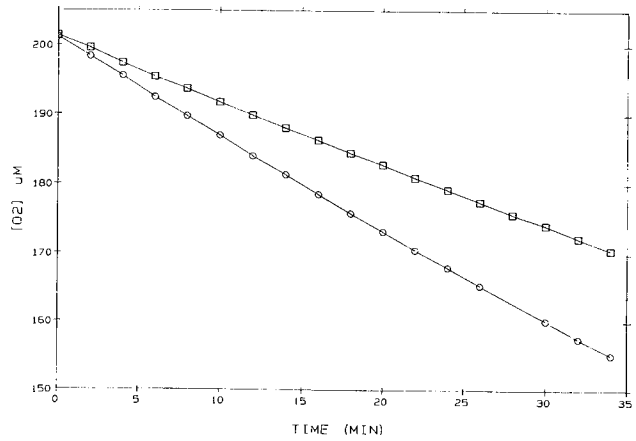

Fig. 5. Oxygen consumption curves for the initiated oxidation of L-157,012 without inhibitor (circles) and with 0.1% (w/w) BHA (squares).

toward a given drug could be ranked. The oxygen uptake curves obtained for oxidizing lovastatin in the presence of alpha-tocopherol, BHA, and propyl gallate are shown in Fig. 6, and the rate constants derived from these data are shown in Table II. The antioxidants were used at the 0.1% (w/w) level since this is representative of the level found in pharmaceutical products (7). All three antioxidants exerted a significant stabilizing effect on the oxidation of lovastatin; most significantly, however, it was found that differences in the effectiveness of these antioxidants could be detected and quantitated. In the present case, the order of antioxidant efficiency based on oxygen uptake rate constants is alpha-tocopherol (53% inhibition) > BHA (44% inhibition) > propyl gallate (33% inhibition). It should be noted that these values, when corrected for differences in molecular weights (i.e., adjusted to an equimolar basis), indicate that alpha-tocopherol is three times more effective than either BHA or propyl gallate. These results are in agreement with other

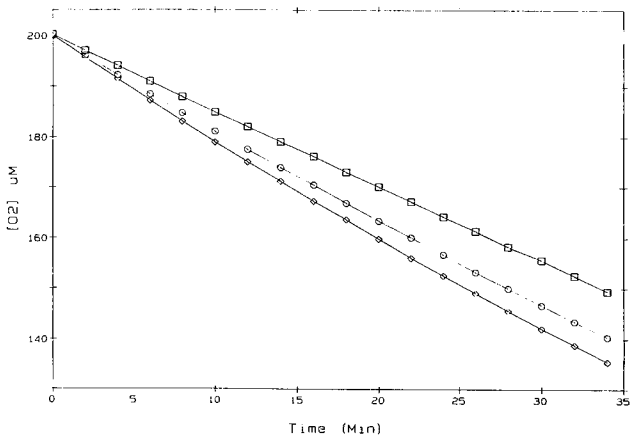

Fig. 6. Oxygen consumption curves for the initiated oxidation of lovastatin inhibited by α-tocopherol (squares), BHA (circles), and propyl gallate (diamonds). Each antioxidant was used at a concentration of 0.1% (w/w) relative to the substrate.

Table II. Effect of Antioxidants on the Initiated Oxidation of Lovastatin at 40°C

Antioxidant[a]	1000 k_i[b]	% inhibition
α-Tocopherol	8.5 ± .6	53
BHA	10 ± 1	44
Propyl gallate	12 ± 1	33
None	18 ± 2	0

[a] The concentration of each antioxidant was 0.1% (w/w) relative to the lovastatin concentration.
[b] In units of min^{-1}.

studies which have shown alpha-tocopherol to be a superior antioxidant to BHA and propyl gallate (8).

CONCLUSIONS

This paper describes the utility of oxygen polarography in studying drug degradation kinetics in solution. The technique differs from conventional accelerated stability testing in two fundamental respects. First, the polarographic technique monitors the rate of oxygen consumption in contrast to the more usual practice of measuring the loss of intact drug. Second, drug decomposition is initiated by supplying free radicals at a constant and reproducible rate. The advantage of this test system is that quantitative stability assessments can be made in a very short time, and the effects of various additives such as antioxidants can be determined. Although our initial studies were confined to pH 7 buffered solutions, it is possible to assess pH effects; in this respect the polarographic method is well suited to formulation development. It should be noted, however, that the polarographic technique is specific for oxidation, and other routes of drug degradation (e.g., hydrolysis) must be assessed by other methods.

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