Pharmaceutical Development and Technology, 4(4), 475–480 (1999)

RESEARCH ARTICLE

Developing an Injectable Formula Containing an Oxygen-Sensitive Drug: A Case Study of Danofloxacin Injectable

Kasra Kasraian, Anna A. Kuzniar, Gabrielle G. Wilson, and Julia A. Wood

Pfizer Central Research, Groton, Connecticut 06340

Received November 11, 1998; Accepted February 5, 1999

ABSTRACT

The purpose of this study was to assess the impact of impurities in formulation components, antioxidants, formulation pH, and processing/packaging on the extent of color change associated with oxidation of danofloxacin injectable. The methods used in this study include reversed-phase HPLC, UV–VIS spectrophotometry, atomic absorption spectroscopy, visual observation, and iodimetric titration for quantification of the antioxidant. The results from this study revealed that trace impurities from two different excipients significantly contributed to color change associated with oxidation. Polyvinyl pyrrolidone (PVP) introduced trace levels of peroxides into the solution. A second excipient also had a significant impact on stability because it introduced trace metal impurities into the product. The minimization of oxygen levels alone in the solution and headspace was not sufficient to completely eliminate the product instability. The addition of an antioxidant, monothioglycerol (MTG), resulted in a formulation less sensitive to processing variables. The impact of pH on the performance of MTG was also studied. At pH 7.5, MTG resulted in significant improvement in stability; however, at pH 6.0 it was not effective as an antioxidant. Process modifications alone may not be sufficient to prevent oxidation. Chemical approaches, such as pH control, addition of an antioxidant, and control of components should be considered first as means of enhancing stability of oxygen-sensitive solutions.

KEY WORDS**:** *Antioxidants; Liquid formulation; Monothioglycerol; Oxidation; Trace impurities.*

INTRODUCTION

Oxidative decomposition is among the most challenging stability problems faced by formulation chemists. Oxidative decomposition in pharmaceutical preparations is typically referred to as autoxidation. Autoxidation of pharmaceuticals is described as a three-step process mediated by free radicals: initiation, propagation, and termination of the reaction to form byproducts (1). The initiation step can be produced by thermal or light-induced decomposition to form a free radical. The propagation of the free radical oxidation requires oxygen. If molecular

Address correspondence to Kasra Kasraian, Central Research Division, Pfizer Inc., Eastern Point Road, Groton, CT 06340. Fax: (860) 441-3972. E-mail: Kasra_Kasraian@groton.pfizer.com

oxygen, which is required for the propagation step, is removed or reduced significantly, one may be able to substantially eliminate or reduce the oxidative process. Propagation will theoretically continue until no drug/active molecule remains to participate in the chain reaction; however, in practice this does not happen because free radicals may combine to form inactive compounds. This is referred to as the termination step. Termination occurs by combining two radicals or by scavenging them using a free radical inhibitor. Autoxidation is catalyzed by temperature, hydrogen ion concentration, trace metals, trace peroxides, or light (2–5).

One approach to minimizing autoxidation of an oxygen-sensitive formulation is through oxygen control during the manufacture process. Possible steps taken to minimize oxygen in a liquid formulation include the control of manufacturing and packaging operations. The use of a nitrogen sparge during formulation compounding can reduce dissolved oxygen substantially. Exposure of the product to oxygen after packaging can be minimized by packaging the product with an inert gas headspace. In many cases, minimizing oxygen alone is not sufficient to prevent autoxidation, because trace levels of oxygen may be enough to initiate this reaction. A chemical approach to stabilization is the addition of an antioxidant to the formulation. Antioxidants protect oxygen-sensitive formulations by one of three mechanisms (6). First, antioxidants may undergo preferential degradation instead of the drug molecule because of higher oxidation potential. Second, antioxidants may inhibit the free radical chain reaction by serving as an acceptor of free radicals. Finally, antioxidants may inhibit the formation of free radicals (e.g., metal sequestering agents).

This paper reviews some of the points to consider when developing an oxygen sensitive liquid formulation. Although many of the variables involved in oxidative degradation have been reported by others, this paper relates the significance of these variables to practical experiences and specific examples that were encountered during the development of such a product. The examples were derived from a recently developed injectable formulation of danofloxacin (see structure in Fig. 1). During the development of this liquid injectable formulation, a color change subsequently determined to be associated with oxidation was observed during long-term product storage. The factors contributing to this color change and approaches used to prevent this color change are discussed in this paper. During the development of danofloxacin injectable, the impact of impurities in formulation components (excipients/packaging), antioxidants, formulation pH, and processing/packaging on the extent of color change was studied. Each of these variables is discussed in this review using specific examples, with the hope that it will serve as ''best practice'' guidance to formulation chemists developing oxygen-sensitive liquid formulations.

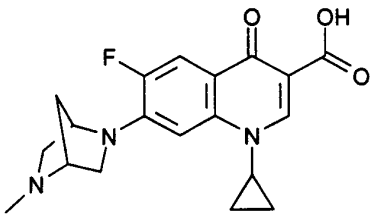

Figure 1. Structure of danofloxacin.

FORMULATION COMPONENT IMPURITIES

Often in the development of pharmaceutical dosage forms there is the need for the use of functional additives. These additives may serve as antioxidants, buffers, bulking agents, chelating agents, antimicrobial agents, solubilizing agents, surfactants, or tonicity-adjusting agents (7). Additives usually provide safe, efficacious, and elegant dosage forms; however, these excipients should be scrutinized because they may in some cases actually contribute to product instability. During the development of danofloxacin injectable, two cases were encountered in which trace impurities in the excipients contributed to the autoxidation of danofloxacin.

The first case involved the excipient polyvinyl pyrrolidone (PVP). During development, it was determined that the presence of PVP in the formulation contributed to the color changes of the product on storage. Further investigation revealed that the stabilization of product color with the removal of PVP was due to trace peroxides present in this component. Polymers, such as PVP, often carry low levels of peroxides. When the peroxide level was controlled in the PVP, the product color change on stability was significantly minimized. Table 1 illustrates how the formulation color stability was impacted when two different sources of PVP were used. Formulations prepared with PVP containing higher levels of peroxides exhibited more color change on stability than those formulations prepared with PVP containing lower levels of peroxides. The significance of trace peroxides on this degradation, apparent by product color changes, was further demonstrated by challenge of the product to varied

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 1

The Effect of Trace Peroxides in PVP and Headspace Gas on Product Color Stability (2 Week Storage at 70°C)

Formulation Containing	Initial Color (Visual Inspection)	Final Color (2 Weeks at 70°C) (Visual Inspection)
PVP (peroxide levels not controlled): Air headspace	Medium yellow	Dark amber (significant color change)
PVP (peroxide levels not controlled): N_2 headspace	Medium yellow	Amber (significant color change)
PVP (peroxide levels controlled to <400 ppm): Air headspace	Medium yellow	Medium yellow (no color change)
PVP (peroxide levels controlled to <400 ppm): N_2 headspace	Medium yellow	Medium yellow (no color change)

headspace (air versus nitrogen purged). The trace peroxides introduced into the formulation through this excipient resulted in more color change than did the presence of an air headspace in the vial. This experience emphasizes the relatively significant effect of trace peroxides on the autoxidation pathway.

The second case involved an additive used as a solubilizing agent. The danofloxacin formulation exhibited varied color stability depending on the supplier of the solubilizing agent used in the formulation. Three different suppliers of the solubilizing agent were included in the study. As shown in Table 2, the product color changed dramatically when the solubilizing agent from vendor A was used in the formulation during a 2-week stress study, whereas product color was not effected when the excipients from vendors B and C were used. It was determined that this variability in color stability was due to trace iron in the excipient. The solubilizing agent obtained from vendor A contained 140 ppm of iron, whereas the product from vendors B and C had iron levels of 13 ppm and 0 ppm, respectively.

As with trace peroxide, minimization of trace iron in the ingoing excipients resulted in an improvement in product stability. Autoxidation, which is a chain reaction that begins with the formation of a free radical, is catalyzed by trace metals and peroxides (6). Most often formulators use chelating agents to prevent catalysis of autoxidation by trace metals; however, in this case the use of a chelating agent was prohibited by the nature of the formulation. Therefore, control of these trace impurities in materials used in formulations that are prone to oxidation is suggested. Furthermore, packaging components (container/closure systems) should also be considered as potential sources of trace metals.

ANTIOXIDANTS

Minimizing oxygen concentration alone is often not sufficient to eliminate completely the possibility of degradation because only trace levels of oxygen can propagate autoxidation. For the danofloxacin formulation, control of processing operations (e.g., use of nitrogen sparging to reduce dissolved oxygen, control of filled vial headspace composition, and thermal effect of terminal sterilization) alone did not completely eliminate the prod-

Table 2

The Impact of Trace Iron on Color Stability (2 Week Stress Study at 30°C/Partially Filled Vials)

Vendor (Iron Content)	Initial Color (Absorbance at 450 nm)	Color After 2 Weeks (Absorbance at 450 nm)	Comment
Vendor A (140 ppm Fe)	0.13 au	0.39 au	Significant color change
Vendor B (13 ppm Fe)	0.13 au	0.13 au	No color change
Vendor C (0 ppm)	0.12 au	0.12 au	No color change

Excipient from vendor A contained 140 ppm iron impurity, excipient from vendor B contained 13 ppm iron, and excipient from vendor C contained 0 ppm (none detected). Note: solution color monitored at 450 nm.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

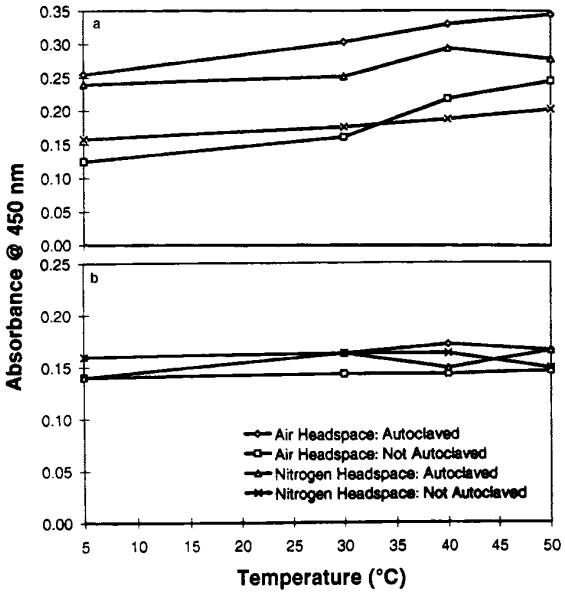

Figure 2. The effect of headspace gas, storage temperature, and terminal sterilization on color stability of formulations (a) without MTG; (b) with MTG as an antioxidant through 6 weeks of storage.

uct oxidation/color change on stability, as exhibited in Fig. 2(a). When the product was manufactured without an antioxidant, the formulation color varied as a function of headspace composition, terminal sterilization, and storage temperature. With the appropriate antioxidant and selection of the antioxidant level, autoxidation of the drug can be inhibited until all of the antioxidant is preferentially consumed. The addition of an antioxidant, in this case monothioglycerol (MTG), resulted in a formulation less sensitive to processing variables, as shown in Fig. 2(b). MTG was selected following a comprehensive screening of potential antioxidants, whereby it provided the desired stability for this multidose product both during shelf-life and the proposed in-use period.

Although it is difficult to accurately predict the efficiency of an antioxidant, initial selection can be based on the difference in redox potential between the drug and antioxidant (8). The best way to assess the effectiveness of an antioxidant is to subject the formulation containing the antioxidant to standard oxidative stress conditions (i.e., addition of peroxides and purging with oxygen). The formulation stability (active ingredient and the antioxidant content) may be assessed over some stability challenge. Because the breakdown product of the antioxidant may have an adverse effect on the stability of the active ingredient, quantitation of the antioxidant and/or its breakdown product(s) during stability storage is essential to the full understanding of the product (3).

Table 3

Effect of Formulation pH on Product Color Stability (2 Week Storage at 70°C)

Formulation pH	Color Change (Visual)
6.6	No color change
7.2	Slight color change
7.7	Significant color change
8.0	Very significant color change

FORMULATION pH

Oxidation of most compounds is minimized at acidic pH (6,9). For this reason, oxygen-sensitive compounds are typically formulated at a lower pH to increase their resistance to oxidation during shelf storage. Development of the danofloxacin injectable formulation followed this pattern. The data in Table 3 demonstrate that as the pH of the formulation is lowered, less color change over stability is exhibited.

Although pH can directly influence the extent of drug oxidation, it may also impact the performance of the antioxidant. Antioxidants are effective in stabilizing oxygen-sensitive drugs when they are preferentially oxidized in place of the drug (6). For the danofloxacin formulation, MTG was ultimately selected as the antioxidant. The impact of formulation pH on the performance of MTG as an antioxidant for danofloxacin was assessed during preformulation. The preformulation studies were done at a concentration of 0.1 mg/ml of danofloxacin, a much lower concentration than the commercial formulation, which is 180 mg/ml.[1] Figures 3 and 4 represent the percent loss of danofloxacin at pH 7.5 and 6.0, respectively. At pH 7.5, MTG has a significant effect on the stability of danofloxacin. At this pH, formulations containing MTG had improved stability relative to formulations not

[1] The percent loss of danofloxacin illustrated in Figs. 3 and 4 is much greater than that observed in the commercial formulation, because at 0.1 mg/ml of danofloxacin, the ratio of drug to oxygen molecules in the headspace is less favorable relative to a 180-mg/ml formulation packaged in the same vial.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

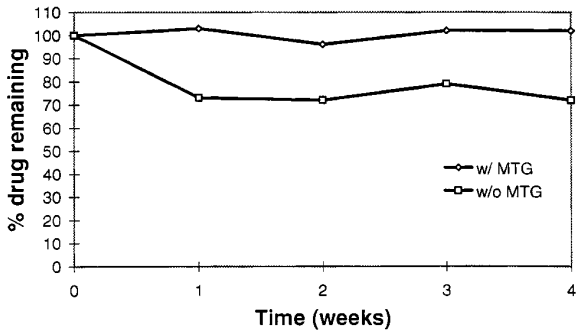

Figure 3. The effect of MTG on danofloxacin stability at pH 7.5 (drug concentration of 0.1 mg/ml; storage temperature of 70°C).

containing MTG (Fig. 3). However, at pH 6.0 there was no apparent effect of MTG on the stability of danofloxacin. Therefore, MTG is more effective as an antioxidant at pH 7.5 than at pH 6.0. This is believed to be because the oxidation potential for the MTG changes as the pH is lowered, making MTG less likely to be oxidized at lower pH. A cyclic voltammogram of MTG as a function of pH is provided in Fig. 5. As illustrated in this voltammogram, MTG is oxidized at a much lower potential at pH 7.5 than at pH 6.0. MTG consumes any residual dissolved oxygen and headspace oxygen much faster at pH 7.5 and hence prevents the oxidation of danofloxacin. In summary, pH may have a direct effect on the oxidation rate of the drug molecule and may impact the oxidation potential of an added antioxidant.

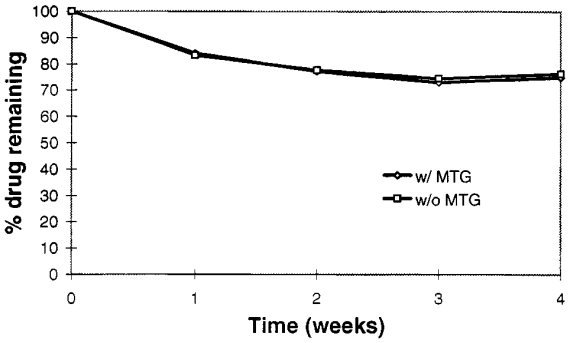

Figure 4. The effect of MTG on danofloxacin stability at pH 6.0 (drug concentration of 0.1 mg/ml; storage temperature of 70°C).

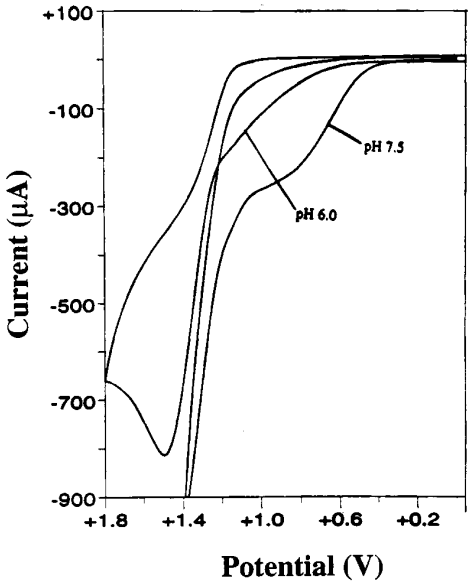

Figure 5. Cyclic voltammogram of MTG as a function of pH.

PACKAGING

The selection of the appropriate container and closure is critical in the development of any dosage form. Aside from the general compatibility concerns and container/closure integrity issues, the package headspace-to-product ratio plays a significant role in the stability of an oxygen-sensitive (liquid) product. For an oxygen-sensitive formulation, several other factors must be taken into consideration. These may include (*a*) light-protected container or package to prevent light-induced catalysis of autoxidation; (*b*) acceptable resealability of closures to prevent leakage and contamination, especially for multiuse vials; and (*c*) maintenance of inert atmosphere (e.g., nitrogen purge of headspace).

All of the above factors played a role in the development of danofloxacin. Most notably, however, the vial size/configuration had a significant impact on the rate of product oxidation. Generally, larger vials provide a lower headspace-to-volume ratio than smaller vials, thus the extent of oxidation is typically less in larger vials (10). Early stability studies on danofloxacin packaged in smaller vials revealed a greater degree of color change than when the same solution was placed in larger vials. An example of the effect of vial size on oxidation is illustrated in Fig. 6. Product packaged with an air headspace

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.