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Inert Gas Sparge Leads to Alternate Reaction Pathway

MIRIAM K. FRANCHINI AND JENS T. CARSTENSEN*†

Bristol-Myers Squibb Pharmaceutical Research Institute, New Brunswick, NJ 08903, and
**University of Wisconsin College of Pharmacy, 425 North Charter Street, Madison, WI 53706 and*
†4713 Tonyawatha Trail, Madison, WI 53716, USA

Abstract

The effect of sparging with an inert gas (argon) was evaluated during the investigation of the solution kinetics of an oxidation-prone amphiphilic drug containing a sulphide moiety.

Samples stored with an air headspace in pH 7 and 8 phosphate buffers at elevated temperatures and in the absence of light degraded to two main products, a sulphoxide and a cinnamic acid analogue. Initially, this appeared to be a sequential mechanism which could be blocked by removing oxygen. Instead, argon-sparge forced the direct degradation to the cinnamate, which was evidenced by the formation of a strong odour of sulphide. In addition, argon-sparged samples remained colourless, while those sparged with oxygen or stored with an air headspace turned yellow and had negligible odour. The half-lives for samples stored in pH 8 buffers at 93°C at an initial drug concentration of 25 mg mL^{-1} were 128 days (argon sparged), 86 days (air headspace), and 65 days (oxygen sparged).

The results indicated that for the drug under study, sparging with an inert gas affected the mechanism as well as the rate of the reaction at elevated temperatures.

It is not uncommon in liquid pharmaceutical preparations to sparge the formulation and/or cap the headspace with an inert gas such as nitrogen in order to retard oxidation. In some cases, a sulphur-containing antioxidant may be added as a scavenger.

We have documented an occasion whereby sparging the drug solution with argon resulted in a slower but alternate pathway of degradation compared with control solutions that were packaged with an air headspace or sparged with oxygen. A malodorous compound not found in the controls evidenced the alternate pathway. While the malodorous product was not positively identified, mass balance and structures of the parent molecule and main degradation products (Figure 1; compound **A**, sulphoxide analogue **B**, cinnamate analogue **C**) suggest a sulphide elimination product.

Correspondence: M. K. Franchini, Bristol-Myers Squibb Pharmaceutical Research Institute, New Brunswick, NJ 08903, USA.

Materials and Methods

Specific experimental details have been published previously (Franchini & Carstensen 1994, 1996, 1999).

Generally, solutions of the disodium salt form of the drug were prepared in pH 7 and 8 phosphate buffers of various molarities and ionic strengths, distributed into glass vials which were then individually sparged with water-saturated argon or oxygen, or left with an air headspace ($\approx$22% oxygen), and placed at 93°C. To minimize the effect of trace metal catalysis, 0·2% EDTA disodium salt (EDTA) was added to the buffers.

Initial drug concentrations were 0·05 mg mL^{-1} (below the critical micelle concentration (cmc) of **A**) and 25 mg mL^{-1} (above the cmc of **A**).

At the appropriate time points, one or two vials from each treatment group were pulled and analysed by HPLC. For analysis, the entire vial contents was dissolved in mobile phase because at later time points the concentration of the degradation products exceeded their solubility at ambient temperatures (the cinnamate precipitating as a white floc, and the sulphoxide oiling out as a yellow oil).

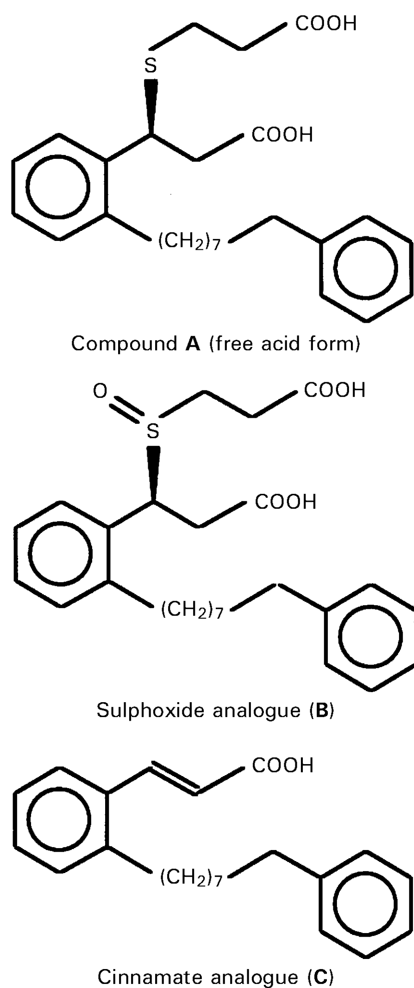

Figure 1. Structures of **A** and its main degradation products, the sulphoxide analogue **B**, and the cinnamic acid analogue **C**.

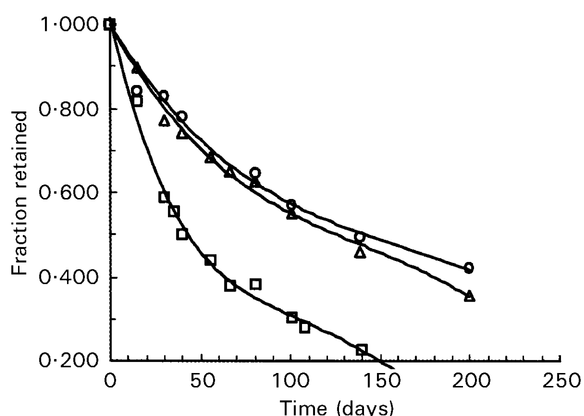

Figure 2. Representative degradation profiles of **A** in pH 8 phosphate buffer (with 0·2% EDTA disodium salt) at 93°C and sparged with argon (○), oxygen (□) or stored with an air headspace (△); initial drug concentration 0·05 mg mL^{-1} (below the cmc of **A**). While the rate of decomposition is not significantly different between argon and air treatment groups, samples sparged with argon remained colourless and smelled of sulphide, while oxygen-sparged and samples stored with an air headspace turned yellow, suggesting a different pathway in the presence of oxygen. Similar trends were observed in 0·1, 0·2, and 0·4 M pH 8 buffers (with EDTA). Curves drawn to guide the eye.

The DSC was a Seiko Model 220C with automatic cooling (liquid N_2). For the experiment, 9·76 mg **A** were heated in a crimped aluminum solid sample pan from 25°C to 280°C at 5°C min^{-1} followed by cooling to 25°C at 40°C min^{-1}. The white solid in the pan was dissolved in mobile phase for analysis by HPLC.

Results and Discussion

When drug solutions in pH 7 phosphate buffer were packaged in ampoules with an air headspace, the addition of 0·1% EDTA disodium salt was observed to slow the degradation significantly compared with samples without EDTA. The drug was manufactured to be free of trace metal contaminants, and so it was concluded that trace metals from the buffer salts were responsible for the enhanced degradation in the absence of EDTA. Therefore, EDTA was included as a metal chelating agent at a level of 0·2% in the studies described herein.

In the presence of EDTA, marginal improvement in stability was obtained by argon-sparge compared with an air headspace (Table 1 (initial drug concentration above the cmc of **A**) and Figure 2 (initial drug concentration below the cmc of **A**)). However, the slight yellowing of the samples stored with an air headspace compared with the colourless solutions obtained under argon-sparge suggested a different reaction mechanism. The yellow colour was identified as the oxidation product of **A** (sulphoxide), and did not appear as a peak in chromatograms of samples that had been sparged with argon.

Oxygen-sparged samples also turned yellow and showed a faster degradation rate than samples stored with an air headspace, however, both oxygen- and air-treatment groups lacked the strong odour associated with the argon-sparged counterparts, again suggesting a different route of degradation. Only at the later time points did the oxygen-and air-treatment groups begin to hint of odour. A second degradation product, which was formed under all three treatment conditions eventually, was identified as the cinnamic acid analogue of **A**.

Table 1. Observed pseudo-zero-order rate constants (k_{obs}) and half-lives ($t_{\frac{1}{2}}$) of data generated at 93°C in pH 8 phosphate buffers of indicated molarity at an initial drug concentration of 25 mg mL^{-1} (above the cmc of **A**) for samples sparged with argon or oxygen, or stored with an air headspace. Solutions sparged with argon remained colourless, while those sparged with oxygen or stored with an air headspace yellowed. Buffers contained 0·2% EDTA.2Na to control for trace metal catalysis. There are no obvious trends with regard to ionic strength or buffer concentration, and so the data for each treatment group have been averaged to provide a mean k_{obs} and half-life.

Treatment group	Buffer concn	k_{obs}[a] $\times 10^3$	$t_{\frac{1}{2}}$	Intercept	r^2	n
Argon sparge	0·1 M	4·60	108·7	0·983	0·982	11
	0·2 M	3·91	127·9	1·003	0·989	11
	0·4 M	4·18	119·6	0·991	0·996	11
	0·4 M	3·70	135·0	1·007	0·997	9
	0·1 M[b]	3·75	133·3	0·987	0·993	10
	0·2 M[b]	4·00	125·0	0·998	0·996	9
	Mean	3·9 ± 0·2	128 ± 7			
Air headspace	0·1 M	7·34	68·1	1·000	0·999	11
	0·2 M	4·37	114·4	0·973	0·989	14
	0·4 M	5·53	90·4	0·996	0·991	11
	0·4 M	6·09	82·1	0·998	0·997	9
	0·1 M[b]	5·60	89·3	1·000	0·999	12
	0·2 M[b]	6·10	82·0	1·004	0·995	9
	Mean	5·8 ± 0·3	86 ± 5			
Oxygen sparge	0·1 M	9·17	54·5	1·004	0·989	12
	0·2 M	6·94	72·0	0·996	0·995	6
	0·4 M	8·32	60·1	0·997	0·997	12
	0·1 M[b]	6·91	72·4	1·000	0·999	10
	0·2 M[b]	7·90	63·3	0·997	0·989	7
	Mean	7·7 ± 0·7	65 ± 6			

[a]$10^3 \times$ (−slope) of the zero-order plot (fraction retained vs days). r^2 is the coefficient of determination. n is the number of time points. [b]indicates the ionic strength of the buffer has been adjusted with KCl to the calculated ionic strength of the 0·4 M pH 8 buffer (final ionic strength = 1·2).

These observations supported a reaction scheme previously proposed (Franchini & Carstensen 1994) whereby compound **A** could either degrade sequentially by an oxidative pathway to the sulphoxide (**B**) and then to the cinnamic acid analogue (**C**), and/or by an anoxic pathway directly to the cinnamic acid analogue. By mass balance, the remaining fragment(s) in the anoxic pathway would include a sulphide, which would account for the malodour.

The existence of an alternate non-oxidative pathway was supported by a DSC experiment whereby solid drug heated to above melting in a DSC pan under nitrogen flow became white and reeked of a sulphide smell, similar to the argon-sparged samples. HPLC analysis of the DSC-treated sample showed that most (87 area percent) of the drug had decomposed to the cinnamate, and only 10·7 area percent remained as intact drug. A third unidentified peak (2·3 area percent), which did not appear in the solution kinetic study samples at pH 7 and 8, was the only other peak detected by HPLC in the DSC-treated sample; no evidence of sulphoxide was noted (Figure 3).

These findings were observed regardless of initial drug concentration. Initial drug concentration was found to affect the order of the reaction, that being zero order above the cmc and close to first order below the cmc (see Carstensen 1998; Franchini & Carstensen 1999).

Conclusions

Many pharmaceuticals that oxidize and some anti-oxidants used in pharmaceutical preparations contain sulphur, and so these findings may be of interest to those attempting to reduce the rate of oxidation by nitrogen-sparge. In addition to being malodorous, and thus not an elegant pharmaceutical product, if a sulphide is formed, it could be toxic. In the case of structures similar to compound **A**, on a more positive note, such a system could be used as a high-yield synthetic route for cinnamic acid analogues (or the corresponding sulphide) using relatively mild conditions.

Finally, it should be remembered that these studies were conducted at an unusually high temperature (93°C) for pharmaceutical systems. It could be that at lower temperatures the alternate pathway would not occur, which could lead to incorrect conclusions being drawn when using accelerated storage conditions to predict room temperature stability of pharmaceutical systems sparged with inert gas.

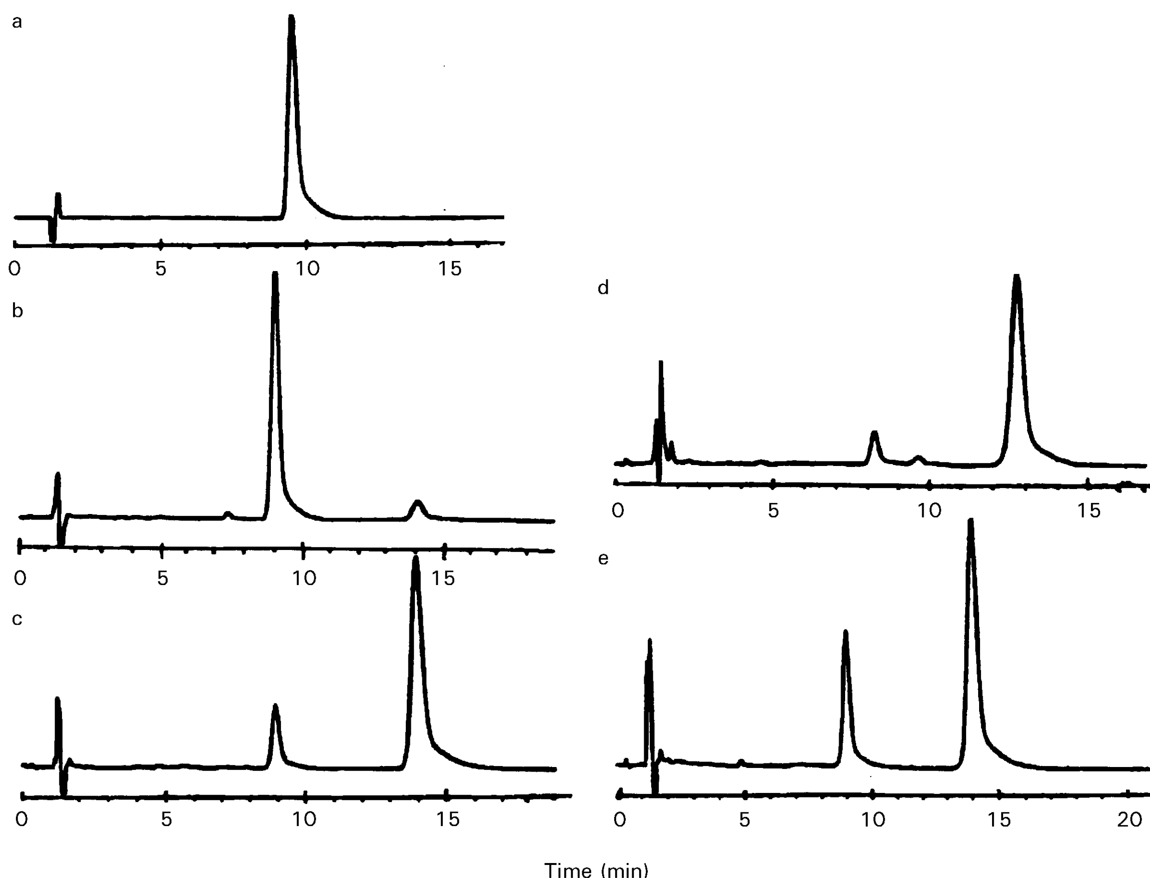

Figure 3. Representative HPLC chromatograms for samples of **A** stored after argon or oxygen sparge or with an air headspace. Elution order is **B** (sulphoxide) at 6–8 min, **A** (parent) at 8–11 min, and **C** (cinnamate) at 12–16 min. Initial drug concentration was 25 mg mL^{-1}, unless otherwise stated. a. Air headspace. Drug in H_2O. Initial drug concentration was 0·25 mg mL^{-1}, stored four years at 4–8°C, no apparent degradation; solution clear and colourless. b. Oxygen-sparged. Drug in 0·4 M pH 8 buffer (with EDTA) stored 10 days at 93°C, 9% degraded; sulphoxide, parent, and cinnamate are the major peaks. c. Air headspace. Drug in 0·2 M pH 8 buffer (with EDTA; KCl to adjust ionic strength to 1·2) stored for 5 months at 93°C, 80% degraded; parent and cinnamate are the major peaks. d. Drug heated by DSC (9·76 mg **A** heated in a crimped aluminum solid sample pan from 25°C to 280°C at 5°C min^{-1} followed by cooling to 25°C at 40°C min^{-1}), dissolved in mobile phase. The first major peak is the parent, followed by an unknown and the cinnamate. e. Argon-sparged. Drug in 0·4 M pH 7 buffer (with EDTA; KCl to adjust ionic strength to 2·2) stored for 5·4 months at 93°C, 74% degraded. The two major peaks are the parent and the cinnamate.

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