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Research Paper

The Effect of Inert Atmospheric Packaging on Oxidative Degradation in Formulated Granules

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Purpose. Oxidative degradation of drug substances in pharmaceutical products is well documented and is thought to occur in many cases via autoxidative processes involving headspace molecular oxygen in the primary package. Reducing the headspace oxygen concentration inside a package could thus be an option for reducing oxidative degradation in pharmaceutical products. The purpose of this study is to examine the effect of headspace oxygen concentration and relative humidity (RH) on the oxidative degradation of a model pharmaceutical formulation.
Methods. Model formulations, including a drug substance known to exhibit oxidative degradation, at two different drug/excipient ratios were packaged in stoppered glass vials maintained at different oxygen concentrations, (from 0% to 20.9%) and headspace relative humidities and were stored at 40°C. The oxidative degradation was quantified as a function of time.
Results. The results clearly show dependence of oxidative degradation on headspace oxygen concentration, relative humidity, drug loading and time.
Conclusions. The results provided insight into the effectiveness of inert atmospheric packaging (IAP) for protecting oxidation-labile products. In light of these observations, a few strategies for practically implementing inert atmosphere packaging are also presented.

KEY WORDS: auto-oxidation; headspace oxygen concentration; headspace relative humidity; inert atmosphere packaging; oxygen scavenger.

INTRODUCTION

One of the most common modes of drug degradation is oxidation (1). Although exact mechanistic details on what governs the promotion of reactions between drug substances and molecular oxygen in pharmaceutical formulations is not fully understood, it is generally thought that many such reactions fall under the category of autoxidation processes (1–3):

Initiation	$In\bullet + R\text{-}H \longrightarrow R\bullet + In\text{-}H$
Propagation	$R\bullet + O_2 \rightarrow ROO\bullet$
	$R\bullet + R\text{-}H \rightarrow R\text{-}H\bullet$
	$R - OO\bullet + R\text{-}H \longrightarrow ROOH + R\bullet$
Termination	$2ROO\bullet \longrightarrow$ Molecular Products

where In • is an unknown radical initiator and R-H can either be the drug substance, excipients, or contaminant. As shown above, whether mediated through excipients or directly with the drug, molecular oxygen is involved in the propagation step of this reaction and is integral to the catalytic cycle responsible for the generation of oxidative degradation of drug substances in pharmaceutical formulations.

Oxidative degradation of the active drug substance in a formulation leads to a lowering of drug potency as well as increased levels of oxidative degradation products, both of which may lead to reduced product shelf life (2). The rate of oxidative degradate formation is dependent to both the structural susceptibility of the specific drug substance to autoxidize and storage (or reaction) conditions such as temperature, humidity, oxygen concentration, and time. Other deleterious effects of oxidative processes that have been noted include product discoloration, changes in dissolution rate/profile, precipitation, and the generation of foul odors and flavors (1). Most importantly, oxidative degradation products generated in the final pharmaceutical product upon storage may also have adverse pharmacological properties, including those related to toxicity or adverse side-effects.

Packaging with an inert gas blanket over the final product seems a prudent option for minimizing degradation in formulations susceptible to oxidation. By removing from the package one of the two key reactants in the autoxidation cycle, important gains can be made in product shelf-life and quality of the product reaching the consumer. Packaging oxygen-sensitive products under an inert atmosphere is a process widely adopted to increase the shelf-life of other oxygen-sensitive commercial products, including foodstuffs and medical devices. While inert atmosphere packaging has taken hold in the parenteral arm of the pharmaceutical industry, there are relatively few examples of solid dosage forms that are packaged under reduced oxygen levels.

The purpose of the research presented here is to determine if a relationship between headspace oxygen concentration and oxidation of formulated drug product existed. A drug product proven to be prone to oxidation was packaged under different % oxygen concentrations and % relative hu-

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Table I. Granule Formulations

Formulation	A (low drug loading)	B (high drug loading)
Compound X	3.3	31.6
Avicel PH 101	90.7	62.4
HPC-EXF	3.0	3.0
Croscarmellose sodium	3.0	3.0
200 Proof EtOH	25[a]	43[a]

[a] Removed during overnight tray drying (ca. 33°C).

midities. The samples were then analyzed periodically to evaluate degradation of samples as a function of the relevant variables.

MATERIALS AND METHODS

The materials and methods used for preparation and packaging of the drug product are presented in this section along with the study design and techniques used to monitor headspace oxygen concentration, relative humidity and drug assay.

Materials

The drug substance X used in this study was prepared at Merck & Co., Inc. Avicel (PH 101) and CROSCARMELLOSE SODIUM used in the formulation were procured from the FMC corporation (Philadelphia, PA, USA), while the HPC-EXF (hydroxypropyl cellulose-EXF Klucel) was obtained from the Hercules/Aqualon Limited (Wilmington, DE, USA). The granulating fluid used was 200 proof ethanol (Fisher Scientific, Fairlawn, NJ, USA). Standards containing various concentrations of oxygen in nitrogen were prepared from double-gravimetrically certified gas cylinders (Scott Specialty Gases, Plumsteadville, PA, USA). Packaging components used in the study were 30 ml amber glass vials (Schott, Lebanon, PA, USA) with 20-mm rubber stoppers and flip-off aluminum crimp seals (West Pharmaceutical Services, Lionville, PA, USA) along with 75 ml HDPE (high-density poly ethylene) bottles (Merck, West Point, PA, USA) with 33-mm caps with FIS (Owens-Illinois, Toledo, OH, USA). Two different kinds of oxygen scavengers were also used in the study: a polymer based RP Grade and an iron based FH-100E (Mitsubishi Gas Chemical America Inc. New York, NY, USA) and silica-gel based desiccant (Sud-Chemie, Belen, NM, USA). Acetonitrile, methanol, 85% phosphoric acid, triethylamine and potassium phosphate (monobasic) used as analytic reagent and solvents in this work were purchased from Fisher Scientific.

Table II. Target Headspace Compositions, Study 1

Target % RH in sealed glass vials	Target % oxygen concentration in sealed glass vials, balance: nitrogen	No. of samples
0	0	6
20	0	6
20	0 (Oxygen scavenger RP grade)	6
20	0.1	6
20	1.0	6
20	3.0	6
20	20.9	6
75	0	6
75	0 (Oxygen scavenger FH grade)	6
75	0.1	6
75	1.0	6
75	3.0	6
75	20.9	6

Table III. Target Headspace Compositions, Study 2

Target % RH in sealed glass vials	Target % oxygen concentration in sealed glass vials, balance: nitrogen	No. of samples
35	0	4
35	0 (Oxygen scavenger FH grade)	4
35	0.1	4
35	1.0	4
35	5.0	4
35	20.9	4
55	0	4
55	0 (Oxygen scavenger FH grade)	4
55	0.1	4
55	1.0	4
55	5.9	4
55	20.9	4

Granule Preparation

Oxidation-prone active ingredient (X) was formulated into granules (drug product) at two different drug loadings using compositions known to be susceptible to oxidation. The formulations used are summarized in Table I.

A 50 g batch of formulations A (low drug loading) and B (high drug loading), respectively were prepared by wet granulation using ethanol as the granulation fluid in a LB Bohle Mini Granulator. The material was screened through a 30 mesh screen into a 500 ml bowl. The mixture was dry blended for about 2 min prior to addition of the granulation fluid. The granulation fluid was added for approximately 3 min; the granulation end-point was determined based on visual observation. The chopper was run at 1000 rpm and the impeller at

Table IV. Formulation Content Uniformity

Replicate	Weight of sample (mg)	Active (area)	Wt corrected area of active[a]
Formulation A			
1	101.91	8820111	8988575
2	102.11	8467825	8646496
3	98.30	8579844	8433987
4	102.14	8837834	9026964
5	101.75	8708823	8861227
	Mean	8682887	8791450
	RSD (%)	1.8	2.8
Formulation B			
1	101.35	7960622	8068090
2	99.18	7784424	7720592
3	100.52	8027524	8069267
4	100.50	7685076	7723501
	Mean	7864412	7895363
	RSD (%)	2.0	2.5

[a] Targeted weight of sample was 100 mg. Calculation for weight corrected area is (weight of sample × 100)* area of active.

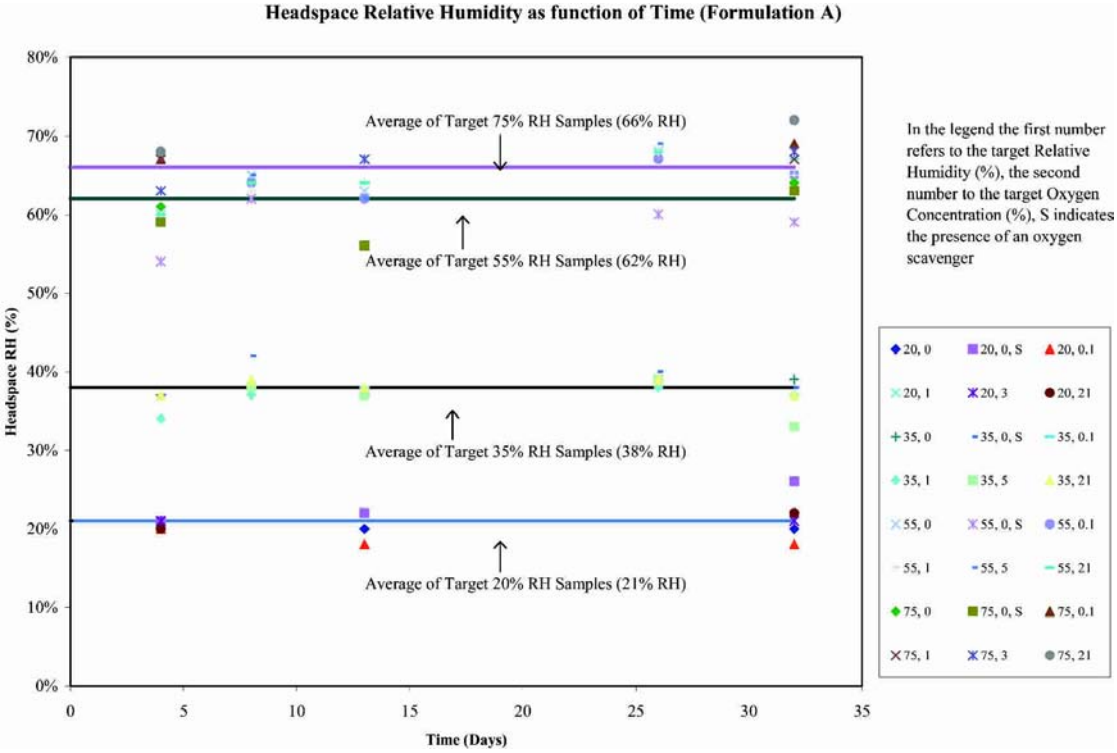

Fig. 1. Headspace relative humidity as a function of time (formulation A).

500 rpm both during dry blending and granulation. The wet granules were then dried at 33°C for approximately 24 h. This was followed by passing the granulation through a 30 mesh screen prior to storage in a sealed glass bottle.

Study 1: Packaged Sample Preparation

The two granule formulations (A and B) were packaged and stored at a variety of oxygen and headspace relative humidity conditions. Approximately 300 mg of formulations A and B were weighed and placed in 30-ml glass vials. An appropriate amount of pre-equilibrated silica-gel desiccant (at different RHs) was placed in the glass vials with the formulation to maintain constant RH after the vials were sealed. The vials were placed in a glove bag and "stoppered" under the appropriate gas compositions. The vials were then removed from the glove bag and an aluminum flip-top cap was

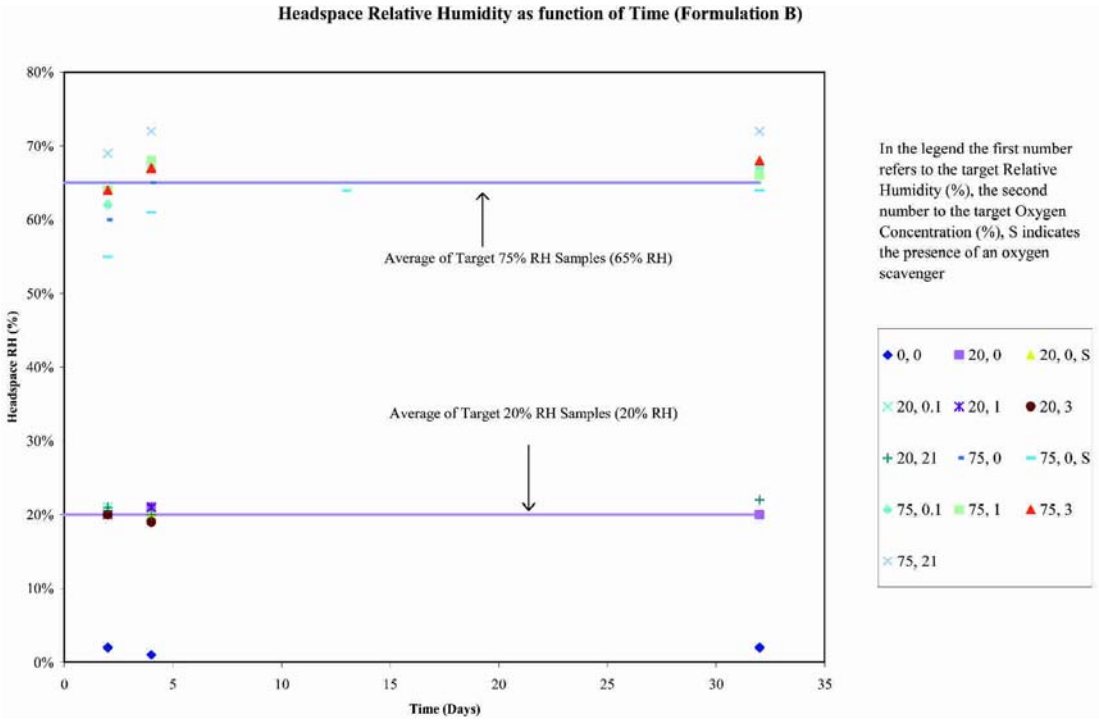

Fig. 2. Headspace relative humidity as a function of time (formulation B).

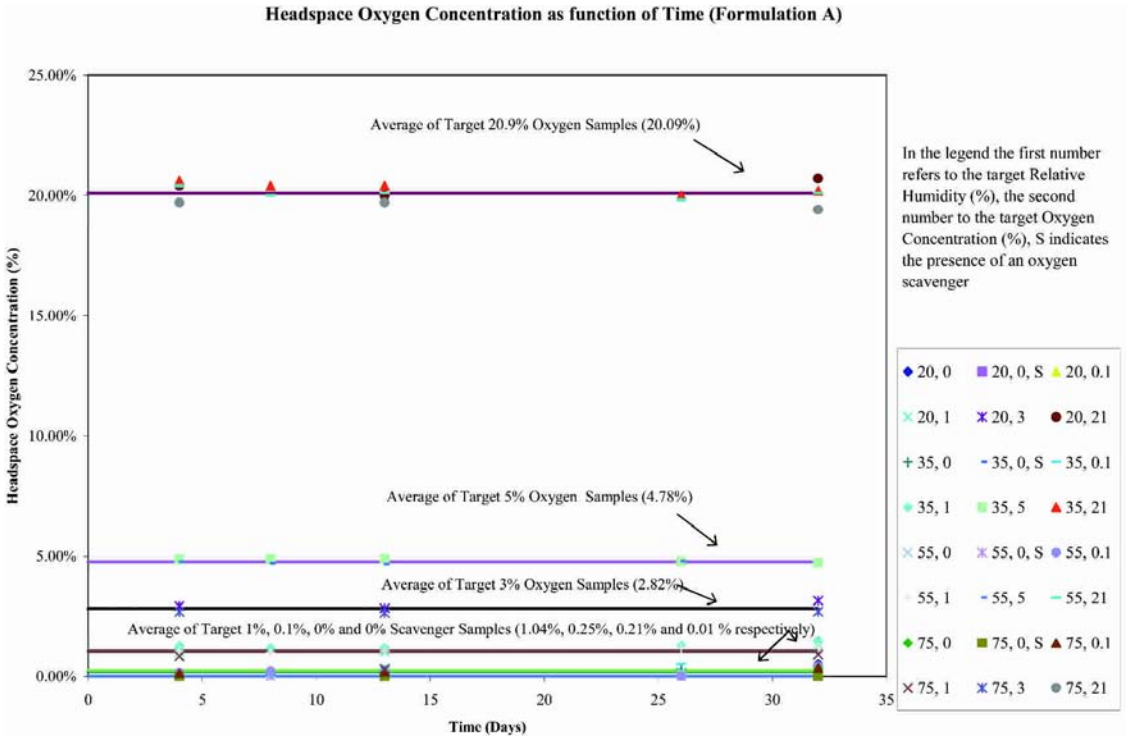

Fig. 3. Headspace oxygen concentration as a function of time (formulation A).

applied to seal the vial. Six control samples of low RH (<2%) were prepared for each formulation by placing two grams of pre-dried desiccant into the vials and stoppering the vials under nitrogen. Oxygen scavenger was included in some of the vials to ensure near-zero concentration as a backup to the vial sealed under pure nitrogen. Based on vendor specifications, the RP Grade oxygen scavengers were used for the low relative humidity conditions and the FH grade oxygen scavenger was used for the higher humidity stations. Details on the various headspace conditions used are summarized in Table II. All samples were placed in a 40°C (±1°C) for accelerated stability testing.

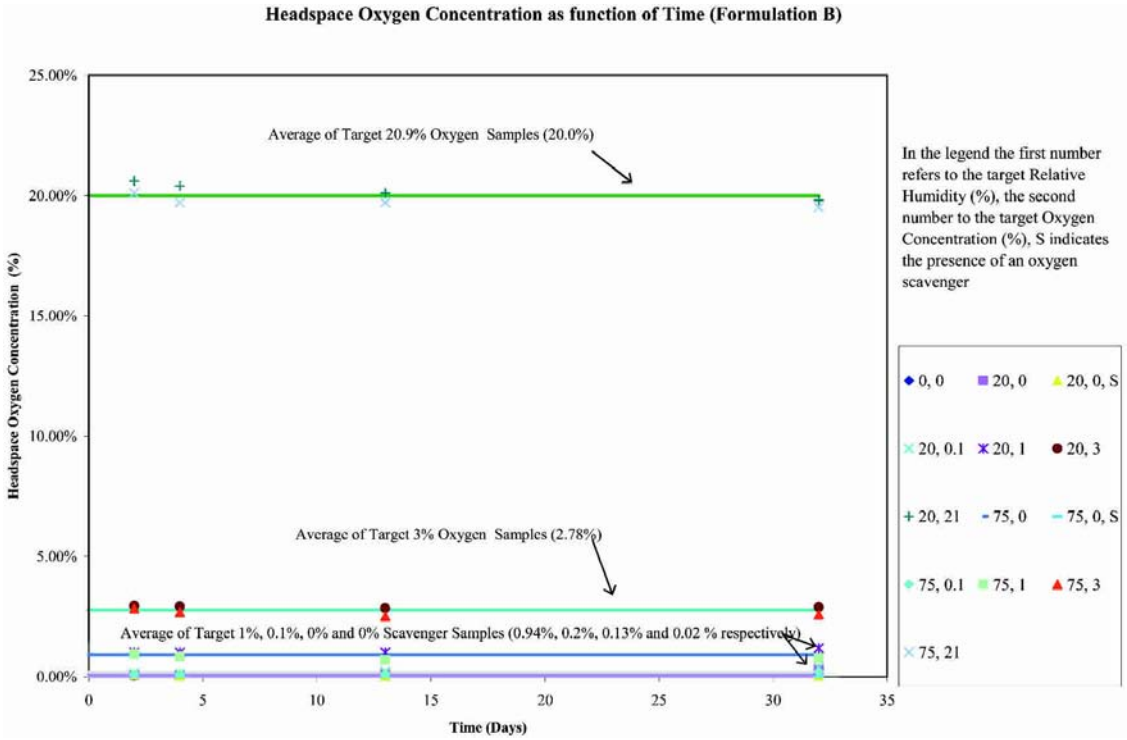

Fig. 4. Headspace oxygen concentration as a function of time (formulation B).

Table V. Impact of Oxygen Scavengers on Headspace Oxygen Concentration: Glass Vials

Nominal conditions		Oxygens readings			
		Time			
%RH	%Oxygen	4 weeks	13 weeks	32 weeks	Mean
Formulation A					
20%	0%	0.06%	0.21%	0.51%	0.26%
20%	0% (RP scavenger)	0.02%	0.02%	0.11%	0.05%
35%	0%	0.10%	0.15%	0.35%	0.21%
35%	0% (FH scavenger)	0.00%	0.00%	0.00%	0.00%
55%	0%	0.11%	0.17%	0.35%	0.19%
55%	0% (FH scavenger)	0.00%	0.00%	0.00%	0.00%
75%	0%	0.06%	0.12%	0.32%	0.17%
75%	0% (FH scavenger)	0.00%	0.00%	0.00%	0.00%
Formulation B					
0%	0%	0.06%	0.12%	0.28%	0.13%
20%	0%	0.06%	0.13%	0.29%	0.14%
20%	0% (RP scavenger)	0.02%	0.02%	0.01%	0.03%
75%	0%	0.06%	0.12%	0.24%	0.12%
75%	0% (FH scavenger)	0.00%	0.00%	0.00%	0.00%

Study 2: Packaged Sample Preparation

Based on results of the first study, a second study was set up with formulation A (low drug loading formulation) to further evaluate the correlation between oxygen concentration, relative humidity and autooxidation. Approximately 100 mg of the granulation was sealed in 30 ml vials under the selected headspace oxygen concentrations and relative humidity conditions. Table III summarizes the headspace conditions examined in the study.

Study 3: Packaged Sample Preparation

The effectiveness of oxygen scavengers in maintaining a low oxygen environment in realistic pharmaceutical packages was also evaluated. HDPE bottles (75 ml) and FH Grade oxygen scavengers were used in this study. Each HDPE bottle contained 5 g of preconditioned desiccant and an oxygen scavenger sachet. The desiccant was preconditioned to produce internal RH of ~33%. Once properly conditioned, the bottles were hand sealed in a glove bag under nitrogen, induction sealed, and stored at 40°C ambient. At predetermined time points, the oxygen concentration was measured. A control sample was also sealed in a similar manner but without an oxygen scavenger.

Table VI. Headspace Oxygen Concentration (%): 75 ml HDPE Bottle with Foil Induction Seal with FH Grade Oxygen Scavenger

Target headspace RH	Bottle	Oxygen concentration (%)					
		Time-point (weeks)					
		0.5	4	8	12	16	24
33%	1	0.02	0.00	0.00	0.00	0.00	0.00
	2	0.02	0.00	0.00	0.00	0.00	0.00
	3	0.02	0.00	0.00	0.00	0.00	0.00
	Avg	0.02	0.00	0.00	0.00	0.00	0.00

Headspace Oxygen Concentration and Relative Humidity Measurement

The % oxygen and the relative humidity in the headspace of the vials were monitored throughout the study. A PBI Dansensor CheckMate 9900 O_2/CO_2 (PBI Dansensor A/S, Ringstëd, Denmark) analyzer equipped with a solid-state zirconia ion-selective electrode for oxygen determination was used for measuring the oxygen concentration (4). The instrument was allowed to warm up for 10 min prior to taking measurements and was calibrated according to the vendor's specification. The instrument was set to withdraw 2 ml of headspace using a small internal diaphragm pump, which fed the headspace sample into a small cell containing the measurement electrode. A lighthouse Instruments FMS-1400 (Lighthouse Instruments, Charlottesville, VA, USA) headspace moisture analyzer equipped with a tunable diode laser source (600 nm) and photodiode detector was used for non-destructive RH determination. The instrument was allowed to equilibrate for 30 min prior to taking measurement. The sample holder was constantly purged with dry nitrogen set at a flow rate of 3 standard liters/minute and measurements were acquired at a sampling rate of 100 Hz. All RH measurements were made at 40°C.

Drug Assay

High-performance liquid chromatography (HPLC) was used for drug assay and degradate quantification. The samples were dispersed in diluent containing 25% acetonitrile/ 75% H_2O and injected onto a Waters Symmetry Shield RP_{18} (250 × 4.6 mm) 5 μm particle size column. The elution was a gradient with mobile starting at 18% acetonitrile/82% 30 mM KH_2PO_4 buffer with 0.1% TEA, pH 2.4. Detection was by UV absorbance at 245 nm. The chromatographic conditions are summarized below.

Column: Waters symmetry shield, RP_{18}, 250 × 4.6 mm, 5-μm particle size
Temperature: 25°C
Flow rate: 1 ml/minute
Detection: UV absorbance at 245 nm
Injection volume: 20 μl
Run time: 60 min
Mobile phase A: 18% acetontrile/82% 30 mM KH_2PO_4 buffer with 0.1% TEA, pH 2.4
Mobile phase B: acetonitrile

Gradient conditions

Time (min)	% Mobile phase A	% Mobile phase B
0	100	0
15	95	5
25	88	12
35	88	12
50	61	39
51	100	0
60	100	0

The drug molecule was chosen due to its propensity for oxidative degradation under both elevated heat and humidity

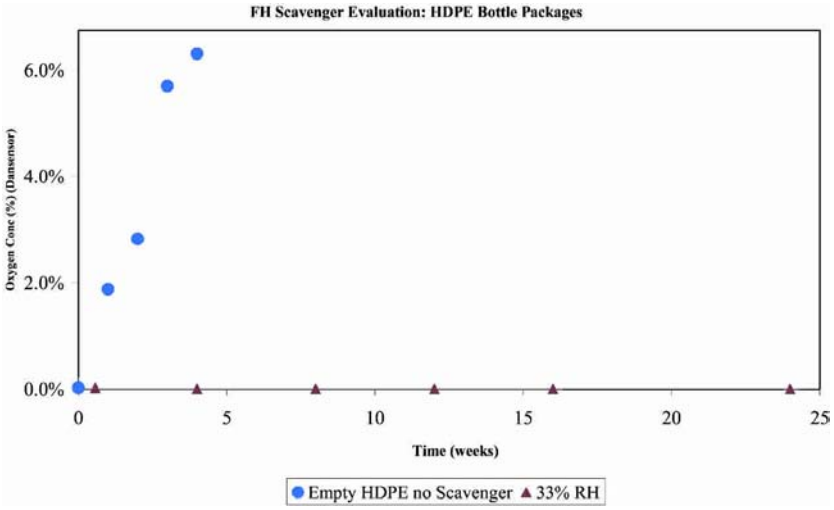

Fig. 5. Impact of oxygen scavengers on headspace oxygen concentration: HDPE bottle package.

conditions. In the solid state, there were five major degradates formed. There were also two dimers formed from both the primary oxidative products and the active, and several smaller unidentified structures only seen under extreme conditions. All degradates (area percentages in the chromatograph) were summed together for analysis purposes, and the response factor for all degradates was assumed to be one. All degradation occurred either on the piperidine or pyridine rings (Appendix 1 details the relevant drug and degradate structures). The primary degradate (M - 4 in the Appendix) was formed by the conjugation of the piperidine ring. There was also N-oxide formation which was observed only on the pyridine ring. All other known degradates were formed on the carbon alpha to the nitrogen through the addition of oxygen to on the piperidine ring. The degradates specified here were confirmed by liquid chromatography (LC)/mass spectroscopy (MS)/mass spectroscopy (MS).

RESULTS

This section presents the results of the various studies and includes: assessment of the suitability of the initial drug product, headspace conditions (relative humidity and oxygen concentration) and observed degradation in the various sample vials as a function of time and impact of oxygen scavenger on headspace oxygen concentration.

Table VII. Total Degradates as a Function of Time (Formulation A)

Nominal conditions		True mean conditions		Total degradates %				
				Time				
%RH	%Oxygen	RH (%)	Oxygen Conc. (%)	4 weeks	8 weeks	13 weeks	26 weeks	32 weeks
20%	0%	20	0.26	0.47	N/A	0.91	N/A	1.9
20%	0% Scavenger	23	0.05	0.47	N/A	0.84	N/A	1.6
20%	0.1%	19	0.27	0.47	N/A	0.9	N/A	1.8
20%	1%	21	1.10	1.06	N/A	0.97	N/A	2.1
20%	3%	21	2.97	0.59	N/A	1.01	N/A	1.4
20%	21%	21	20.37	0.55	N/A	1.18	N/A	2.4
35%	0%	38	0.21	0.94	0.61	1.4	N/A	1.6
35%	0% Scavenger	39	0.00	3.54	0.34	0.9	0.41	0.67
35%	0.1%	38	0.33	1.36	0.93	1.9	2.24	N/A
35%	1%	37	1.29	4.61	1.65	2.4	2.75	3.7
35%	5%	37	4.82	5.78	2.22	2.8	3.73	4.56
35%	21%	38	20.32	8.14	2.47	2.9	4.65	5.47
55%	0%	62	0.19	0.92	0.49	1.3	0.95	0.95
55%	0% Scavenger	60	0.00	0.58	0.43	1.2	0.66	0.41
55%	0.1%	63	0.19	0.73	1.78	2.1	1.86	2.59
55%	1%	63	1.09	3.10	8.41	7.1	11.61	13.2
55%	5%	62	4.74	4.56	11.27	10.7	16.96	N/A
55%	21%	62	20.08	4.64	12.29	10.8	19.67	19.48
75%	0%	64	0.17	0.47	N/A	0.41	N/A	2.5
75%	0% Scavenger	63	0.00	3.07	N/A	0.75	N//A	1.8
75%	0.1%	68	0.22	0.89	N/A	1.37	N/A	2.8
75%	1%	67	0.68	3.55	N/A	9.58	N/A	11.2
75%	3%	66	2.67	5.19	N/A	14.32	N/A	11.5
75%	21%	70	19.60	7.59	N/A	19.89	N/A	15.4

Table VIII. Total Degradates as a Function of Time (Formulation B)

Nominal conditions		True mean conditions		Total degradates %		
%RH	Oxygen conc. (%)	RH (%)	Oxygen conc. (%)	4 weeks	13 weeks	32 weeks
0%	0%	1	0.13	0.31	0.76	1.3
20%	0%	20	0.14	0.29	0.73	1.0
20%	0	(RP 20 Scavenger)	0.03	0.30	0.73	0.6
20%	0.1%	21	0.23	0.26	0.78	0.6
20%	1%	21	1.05	0.32	0.70	0.6
20%	3%	19	2.90	0.32	0.74	0.6
20%	21%	21	20.23	0.32	0.77	0.7
75%	0%	63	0.12	0.30	0.66	0.8
75%	0	(FH 61 Scavenger)	0.00	0.26	0.65	0.7
75%	0.1%	65	0.16	0.30	0.84	1.2
75%	1%	66	0.82	0.73	2.36	2.1
75%	3%	66	2.65	0.89	2.82	2.8
75%	21%	71	19.75	1.08	3.66	3.1

Content Uniformity of Initial Granules

Content uniformity was determined using five replicate preparations of initial granules for both formulations by HPLC. There was no degradation observed on the initial analysis. The results are summarized in Table IV. Both formulations exhibited reasonable content uniformity.

Headspace Conditions

The oxygen concentration and the relative humidity in the headspace of the samples were monitored throughout the study and the results are summarized in Figs. 1 through 4. The headspace in the vials indeed stayed at a reasonably constant RH (less than 5% variation in all cases) over the length of the study. Note that the target RH does not completely agree with the actual value in some cases; this however, has no impact on the interpretation of the data. The headspace oxygen concentration also remained essentially constant over the length of the study.

Evaluation of Oxygen Scavenger

Oxygen scavengers were effective in maintaining a low oxygen concentration both in the glass vials used in studies 1 and 2 (for both RP grade and FH grade oxygen scavengers) as well as HDPE bottle packages in study 3 (for the FH grade oxygen scavengers). Table V compares the headspace oxygen concentration in samples from studies 1 and 2 with and without oxygen scavengers, while Table VI and Fig. 5 highlight the performance of the oxygen scavenger in a HDPE bottle package. The data clearly indicates that the FH grade oxygen scavenger can maintain a virtually zero oxygen concentration environment in an HDPE bottle package for a prolonged time period.

Observed Drug Assay/Total Degradation

Samples were assayed by the HPLC. Tables VII and VIII below provide a summary of degradate growth along with the average headspace condition. The next section discusses the implications and conclusions that can be drawn from this data.

DISCUSSION

The results and data presented above suggest that the relative humidity, oxygen concentration, time and drug load all affect the ultimate stability of the drug product. Furthermore, there seems to be significant interaction between these different factors. To help guide the ensuing discussion, the various experiments and results are summarized in Table IX.

Table IX. Summary of Experiments and Corresponding Data/Results

Experiments	Data/results
Studies 1 and 2: Study 1 included vials containing both low and high drug loading formulations stored at various constant oxygen and relative humidity stations for probing the impact of headspace relative humidity, oxygen concentration, and drug loading on product degradation. Study 2 was an extension of Study 1.	Figures 1 through 4 present confirmation of the ability of the packaging system in maintaining constant temperature and relative humidity environment over the length of the study. Figures 6 through 9 and Tables VII and VIII present the impact of headspace relative humidity, oxygen concentration, and time on observed degradation. Figure 10 presents the impact of drug loading on observed degradation. Table V presents the impact of oxygen scavengers on oxygen concentration in the headspace of the vials. Table X presents the impact of drug-excipient ratio on observed degradation. Table XI presents the impact of the amount of oxygen on observed degradation.
Study 3: Study 3 was for probing the effectiveness of oxygen scavenger for reducing oxidation in the HDPE bottle.	Figure 5 and Table VI present the impact of oxygen scavenger on headspace oxygen concentration in an HDPE bottle package.

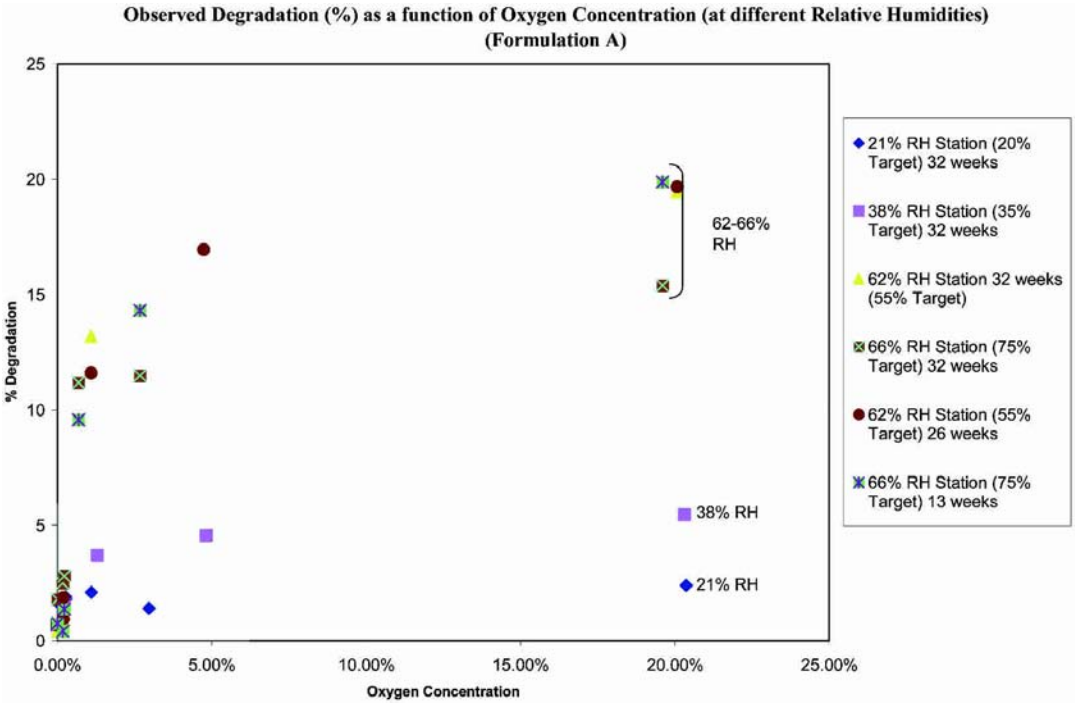

Fig. 6. Observed degradation as a function of oxygen concentration (formulation A).

Effect of Relative Humidity and Oxygen Concentration

Increasing either relative humidity or oxygen concentration does lead to higher degradation. The effects of increasing oxygen concentrations are more pronounced at higher relative humidities (Figs. 6 and 8) [or the effects of increasing relative humidity are more pronounced at higher oxygen concentrations (Figs. 7 and 8)]. Figure 9 highlights the combined impact of oxygen concentration and RH on observed degradation for formulation A. At low RH (the nominal 20% station), the granules were reasonably stable at all oxygen concentrations and there was no dependence of observed degradation on the oxygen concentration. At the intermediate (the target 35% RH, actual 38% RH station) and higher (the target 55% RH, actual 62% and the target 75% RH, actual 66% RH stations) relative humidities, there appears to be a strong asymptotic dependence of the observed degradation on the oxygen concentration; samples at higher RHs showed a much higher loss in assay relative to the intermediate RH samples. Furthermore, at intermediate and

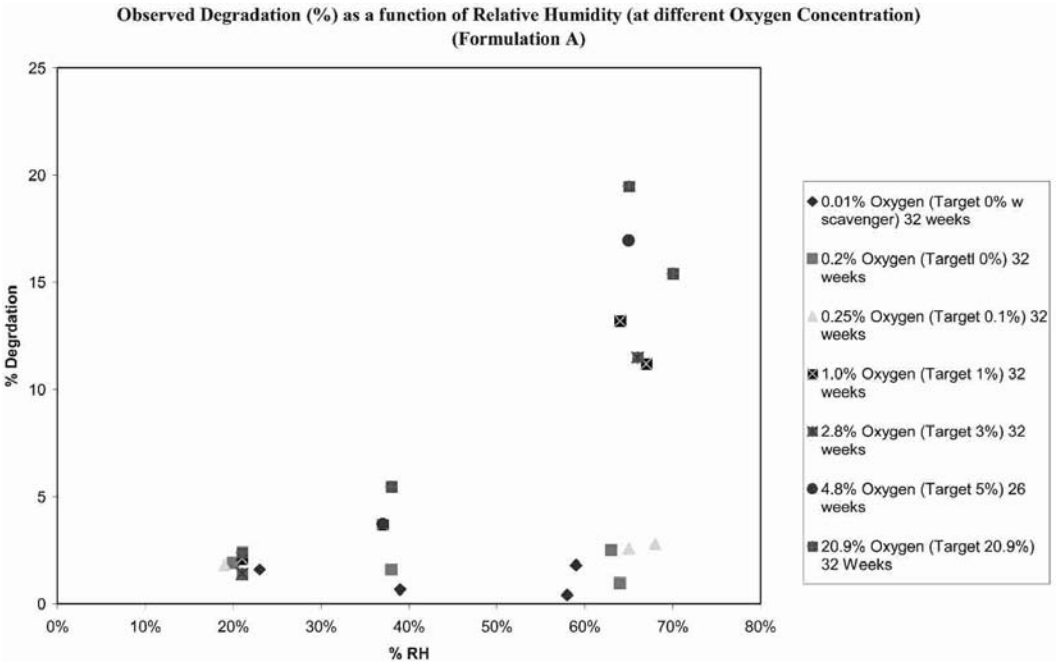

Fig. 7. Observed degradation as a function of relative humidity (formulation A).

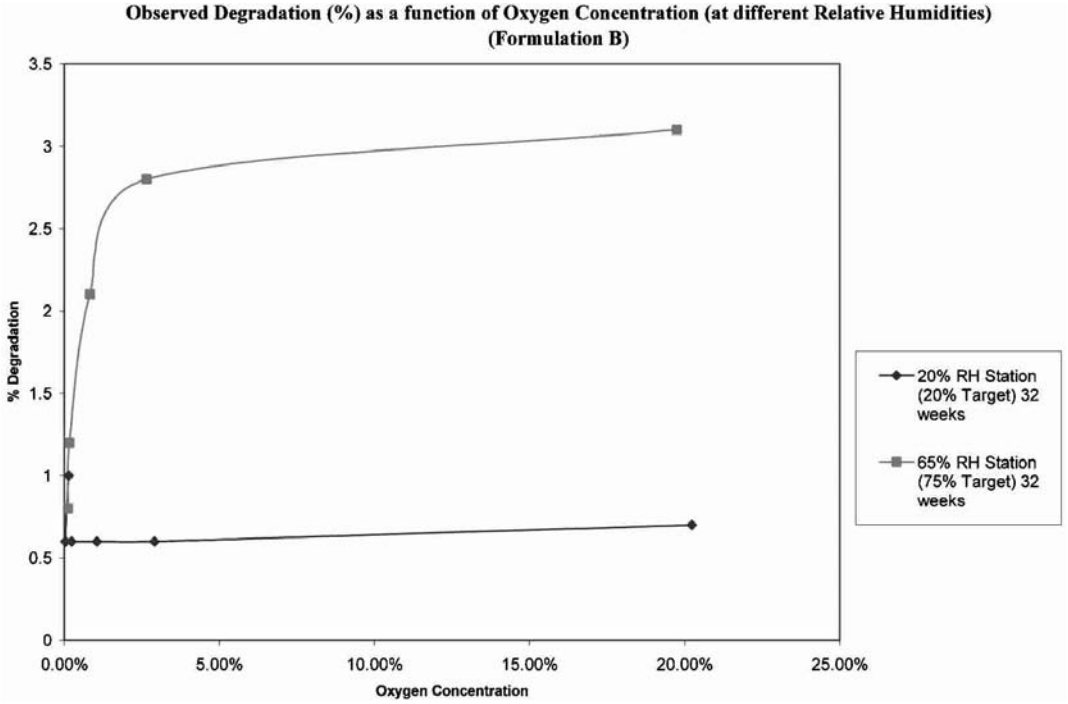

Fig. 8. Observed degradation as a function of oxygen concentration (formulation B).

high RHs, there appears to be a pronounced difference between the samples stored at 0.25% oxygen (the target 0.1% oxygen station) and less vs. samples stored at 1% oxygen and higher with significantly higher loss in assay above 1% oxygen.

The need for relatively low concentrations of oxygen to stave off degradation suggests that molecular oxygen is probably involved in the oxidation reaction but is not rate-limiting once the concentration of oxygen is 1% or higher.

The significant dependence of the assay-loss on RH and the increase in degradation at higher relative humidities might imply that the reaction occurs in sorbed moisture layers on the surface of the crystals. Similar observations have been made on a variety of drug products in the past (1,3). The presence of water is expected to induce higher mobility in the solid state, thus greater interaction between oxidation initiators, potential catalytic impurities such as metal ions and the drug product.

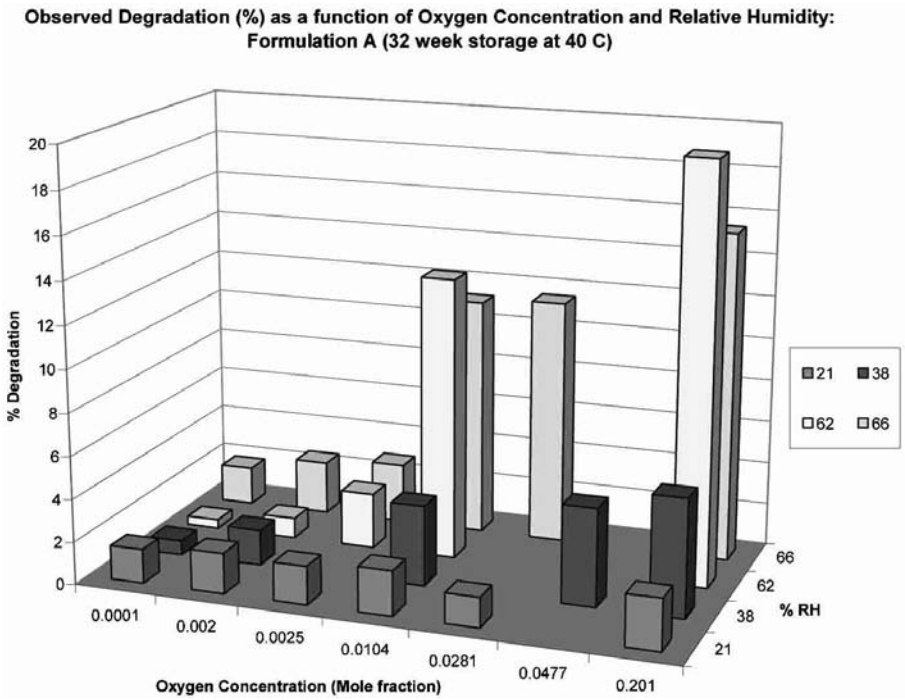

Fig. 9. Observed degradation as a function of oxygen concentration and relative humidity (formulation A).

Effect of Drug Load

As is often observed, much higher loss in assay was seen for the lower drug loading formulation (Formulation A) relative to the high drug loading formulation (Formulation B). Comparison between the two formulations at the high RH station (65%) storage at 32 weeks is shown in Fig. 10 below. The loss in assay with oxygen concentration profiles are similarly asymptotic in both cases, though the asymptote for the high drug loading formulation (formulation B) is about 4 times lower than the low drug loading formulation (formulation A) on a relative scale.

One explanation for the above difference could be that the oxidation initiators or catalysts originate from the excipients. Peroxide impurities in excipients, especially polymeric excipients, are a major source of oxidation in pharmaceutical formulations (3). Table X lists the moles of drug present, moles of drug degraded, ratio of the moles degraded relative to the amount of excipient present for the two formulations at the high RH stations. The higher drug loading formulation interacts with less excipient per mole of drug and also shows less degradation as % of the initial.

An alternate hypothesis for the observed differences could be differing relative amounts of amorphous drug in the two formulations. Because most drug substances are typically less stable in the amorphous form than in a crystalline form (5) one would anticipate more degradation in a formulation with higher amorphous content. The formulations were wet granulated in ethanol and the drug is sparingly soluble in ethanol (4.03 mg/ml). The maximum potential amorphous content generated for the two formulations was estimated by multiplying the amount of ethanol used for each formulation with the solubility of the drug in ethanol. Based on this calculation the maximum amount of amorphous drug in formulation A could be as much as 7.7% of the total drug in the formulation, while for formulation B the maximum amount of amorphous drug could only be 1.3% of the total drug in the formulation.

Effect of Amount of Oxygen in Headspace

Formulation samples stored at 62% and 66% RH (the target 55% and 75% RH) stations provide direct comparison of the effect of amount of oxygen in the headspace relative to the moles of drug in the system. Table XI lists the moles of drug present, degraded as well as the moles of oxygen per mole of drug present in the vials for the two cases. For the 62% RH (the target 55% RH Station) samples 100 mg of sample were stored per 30 ml vial while for the 66% RH station (the target 75% RH station) 300 mg of sample were stored per 30 ml vial. Despite three times as many moles of oxygen available per mole of drug in the 62% RH station vials there was little, if any, difference between the two stations in terms of the observed degradation. This suggests that the oxygen concentration and not the amount of oxygen present in the system is important.

Effect of Time

More degradation was observed in almost all stations with increasing time; however, no clear reaction kinetics pattern was discernible. There does however appear to be a slowdown in the degradation rate at higher extent of degradation. This observation is consistent with the hypothesis that depletion of a key component required for oxidation (potentially initiation sites where the drug and excipient are in close contact or amorphous drug in the formulation) eventually slows down the reaction.

CONCLUSIONS

The study presented the impact of headspace relative humidity, headspace oxygen concentration, drug loading and time on oxidative degradation in a formulated drug product

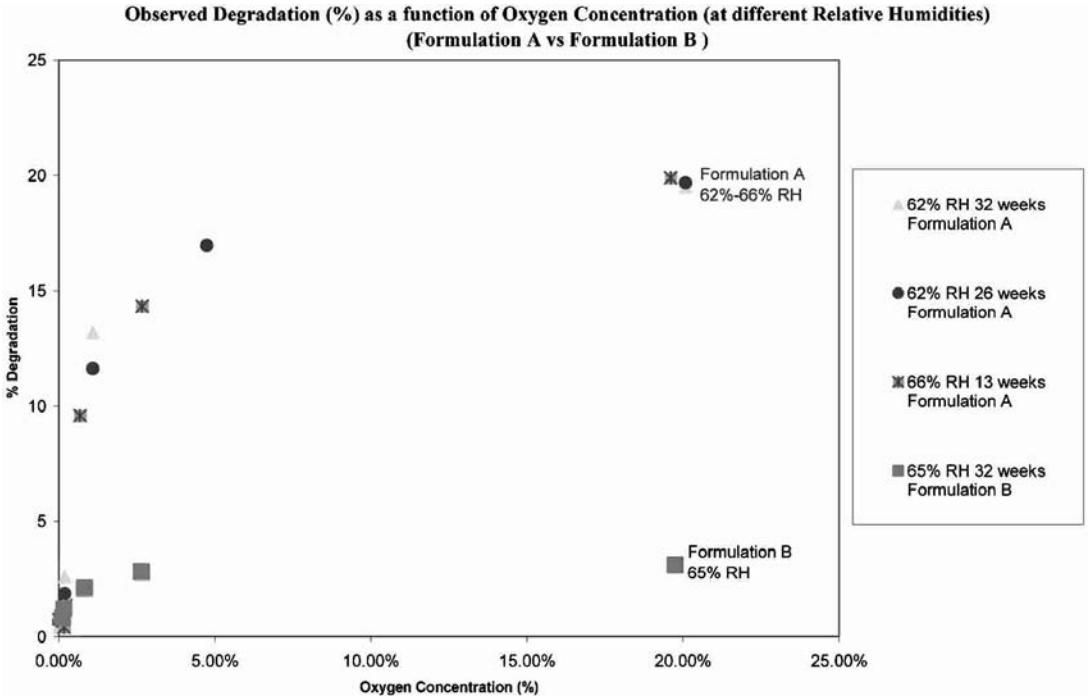

Fig. 10. Impact of drug loading on observed degradation as a function of oxygen concentration.

Table X. Impact of Drug-Excipient Ratio on Observed Degradation

	RH %	Oxygen %	Degradation %	μmoles of drug per vial	μmoles of drug degraded per vial	Milligrams of excipient per μmole of drug	μmoles of drug degraded per gram of excipients
Formulation B	61%	0.01%	0.7	138	0.97	1.67	4.18
(31.6% drug load),	63%	0.12%	0.8	138	1.10	1.67	4.78
high RH (65%),	65%	0.16%	1.2	138	1.66	1.67	7.17
station 32 weeks	66%	0.82%	2.1	138	2.90	1.67	12.5
	66%	2.65%	2.8	138	3.86	1.67	16.7
	71%	19.75%	3.1	138	4.28	1.67	18.5
Formulation A	61%	0.00%	0.41	5	0.02	19.5	0.21
3.3% drug load),	64%	0.19%	0.95	5	0.0475	19.5	0.487
high RH (62%),	65%	0.19%	2.59	5	0.13	19.5	1.33
station 32 weeks	64%	1.09%	13.2	5	0.66	19.5	6.77
	65%	4.74% (26 weeks)	16.96	5	0.848	19.5	8.70
	65%	20.08%	19.48	5	0.974	19.5	9.99

known to be susceptible to oxidation. The data from these experiments clearly show the significant dependence of oxidative degradation on headspace relative humidity and to a lesser degree the role of % oxygen concentration in either aiding or diminishing that effect. Significantly higher relative loss in assay was seen at lower drug loadings and more degradation was observed in almost all stations with increasing time; though no clear reaction kinetics pattern was discernible.

Reducing the headspace oxygen concentration does improve stability, though significant protective benefits are seen only at very low oxygen concentrations (less than 0.25%). For products where oxidative degradation is a concern and where RH control is not a convenient option, one can envision the use of inert atmospheric packaging as a possible protective strategy. These cases could include products where low RH needs to be avoided due to other concerns (e.g., for products encapsulated in gelatin), as low RHs can result in product embrittlement. For blister packages, achieving low internal RH can sometimes imply strict control of the manufacturing, bulk storage, shipping and final packaging areas which can add considerable complexity to the process; in such cases, IAP might again be an option. Even when RH control is an option, the synergistic use of RH and oxygen control is expected to be beneficial. The use of oxygen scavengers in bottles as well as inert atmospheric packaging foil-foil blister lines could be options for the achieving pharmaceutical packages with low oxygen concentrations.

ACKNOWLEDGMENTS

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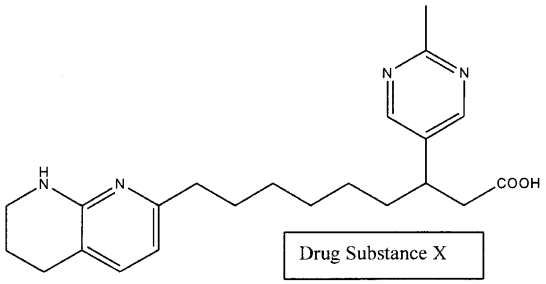
Table XI. Impact of Drug-Oxygen Amount Ratio on Observed Degradation

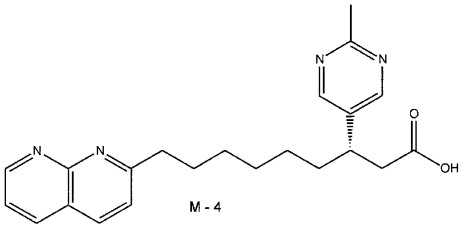
	RH %	Oxygen conc. %	Degradation %	μmoles of drug per vial	μmoles of drug degraded per vial	μmoles of oxygen per vial	μmoles of oxygen per μmole of drug	μmoles of drug degraded per μmole of oxygen present
Formulation B	61	0.00	0.7	138	0.97	0.00	0.00	NA/∞
(31.6% drug load),	63	0.12	0.8	138	1.10	1.4	0.01	0.79
high RH (actual 66%),	65	0.16	1.2	138	1.66	1.87	0.01	0.89
target 75%), station	66	0.82	2.1	138	2.90	9.58	0.07	0.30
32 weeks (~300 mg	66	2.65	2.8	138	3.86	31	0.22	0.12
formulation per vial)	71	19.75	3.1	138	4.28	231	1.67	0.02
Formulation A	58	0.00	0.41	5	0.02	0.00	0.00	NA/∞
(3.3% drug load),	64	0.19	0.95	5	0.0475	2.22	0.44	0.02
high RH (actual 62%,	65	0.19	2.59	5	0.13	2.22	0.44	0.06
target 55%), station	64	1.09	13.2	5	0.66	12.7	2.55	0.05
32 weeks (~100 mg	65	4.74	16.96	5	0.848	55.4	11.07	0.02
formulation per		(26 weeks)						
vial)	65	20.08	19.48	5	0.974	235	46.91	0.00
Formulation A	59	0.00	1.8	15	0.27	0.00	0.00	NA/∞
(3.3% drug load),	63	0.17	2.5	15	0.375	1.99	0.13	0.19
high RH (actual 66%,	68	0.22	2.8	15	0.42	2.57	0.17	0.16
target 75%), station	67	0.68	11.2	15	1.68	7.94	0.53	0.21
32 weeks (~300 mg	66	2.67	11.5	15	1.73	31.2	2.08	0.06
formulation per vial)	70	19.60	15.4	15	2.31	229	15.26	0.01

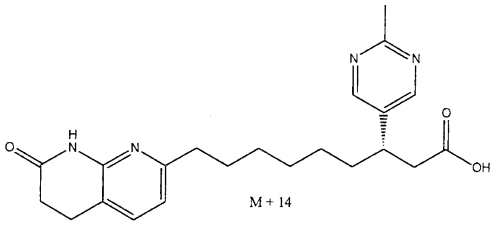
APPENDIX
DRUG AND DEGRADATE STRUCTURES

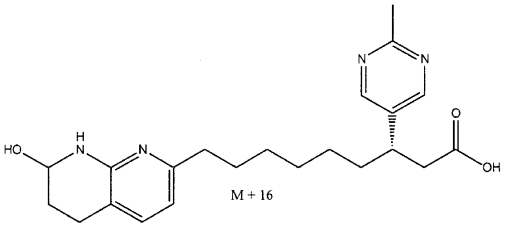
Drug Structure

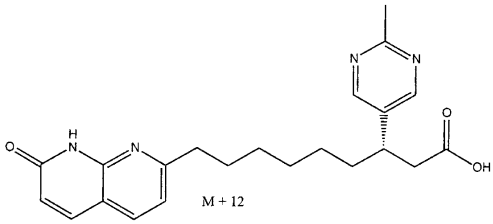


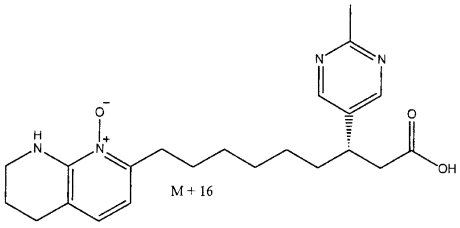
Degradate Structures

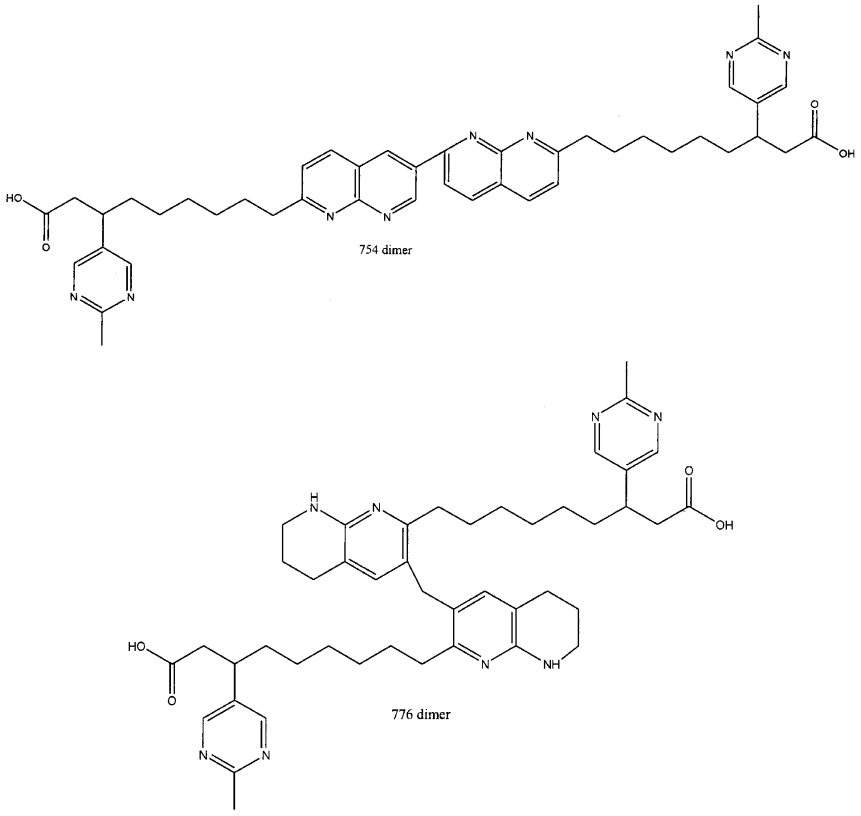












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