Solvent Effects on the AIBN Forced Degradation of Cumene: Implications for Forced Degradation Practices

ERIC D. NELSON,[1] GINA M. THOMPSON,[2] YE YAO,[1] HOLLY M. FLANAGAN,[2] PAUL A. HARMON[2]

[1]Transform Pharmaceuticals, Inc., Lexington, Massachusetts 02421

[2]Merck and Company, Inc., West Point, Pennsylvania 19486

Received 31 January 2008; revised 6 June 2008; accepted 10 June 2008

Published online 11 July 2008 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.21489

ABSTRACT: Solvent effects on the AIBN and ACVA forced degradation of cumene are explored. The degradant formation rates of the three cumene oxidative degradants, cumene hydroperoxide, acetophenone, and 2-phenyl-2-propanol are reported. The relative abundance and ratios of these three degradants provide insight into the fate of the peroxy radical oxidants generated by the forced stress system, and suggest that alkoxy radicals are actually a significant source of the observed reactivity. The presence of even 1% methanol in the forced stress solvent significantly quenches this alkoxy radical reactivity, dramatically reducing the overall degradation rate and leaving cumene hydroperoxide as the major product of the oxidation reaction. The origin of this significant solvent effect on the oxidation product distribution is shown to be related to the preferential H-atom abstraction from methanol and its trace impurities by any alkoxy radicals present in the reaction solution. The implications for these observations are explored with the intent of producing more predictive oxidative forced stress experiments. © 2008 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 98:959–969, 2009

Keywords: chemical stability; chromatography; high-performance/pressure liquid chromatography (HPLC); stability; preformulation

INTRODUCTION

Forced degradation is a regulatory requirement to demonstrate the selectivity of stability indicating analytical methods.[1] It is also an important tool that helps give early definition to what a method should be selective for. However, there is little specific regulatory guidance on how forced degradation experiments should be conducted, leading to a wide diversity of practices.[2] Practices for oxidative forced degradation are particularly subject to interpretation.[3] Recently, there has been a resurgence of interest in evaluating how the meaningfulness and predictive power of oxidative forced degradation can be increased and improved.[4,5] A survey of industry practices in 2003 suggested a wide variety of oxidation conditions, including a growing trend of the use of azonitrile radical initiators such as AIBN (2, 2′-azobisisobutyronitrile) and ACVA (4,4′-azobis-4-cyanovaleric acid) to purposefully induce peroxy radical oxidation of drug substance in solution.[2]

The prevalent pharmaceutical industry practice for AIBN and ACVA forced degradation is to conduct the forced degradation in acetonitrile/water solutions.[2] This practice arises both from convenience, since acetonitrile is the favored solvent for reversed phase HPLC, and also from the early observations by Boccardi that AIBN forced degradation of tetrazepam occurs faster in acetonitrile than in alcohols.[6] Since the work of

Correspondence to: Eric Nelson (Telephone: 781-674-7910; Fax: 781-863-7208; E-mail: enelson3@tpius.jnj.com)

Journal of Pharmaceutical Sciences, Vol. 98, 959–969 (2009)

Boccardi, however, the choice of solvent system has been an unexamined practice in oxidative forced degradation. However, as Boccardi rightly concluded in his initial comments on the solvent effects on AIBN forced degradation, the solvent effects of greatest significance are the competitive reactions of solvent molecules with the active radical species purposely formed in the stress solution. The results of competitive solvent reactions do more than merely moderate the rate of reactions—they can impact the speciation of radicals in the stress solution, and consequently the product distribution that is observed. Of particular importance, is the tendency for tertiary peroxy radicals (such as those formed by AIBN decomposition under oxygen environments) to disproportionate into more aggressive alkoxy radicals.[7] The disproportionation is second order with respect to peroxy radicals[7] and the formation of alkoxy radicals through this mechanism is very sensitive to the steady-state peroxy radical concentration. This is much more than an academic question, since peroxy radicals are >20 kcal/mol less aggressive hydrogen atom abstractors, and thus possess a distinct selectivity in their sites of radical abstraction[8] and the resultant degradation products. In this work, we report a significant methanol solvent effect on the AIBN and ACVA forced stress of cumene. The effect is shown to be the result of the quenching of alkoxy radicals formed from AIBN-derived peroxy radical disproportionation. The implications of these observations for the general practice of oxidative forced degradation experiments are discussed. These observations are particularly relevant to drug-sparing forced degradation experiments, as the disproportionation of the desired peroxy radical oxidizing species into alkoxy radicals are more likely when low drug concentrations are used.[4]

EXPERIMENTAL

HPLC grade solvents were purchased from Fisher Scientific (Philadelphia, PA). AIBN, ACVA, cumene, cumene hydroperoxide, acetophenone, 2-phenyl-2-propanol, formic acid, and formaldehyde were purchased from Sigma–Aldrich (St. Louis, MO). All chemicals were used as received.

Solutions of cumene were prepared at ~0.1 mg/mL concentrations (~0.8 mM) together with 5 mM AIBN or ACVA in various solvent compositions. The oxidative degradants of cumene were separated by a gradient reversed phase HPLC method pumping at 1.0 mL/min from 10:90 to 90:10 acetonitrile/water in 40 min on a Waters Symmetry C18 (5 μm particles, 4.6 mm ID × 250 mm length) column. Detection was by UV absorption at 210 nm. Degradation kinetics were measured by placing the HPLC sample tray at 40°C and measuring active and degradant levels as a function of time of injection over the course of several days of repeated sample injections. Control samples were prepared of the cumene hydroperoxide, acetophenone, and 2-phenyl-2-propanol and were included in chromatographic experiments to confirm the retention times and stability of these species under the experimental conditions.

Formaldehyde, formic acid, and formate esters in methanol-containing AIBN forced degradation solutions were measured by a slight modification of the HS-GC approach of del Barrio et al.[9] Samples were diluted with methanol solution of p-toluenesulfonic acid (1% w/w) and incubated at 60°C for 15 min to form methyl formate and dimethoxymethane from any formaldehyde, formic acid, or formate esters present in the sample. Separation was achieved using a Phenomenex ZB-WAX column, 30 m long with a 0.32 mm i.d. and 0.5 μm film thickness. The carrier gas was helium and was set at a constant flow rate of 2.5 mL/min. The injector was maintained at 170°C with a split ratio of 10:1 and split flow of 25 mL/min. The headspace sample and standard solutions were equilibrated at 60°C for 15 min. The loop and transfer line was set at 120°C. The column oven temperature was set at 30°C for 5 min. MSD was performed at 280°C with either full scan for identification or with selected ion mode for quantitative analysis. The qualifying ion is m/z 31 for formic acid and m/z 45 for formaldehyde. Quantitation is versus a formaldehyde or formic acid external standard and all samples are blank corrected with reference to stress solutions without added methanol.

RESULTS

When cumene is subjected to AIBN forced degradation in acetonitrile solutions at 40°C and low solution concentrations of both cumene and AIBN under the conditions reported by Harmon et al.[5] it reacts slowly (~1.5%/day) to form acetophenone, 2-phenyl-2-propanol, and cumene hydroperoxide (Fig. 1, Scheme 1). Relative to drug molecules with known sensitivity to

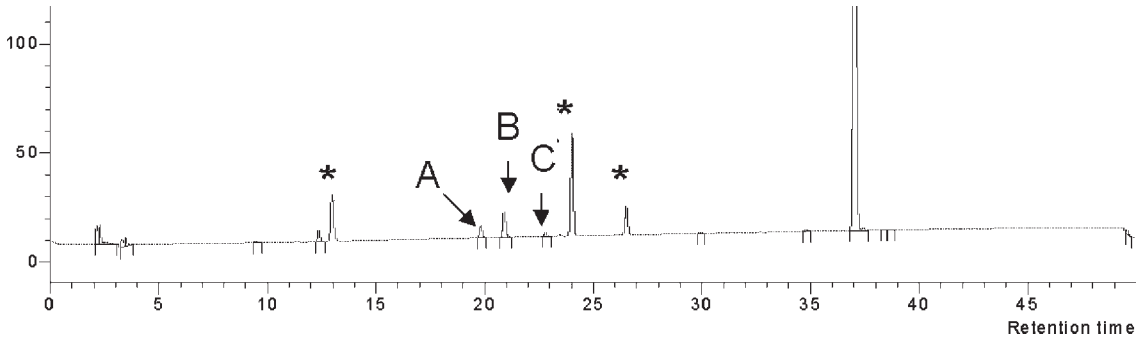
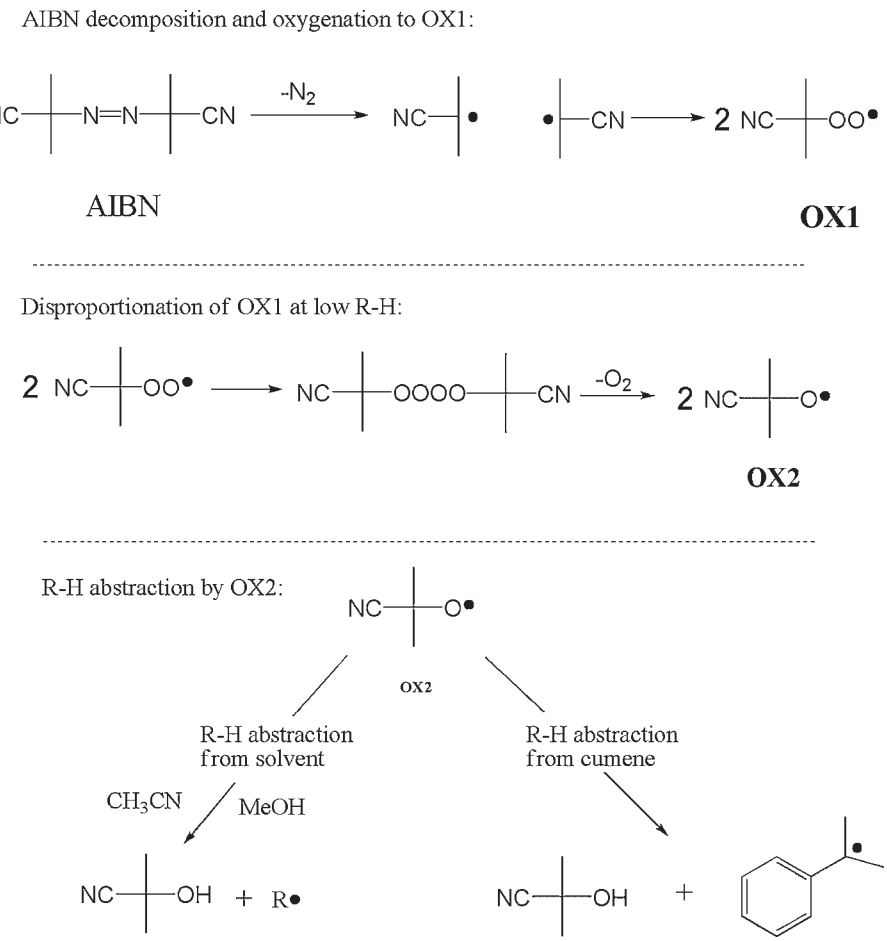


Figure 1. Representative chromatogram of a 0.1 mg/mL cumene solution stressed for 3 days in a 50% acetonitrile solution of 5 mM AIBN. Cumene degradants 2-phenyl-2-propanol (A), acetophenone (B), and cumene hydroperoxide (C) are observed at retention times ranging from 19 to 23 min. A truncated cumene peak is seen at 37 min. Peaks marked with * are present in the AIBN blank.

oxidative degradation, this degree of degradation response to AIBN forced degradation is relatively small.[5] Minor solvent effects are observed on the degradation rates of cumene in acetonitrile solutions of AIBN and ACVA as the aqueous fraction is changed. However, very little effect is observed on the product branching ratios, and the qualitative conclusions of the forced stress experiment is thus relatively insensitive to the amount of water present in the reaction solvent. However,



Scheme 1. Competing radical reactions in the AIBN-initiated oxidation of cumene.

when acetonitrile is replaced with methanol, the overall rate of degradation decreased markedly and cumene hydroperoxide becomes the major rather than the minor observed product. Similar quenching of the 2-phenyl-2-propanol and acetophenone formation rates is also observed in isopropanol and THF solutions. This cumene hydroperoxide-dominated product distribution was also observed when excess *tert*-butyl hydroperoxide was added to the forced degradation solution according to the method of Courtneidge et al.[10] to force the steady state free radical population in solution to tertiary peroxy radicals.

When the forced stress experiment is conducted in a mixed solvent system of acetonitrile and methanol, the product distribution is similar to that obtained using methanol only (Figs. 2 and 3, Tab. 1), even at a composition of 99% acetonitrile and 1% methanol. The total degradation rate decreases fivefold from 0% to 1% methanol (Fig. 4). There is a slight trend toward less overall reactivity with decreasing proportions of methanol, but cumene hydroperoxide remains the major degradation product as long as some methanol is present. The ratio of acetophenone to 2-phenyl-2-propanol decreases steadily from 3:1 to 1:1 as the methanol concentration is increased from 0% to 5% (Fig. 5). This latter observation reflects the favoring of the kinetic product distribution of H-abstraction over unimolecular dissociation of the tertiary alkoxy radical.

When the concentration of cumene is increased from the initial API-sparing 0.1 mg/mL concentrations to 5 mg/mL (the effective solubility limit under these conditions), a shift in the product distribution is observed that mirrors the effect of methanol cosolvent (Fig. 6). As a function of this 50-fold range of cumene concentrations, the relative rates of cumene hydroperoxide formation increase, with corresponding decrease in the acetophenone and 2-phenyl-2-propanol formation rates. The sum of the three reaction rates is approximately proportional to cumene concentration from 1 to 5 mg/mL, but decreases by 40% on a relative basis from 0.1 to 1 mg/mL.

Measurement of formic acid and formaldehyde levels in the forced stress solutions as a function of methanol concentration and time reveals that the methanol co-solvent is oxidized to both species during the forced stress experiment (Figs. 7 and 8). Production of both species is approximately linear with methanol concentration, and formaldehyde is formed at approximately twice the rate of formic acid across all solvent compositions.

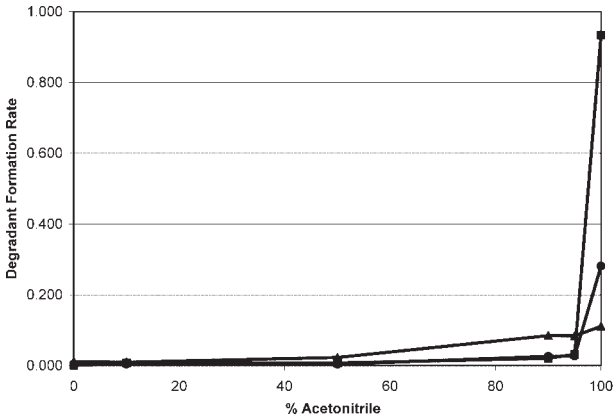


Figure 2. Degradant formation rates as a function of solvent composition for 2-phenyl-2-propanol (●), acetophenone (■), and cumene hydroperoxide (▲). Stress solutions are 5 mM AIBN and are stored at 40°C. The solvent composition is listed as % acetonitrile, and the remainder is methanol.

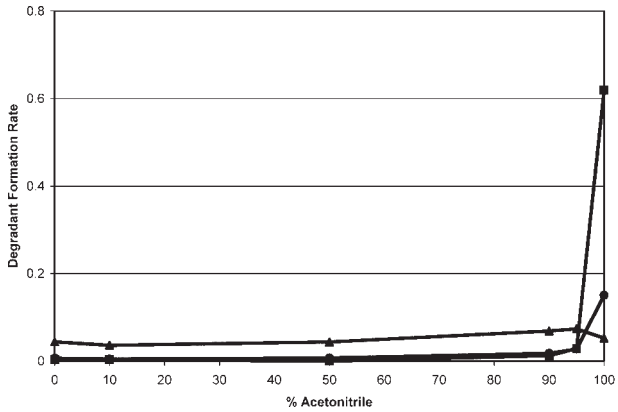


Figure 3. Degradant formation rates as a function of solvent composition for 2-phenyl-2-propanol (●), acetophenone (■), and cumene hydroperoxide (▲). Stress solutions are 5 mM ACVA and are stored at 40°C. The solvent composition is listed as % acetonitrile, and the remainder is methanol.

DISCUSSION

The upper portion of Scheme 1 shows the thermal decomposition of AIBN and subsequent oxygenation to yield the 2-cyanopropyl peroxy radical (OX1 in Scheme 1). This is the "desired oxidant" which defines the appropriate reactivity for the subject oxidative stress test. The experimental conditions outlined here assume that very little drug substance (modeled here as cumene) is

Table 1. Relative Reaction Rates from AIBN Forced Degradation of Cumene Depicted in Figure 2

% Methanol	% Acetonitrile	2-Phenyl-2-Propanol	Acetophenone	Cumene Hydoperoxide	Total
100	0	0.005	0.000	0.012	0.018
90	10	0.004	0.008	0.010	0.022
50	50	0.004	0.008	0.025	0.037
10	90	0.028	0.022	0.091	0.141
5	95	0.029	0.035	0.090	0.154
0	100	0.301	1.000	0.119	1.420

All rates are normalized to the fastest individual degradation rate.

available to the pharmaceutical scientist for oxidative stressing. Thus the cumene concentration is low, 0.1 mg/mL (0.60 mM); while the AIBN "initiator" is much more concentrated (5 mM). In addition, cumene and many dilute drug substances are only mildly reactive toward peroxy radicals such that only a few percent of the starting substrate is oxidized over the 2- to 3-day test period as described in Figure 6. Given the known temperature dependence of AIBN decomposition,[11] and our own measurements, over the 3-day test period about 10% of the 5 mM AIBN will decompose as in Scheme 1. Even assuming a 50% oxygenation rate, it is clear the ratio of moles of 2-cyanopropyl peroxy radicals formed to moles cumene or substrate oxidized is large. Since acetonitrile and methanol are largely unreactive toward peroxy radicals due to the endothermicity of the H-atom abstraction reaction,[12] and considering the rapid disproportionation rates of peroxy radicals, the subject oxidative stress test conditions will lead to a significant amount of 2-cyanopropyl peroxy radical disproportionation. Since these are tertiary peroxy radicals, they cannot undergo Russell termination[7] and formation of 2-cyanopropyl alkoxy radicals as shown in the center portion of Scheme 1 follows.[8]

The 2-cyanopropyl alkoxy radical (OX2 in Scheme 1) is ca. 20 kcal/mol stronger H-atom abstractor than the desired peroxy radical oxidant (OX1), and can thus exhibit a significantly decreased selectivity compared to OX1. This can be a major problem for the subject oxidative stress test which aims to predict peroxy radical oxidative susceptibility. The lower portion of Scheme 1 depicts the two competing reaction pathways available to OX2, hydrogen atom abstraction from solvent or H-atom abstraction from substrate. Scheme 2 depicts the oxidation of cumene under the subject experimental conditions. Cumene has a single benzylic, tertiary C–H hydrogen atom which is known to be mildly reactive toward peroxy radical.[8] The upper portion of Scheme 2 shows the cumene benzylic H-atom abstraction by

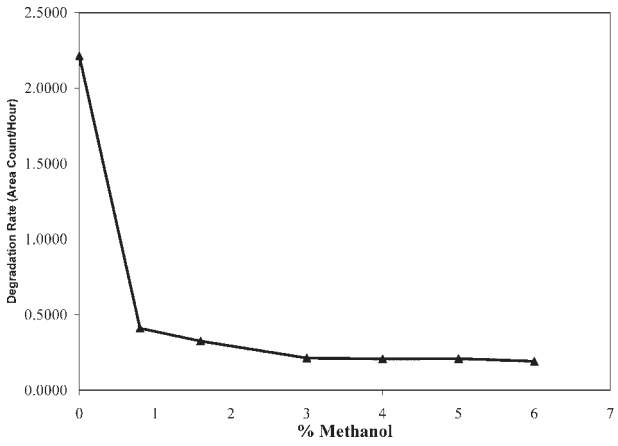


Figure 4. Total degradant formation rates as a function of solvent composition. Stress solutions are 5 mM AIBN and degradation kinetics were collected at 40°C over three days in a single combined experiment.

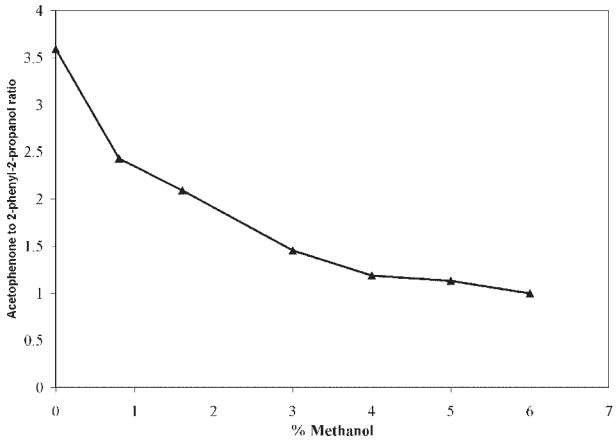


Figure 5. Ratio of acetophenone to 2-phenyl-2-propanol formation rates as a function of solvent composition. Stress solutions are 5 mM AIBN and degradation kinetics were collected at 40°C over 3 days in a single combined experiment.

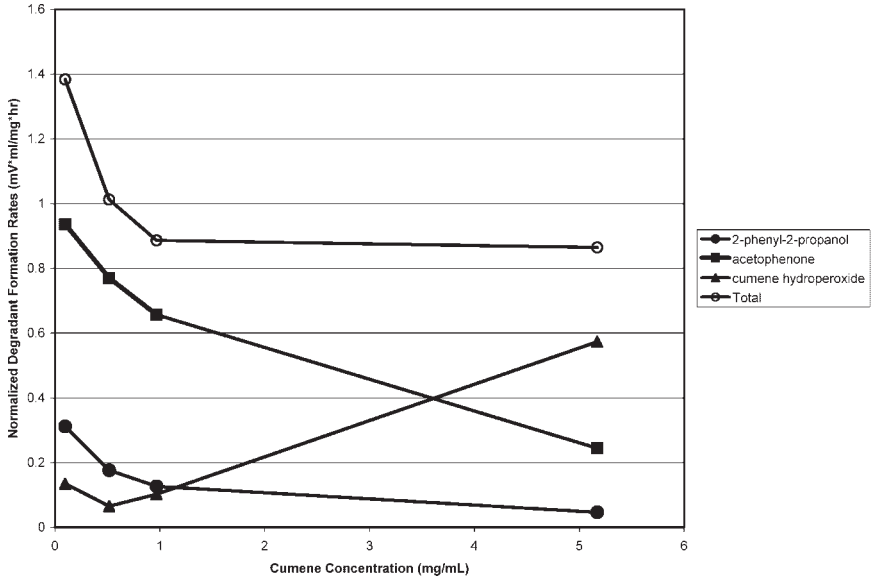


Figure 6. Normalized degradant formation rates as a function of cumene concentration for 2-phenyl-2-propanol (●), acetophenone (■), and cumene hydroperoxide (▲) and the sum of all three degradants (○). Stress solutions are 5 mM AIBN in 50% acetonitrile and are stressed at 40°C. Degradation rates are normalized by cumene concentration.

OX1, OX2 (if present), or by cumene hydroperoxy radical. The resulting carbon centered radical rapidly oxygenates at a rate controlled only by diffusion to give cumeme peroxy radical. Cumene peroxy radical can then either (1) abstract a hydrogen atom from another cumene molecule to form cumene hydroperoxide, or (2) disproportionate. The two most likely peroxy radicals involved in the disproportionation are the 2-cyanopropyl peroxy radical or another cumeme peroxy radical, both of which are tertiary. The resulting tetroxide intermediates cannot undergo Russell termination[7] and thus the cumene alkoxy radical shown in Scheme 2 is generated.[8] The cumene alkoxy radical in turn has two competing pathways of further reaction, (1) abstraction of a hydrogen

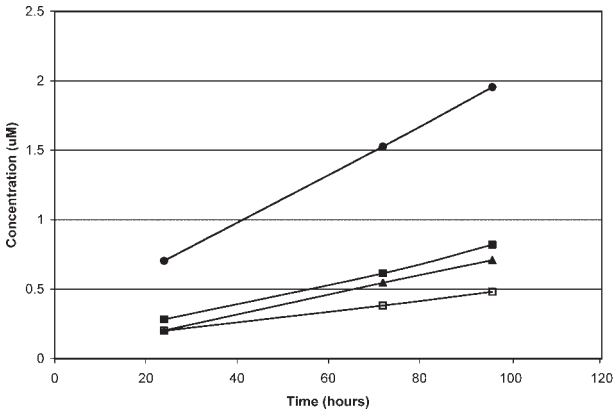


Figure 7. Formaldehyde levels in μM as a function of time for 5 mM AIBN solutions stored at 40°C. Solvent compositions are 50% acetonitrile/50% methanol (●), 90% acetonitrile/10% methanol (■), 95% acetonitrile/5% methanol (▲), and 99% acetonitrile/1% methanol (□). Detection is by GC/MS SIM versus a calibration curve with linear response across the range of quantitation.

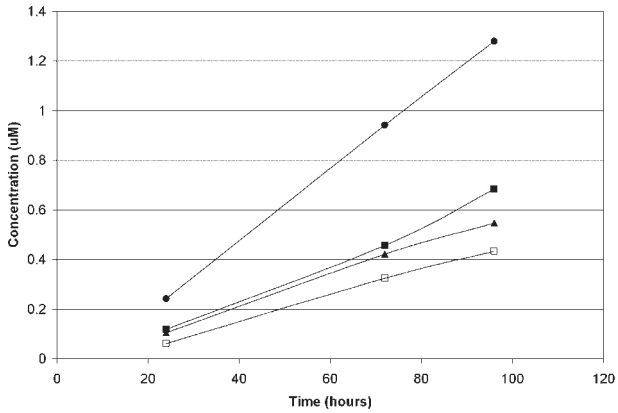
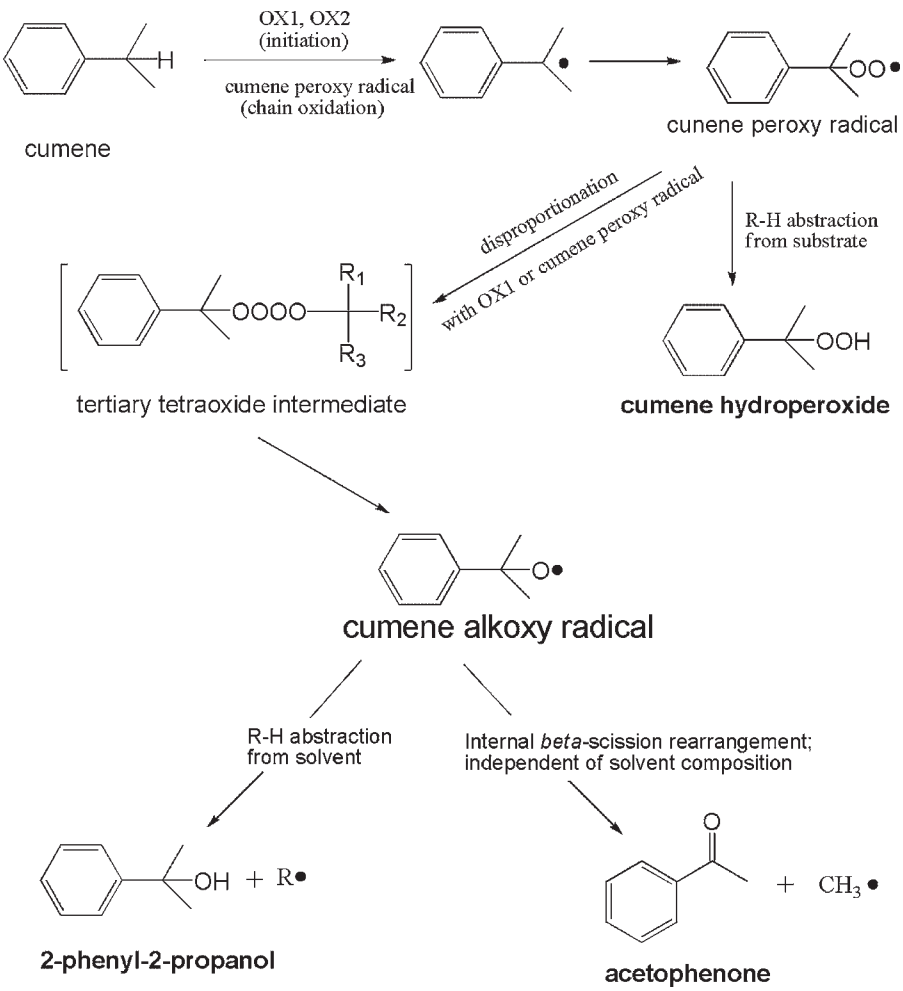


Figure 8. Formic acid levels in μM as a function of time for 5 mM AIBN solutions stored at 40°C. Solvent compositions are 50% acetonitrile/50% methanol (●), 90% acetonitrile/10% methanol (■), 95% acetonitrile/5% methanol (▲), and 99% acetonitrile/1% methanol (□).Detection is by GC/MS SIM versus a calibration curve with linear response across the range of quantitation.



Scheme 2. Mechanism for the formation of cumene oxidative degradants.

atom from solvent to form 2-phenyl-2-propanol, or (2) an internal β-scission rearrangement to give acetophenone and liberation of a methyl radical.[8] This β-scission is similar to the well studied β-scission of tert-butoxy radical.[13–15] Since the β-scission in Scheme 2 proceeds at a fixed rate which is not likely to be sensitive to small changes in solvent composition, the ratio of acetophenone to 2-phenyl-2-propanol formed is an "internal clock" which monitors the relative abundance of H atoms which are abstractable by the cumene alkoxy radical.

Schemes 1 and 2 rationalize the data in Figures 1–6 when one hypothesis is made, that is, that methanol is a facile H-atom donor to alkoxy radicals (OX2 in Scheme 1) compared to acetonitrile. Thus, even low levels of methanol can quench alkoxy radical activity. This facile H-atom donation of methanol to the OX2 alkoxy radical is supported by Figure 5 which shows the same facile H-atom donation by methanol to the cumene alkoxy radical. At 0% methanol (100% acetonitrile), the acetophenone to 2-phenyl-2-propanol ratio is 3.5. Addition of only about 2% methanol by volume doubles the H atom abstraction rate (of cumene alkoxy radical) from the solvent thus lowering the acetophenone to 2-phenyl-2-propanol ratio to near 1.75. Thus by volume methanol donates H-atoms approximately 50 times faster than acetonitrile (ca. 35-fold faster on a molar basis). We interpret the large drop in the oxidative degradation rates upon addition of only a few percent methanol (Fig. 4) as evidence that the strong alkoxy radicals (OX2) account for as much as 90% of the observed oxidation of cumene in the absence of methanol. Even 1% methanol allows for rapid quenching of OX2 to the alcohol (Scheme 1) preventing reaction with cumene. Thus a strong

OX2 alkoxy radical is "converted" to a methanol peroxy radical, which has the desired reactivity. In Figures 2 and 3, the large increase in cumene oxidation products on the right hand side of each figure is again oxidation by OX2 due to the lack of any methanol present.

The AIBN stress system is aimed at producing a peroxy radical stress environment simulating autoxidation in pharamaceutical formulations. However, it is clear from the observations described above that the forced stress conditions we have examined in this work (5 mM AIBN in acetonitrile and ~0.1 mg/mL cumene incubated at 40°C) actually represent oxidation by alkoxy radicals (OX2) due to the prevalence of disproportionation reactions over peroxy radical chain propagation reactions. It is only when the rate of H-atom abstraction from cumene is significant relative to disproportionation rates that the undesirable alkoxy radical contributions to the overall chemistry can be avoided. Because disproportionation is second order with respect to steady state tertiary peroxy radical concentrations, they will be very sensitive to even small perturbations in the initiation or propagation rates and also to the rates of solvent reactions that result in the formation of non-tertiary peroxy radicals. These rates can be easily manipulated by changing the concentration of initiator or oxidative substrate or solvent composition. In Figure 6 we show the consequence of changes in cumene concentration over a range of concentrations from 0.1 to 5 mg/mL of cumene. As expected, the product distribution of the reaction reveals an increase in the reaction product associated with propagation (cumene hydroperoxide) and decreases in the relative formation of the two degradation products associated with disproportionation (acetophenone and 2-phenyl-2-propanol) as the cumene concentration is increased 50-fold. However, even at the high end of this range, the ratio of cumene hydroperoxide to the two termination products is only approximately 2:1. In comparison, even 1% methanol in the reaction solution creates a more dramatic shift toward propagation over disproportionation. The significant effect of methanol addition is believed to be the result of its sacrificial reaction with alkoxy radicals. In the absence of alkoxy radicals, the rate of cumene peroxy radical formation is reduced, and this in turn reduces the rate of disproportionation of the tertiary cumene peroxy radicals present in the system. Additionally, the methanol reactions produce non-tertiary peroxy radicals that may participate in Russell termination to non-radical products, further reducing the steady state peroxy radical population and the rates of alkoxy radical formation.

Participation of Methanol in the Oxidation Chemistry

The facile H-atom donation of methanol to OX2 in Scheme 1 is directly demonstrated by the observation of small amounts of both formaldehyde and formic acid in the forced stress solutions (Figs. 7 and 8). The presence of both species represents H atom donation of methanol molecules by OX2 and the cumene alkoxy radical (Scheme 2) formed in the stress solution. There are a number of mechanisms to explain the formation of both species, all centering on the initiating steps of radical abstraction from methanol and oxygen addition to form a peroxy radical. Ilan et al.[16] have shown that the peroxy radical of methanol can decompose directly into $HO_2\bullet$ and formaldehyde. Likewise, once the methanol peroxy radical abstracts a hydrogen atom from a donor molecule to form $HOOCH_2OH$, this species is at equilibrium with formaldehyde and hydrogen peroxide in aqueous solution.[17] Formic acid may be formed either by Russell termination[7] of methanol peroxy radical or by the subsequent oxidation of formaldehyde. Although the peroxy radicals resulting from these methanol product radicals presumably participate in propagation reactions with selectivity similar to those of any other peroxy radicals, the ability of these primary peroxy radicals to undergo Russell termination may help to prevent the formation of further alkoxy radicals.[18,19]

The observations presented in this work indicate that methanol is a significantly better H-atom donor towards alkoxy radicals than toward acetonitrile. This claim appears at first to be in conflict with the similar C–H bond dissociation energies of these two molecules.[12] In fact, the relatively nonpolar methyl radical has been shown to have similar hydrogen abstraction rates toward both molecules,[20] as predicted by this thermochemistry. However, the situation is markedly different for the reaction of highly nucleophilic radicals, such as peroxy and alkoxy radicals. Hendry et al.[21] have reported the hydrogen abstraction rates between the *tert*-butoxy radical and a number of common organic compounds, and cyanoalkanes such as acetonitrile are among the slowest reactions reported—

more than an order of magnitude slower than ethers and only slightly faster than alkanes. A number of groups have taken advantage of the relative inertness of acetonitrile with respect to oxidation by polar radicals to use it as a reaction solvent. Karki et al.[22] used acetonitrile as one of several inert solvents for the measurement of *tert*-butoxy radical lifetimes with respect to β-scission. Likewise, acetonitrile has been used as an "inert" reaction solvent in the measurement of methanol oxidization by triplet anthraquinone.[23] Further, it has been shown that triplet benzophenone was >2000-fold more active toward hydrogen abstraction from acetonitrile than from methanol.[24]

In cases where radical polarity is significant, the barrier height of the H-atom abstraction reaction may be controlled by polar stabilization of the transition state rather than intrinsic thermochemistry. Donahue[25,26] and Donahue et al.[27] have described how the kinetics of the reactions of nucleophilic radicals are strongly influenced by ionic valence states in the transition state region, and barrier heights are strongly correlated with the ionization energy of the product radical. From this perspective, methanol and acetonitrile are actually quite different as hydrogen atom donors. Methanol can be considered to be a relatively good hydrogen atom donor toward nucleophilic radicals by virtue of the 7.6 eV ionization energy of the hydroxymethyl radical product.[28] In contrast, acetonitrile is a poorly matched donor toward nucleophilic radicals, based on the significantly higher 10 eV ionization energy of the cyanomethyl radical.[28] The importance of polar effects on the kinetics of hydrogen abstraction from acetonitrile have been further illustrated by Paul and Roberts[29] in the demonstration that, although the intrinsic rate of hydrogen abstraction from acetonitrile by the *tert*-butoxy radical was quite slow, the reaction rate could be increased significantly by the intermediacy of polarity reversal catalyst trimethylamine-2,3-dimethyl butan-2-yl borane complex.

We have focused the above discussion on the alkoxy radical quenching effects of methanol itself, the addition of methanol implies as well the presence of trace impurities. Previous literature reports of methanol impurities have included alcohols,[30] esters,[30] ketones,[30] amines,[31] hydrocarbons,[30] and ethers.[30] The specifications of even high-purity HPLC grade methanol allow for ppb-ppm levels of aldehydes, ketones, and organic acids. Although these levels of trace impurities are quite small, many potential impurities are actually much more thermodynamically favorable hydrogen atom donors that methanol itself, and they may thus be collectively significant as a contribution to the quenching of alkoxy radicals. We have not endeavored in this work to separate and quantitate the collective or individual methanol impurities on the overall observed reactivity. Instead we have used the purest commercially available grades of methanol for our experiments, and we feel that these practices are representative of general forced degradation practices across the pharmaceutical industry.

Implications for Forced Degradation Practices

The general observations that we have reported in this work about the relative contributions of peroxy and alkoxy radicals to the azonitrile forced degradation of cumene under various experimental conditions provide valuable insights into the important experimental variables of these experiments. It is clear from this understanding of the representative example of cumene that many drugs which are unreactive or weakly reactive toward hydrogen abstraction by peroxy radicals, may be unintentionally exposed to harsher alkoxy radical stress by virtue of the disproportionation of peroxy radicals in the absence of facile and abundant H-atom donors. Such false peroxy radical reactivity is especially concerning in that it may lead to false assignment of a stable compound as oxidatively sensitive and the tracking of unrealistic degradants. In fact, Watkins et al.[32] have recently reported such a cautionary tale in the observation of 2-cyano-2-propanoxy radical addition products during AIBN forced degradation in acetonitrile solutions. Consistent with the observations of this study, when 5–10% methanol is added to the reaction solvent, the reaction pathways attributable to alkoxy radicals are quenched. Alkoxy radical degradants of this type cannot reasonably be considered to be representative of the degradation of solid oral dosage forms, and their presence in early forced degradation screens serve only to decrease the clarity and value of this data. The addition of small amounts of methanol (≥5%) to the stress solution provides a simple and convenient experimental fix to filter out reactivity attributable to alkoxy radical chemistry that is not relevant to the prediction of autoxidative degradation profiles.

Although the presence of methanol has the desirable effect of inhibiting alkoxy radical reactions, use of methanol may introduce new degradants into the observed forced degradation profile via non-oxidative pathways. The simplest of these additional degradation pathways is the direct reaction of methanol with drugs containing carboxylic acid, ester, or amide functional groups to produce methyl ester products.[33] In such cases it is often sufficient to understand the potential for esterification, and to conduct control experiments with a methanolic solvent system to identify the chromatographic retention times associated with direct methanol reaction. The trace formaldehyde and formic acid formation during the AIBN/ACVA forced degradation shown here pose a more subtle source of additional degradation chemistry, especially when the drug contains a primary or secondary amine functional group.[33] Control experiments with methanol under non-oxidizing conditions are unlikely to show formation of formaldehyde or formic acid degradants. However, other control experiments explicitly incorporating formadehyde or formic acid may suffice to clarify the identity of the additional peaks.

CONCLUSIONS

We have shown in this work that the most commonly used AIBN/ACVA oxidative forced degradation conditions[2] have a potential to produce undesired alkoxy radicals instead of the desired peroxy radical oxidants. However, the use of even small amounts of methanol as a reaction additive prevents this effect. If one accepts the premise that the purpose of such stress solutions is to mimic peroxy radical chemistry,[4–6] then it follows that it is prudent to select a solvent system that is not prone to this issue. We have found that acetonitrile/methanol/water systems serve this role well, and commonly use 5% methanol in control experiments to quench alkoxy radical activity of the AIBN forced degradation experiment. Likewise small amounts of ethanol, 2-propanol, or THF may be substituted for methanol with similar results. For very oxidizable drugs at high solution concentrations, the primary peroxy radical reactivity will dominate, regardless of solvent composition, as disproportionation rates will be small compared to oxidation of substrate by peroxy radicals. However, solvent choice is an important consideration for drugs with moderate or low reactivity toward peroxy radical and experimental conditions with low drug concentration in the stress solution.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Robert A. Reed for encouraging the research collaborations that ultimately led to this work. The authors also wish to thank Dr. Steve Baertschi and Dr. Michael Watkins of Eli Lilly for useful discussions.

REFERENCES

1. FDA. 2003. "Guidance for Industry Q1A(R2) Stability Testing of New Drug Substances and Products," Federal Register 68(225) (November 21) 65717.
2. Alsante KM, Martin L, Baertschi SW. 2003. A Stress Testing Benchmarking Study. Pharm Tech 27:60–72.
3. Reynolds DM, Facchine KL, Mullaney JF, Alsante KM, Hatajik TD, Motto MG. 2002. Available guidance and best practices for conducting forced degradation studies. Pharm Tech 26:48–54.
4. Nelson ED, Harmon PA, Szymanik RC, Teresk MG, Li L, Seburg RA, Reed RA. 2006. Evaluation of solution oxygenation requirements for azonitrile-based oxidative forced degradation studies of pharmaceutical compounds. J Pharm Sci 95:1527–1539.
5. Harmon PA, Kosuda KM, Nelson ED, Mowery MA, Reed RA. 2006. A novel peroxy radical based oxidative stressing system for predicting oxidative instability of active pharmaceutical ingredients. J Pharm Sci 95:2014–2028.
6. Boccardi G. 1994. Autoxidation of Drugs : Prediction of Degradation Impurities from Results of Reaction with Radical Chain Initiators. Il Farmaco 49:431–435.
7. Russell GA. 1957. Deuterium-isotope effects in the autoxidation of aralkyl hydrocarbons. Mechanism of the interaction of peroxy radicals. J Am Chem Soc 79:3871–3877.
8. Ingold KU. 1969. Peroxy Radicals. Acc Chem Res 2:1–9.
9. del Barrio MA, Hu J, Zhou P, Cauchon N. 2006. Simultaneous determination of formic acid and formaldehyde in pharmaceutical excipients using headspace GC/MS. J Pharm Biomed Anal 41:738–743.
10. Courtneidge JL, Bush M. 1992. Initiated, tert-butyl hydroperoxide-loaded, low-temperature autoxidation of alkenes: A chemoselective synthesis of allylic hydroperoxides, allowing analysis of the regioselectivity of hydrogen atom abstractions from some unsymmetrically substituted substrates. J Chem Soc Perkin Trans I 12:1531–1538.

11. Dixon KW. 1999. "Decomposition rates of organic free radical initiators". In: Brandrup J, Immergut EH, Grulke EA, editors. Polymer handbook, 4th edition. New York: Wiley. p. II/12.
12. McMillen DF, Golden DM. 1982. Hydrocarbon bond dissociation energies. Ann Rev Phys Chem 33:493.
13. Cadman P, Trotman-Dickenson AF, White AJ. 1971. Kinetics and pressure-dependence of the t-butoxyl radical decomposition. J Chem Soc 14: 2296–2298.
14. Rauk R, Boyd RJ, Boyd SL, Henry DJ, Radom L. 2003. Alkoxy radicals in the gaseous phase: β-scission reactions and formation by radical addition to carbonyl compounds. Can J Chem 81:431–442.
15. Tsentalovich YP, Kulik LV, Gritsan NP, Yurkovskaya AV. 1998. Solvent effect on the rate of β-scission of the tert-butoxyl radical. J Phys Chem A 102:7975–7980.
16. Ilan Y, Rabani J, Henglein A. 1976. Pulse radiolytic investigations of peroxy radicals produced from 2-propanol and methanol. J Phys Chem 80:1558–1562.
17. Marklund S. 1971. The simultaneous determination of bis (hydroxymethyl)-peroxide (BHMP), hydroxymethylhydroperoxide (HMP), and H2O2 with TITANIUM (IV). Equilibria between the peroxides and the stabilities of HMP and BHMP at physiological conditions. Acta Chem Scand 25:3517.
18. Russell GA. 1955. The competetive oxidation of cumene and tetralin. J Am Chem Soc 77:4583–4590.
19. Bolland JL, Cooper HR. 1954. The Photo-sensitized oxidation of ethanol. Proc Royal Soc 225A:405.
20. Gray P, Herod AA, Jones A. 1971. Kinetic data for hydrogen and deuterium atom abstraction by methyl and trifluoromethyl radicals in the gaseous phase. Chem Rev 71:247–294.
21. Hendry DG, Mill T, Piszkiewicz L, Howard JA, Eigenmann HK. 1974. A critical review of H-atom transfer in the liquid phase: Chlorine atom, alkyl, trichloromethyl, alkoxy, and alkylperoxy radicals. J Phys Chem Ref. Data 3:937–978.
22. Karki SB, Treemaneekarn V, Kaufman MF. 2000. Oxidation of HMG-CoA reductase inhibitors by tert-botoxyl and 1,1-diphenyl-2-picrylhydrazyl radicals: Model reactions for predicting oxidatively sensitive compounds during preformulation. J Pharm Sci 89:1518–1524.
23. Kúti Z, Gál D. 1992. On the mechanism of methanol oxidation sensitized by anthraquinone. Ber Bunsenges Phys Chem 96:1843–1847.
24. Yousry MA, Naguib CS, Cohen SG, Young MA. 1987. Photoreduction of benzophenone by acetonitrile: Correlation of rates of hydrogen abstraction from RH with the ionization potentials of the radicals R. J Phys Chem 91:3033–3036.
25. Donahue NM. 2003. Reaction barriers: Origin and evolution. Chem Rev 103:4593–4604.
26. Donahue NM. 2001. Revisiting the hammond postulate: The role of reactant and product ionic states in regulating barrier heights, locations, and transition state frequencies. J Phys Chem A 105:1489–1497.
27. Donahue NM, Clarke JS, Anderson JG. 1998. Predicting radical-molecule barrier heights: The role of the ionic surface. J Phys Chem A 102:3923–3933.
28. Lias SG, Liebman JF. 2005. Ion energetics data. In: Linstrom PJ, Mallard WG, editors. NIST chemistry WebBook, NIST standard reference database number 69. Gaithersburg MD, 2089: National Institute of Standards and Technology. http://webbook.nist.gov.
29. Paul V, Roberts BP. 1987. Polarity reversal catalysis of hydrogen atom abstraction reactions. J Chem Soc Chem Commun 17:1322–1324.
30. Wang Q. 1987. Analysis for trace impurities in methanol. Shiyou Huangong 16:654–657.
31. Deshmukh BK, Coetzee JF. 1984. Characterization of reactive impurities in methanol, ethanol, and 2-propanol by monitoring the activities of added ionic probes with ion selective electrodes. Anal Chem 56:2373–2378.
32. Watkins MA, Harmon PA, Pitzenberger SM, Van Nostrand V, Bass LC, Templeton AC. Revision to the AIBN stress testing method: Identification and elimination of alkoxyradical-mediated drug degradation artifacts (in preparation).
33. Baertschi SW, Alsante KM. 2005. Stress testing: The chemistry of drug degradation. In: Baertschi SW, editor. Pharmaceutical stress testing, drugs and the pharmaceutical sciences, Vol. 153. Boca Raton: Taylor and Francis. Chapter 3.