Protection of Oxygen-Sensitive Pharmaceuticals with Nitrogen

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Abstract □ A conventional procedure for incorporating nitrogen into a vial, in order to prevent degradation of the active ingredient by aerobic oxidation, was unsuccessful. It was found that the best method for introducing nitrogen into a container is to perform the operation in a nitrogen atmosphere within a closed system. Variables associated with the method are discussed.

Keyphrases □ Pharmaceuticals—oxidation protection □ Nitrogen layering—oxidation protection □ Oxygen content—nitrogen layered vials □ Hood systems—nitrogen layering □ Diagram—nitrogen layering

Oxygen-sensitive liquid pharmaceuticals are usually protected from degradation by two methods. These are: (*a*) incorporating antioxidants and/or chelating agents into the formulation, and (*b*) maintaining the product in an inert atmosphere. Although much information is available with regard to the former technique (1–5), the latter one has received considerably less attention. In his study on excluding air from oxygen-sensitive parenterals, Wheeler (6) measured the oxygen content of a nitrogen-sparged system using paper impregnated with enzymes and a reduced chromagen. The addition of glucose to the paper produced a color change in the presence of oxygen. Using this method, he was able to evaluate rubber closures with regard to their ability to transmit oxygen into the container. He also demonstrated that the pullaway type of sealer was superior to the melt-down type for sealing nitrogen-sparged ampuls. To the best of the authors' knowledge, however, no study of the factors associated with creating an inert atmosphere in vials has been published. It is the purpose of this paper to discuss experiences in this area.

Perhaps the paucity of information on this subject is due to the difficulty in quantitatively evaluating the oxygen content of a purged system. The method employed by Wheeler has the disadvantage of not being readily applicable as a routine checking procedure in parenteral production work. Although gas chromatography is an alternate possibility, cost and manipulative difficulties detract from its potential use in a packaging operation. This study employed an oxygen analyzer[1] equipped with a head-space sampler. Although the head-space sampler required some modification, this equipment was found to be relatively inexpensive, quantitative, rapid, and readily adaptable to production facilities. Presently, units of this type are being employed routinely in the parenteral packaging area.

Although inert atmospheres may be created by a number of gases, the two most commonly employed are nitrogen and carbon dioxide. Because of the latter's effect on solution pH, only nitrogen was used in these studies. However, many of the principles demonstrated are sufficiently general as to be readily translatable to other gases.

EXPERIMENTAL

The oxygen analyzer was standardized against atmospheric oxygen. The solid plunger supplied with the head-space sampler, was replaced with the specially designed syringe needle shown in Fig. 1. Most dimensions in Fig. 1 are not critical, and may be varied to accommodate a particular package or head-space volume. However, the wide position of the modified plunger which fits snugly within the head-space sampler, should be the same size as that supplied with it.

All vials used in this study, with the exception of a few special laboratory batches, were filled on a filling machine.[2]

DISCUSSION

A parenteral product which had been developed, was found to degrade by reaction with atmospheric oxygen. The nature of the formulation was such that an antioxidant could not be incorporated into the system. Using accelerated conditions, initial experiments with filled vials led to the conclusions that: (*a*) vials containing an atmosphere of air were not stable; (*b*) vials carefully layered with nitrogen in the head space were stable; and (*c*) an attempt to protect the system by layering nitrogen with a conventional production method was unsuccessful. These data are shown in Table I. The conventional method referred to above consisted of flowing nitrogen onto the surface of the liquid followed by insertion of the closure. The apparent lack of complete protection demonstrated by this technique caused the authors to examine more carefully the overall concept of nitrogen protection of a parenteral.

Creation of an Inert Atmosphere—It is well known that the major adverse effect to liquids containing oxygen-sensitive materials is due to the air (oxygen) in the head space of the container. It follows then, that displacement of the air with an inert gas, such as nitrogen, will remedy this condition. However, what has not been generally appreciated is the fact that nitrogen, being lighter than air may, in an attempt to equilibrate with the surrounding atmosphere, diffuse out of the container at a rapid rate. The net result is that some or all nitrogen delivered to a container is replaced with air in the interim between layering and capping. For many years the pharmaceutical industry at large has empirically tried to replace the head-space air within vials by nitrogen layering. The assumption made in most cases was that nitrogen delivered to the head space would displace the air present and remain undisturbed leaving an inert atmosphere in contact with the capped solution. Since, until recently, no simple monitoring device has been available for evaluating the efficiency of this procedure, this hypothesis has essentially gone unchallenged.

With the development of electrode oxygen analyzers, it was possible to investigate this technique more completely. While a sampler is available for determining the oxygen level in the head space of containers made with rigid caps, this principle has not been applied by the pharmaceutical industry to vials, possibly because of the resiliency of the elastomer closure, which, in effect, seals itself

[1] Beckman model 777, Beckman Instruments, Inc., Fullerton, Calif.

[2] Popper FS1.

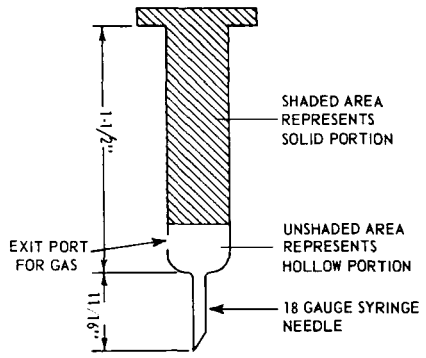

Figure 1—*Modified plunger for head-space sampler.*

around the sampling plunger preventing sample removal. The solid plunger commercially supplied with the head-space sampler has been replaced with a specially designed syringe needle (Fig. 1) so that the elastomer closure on vials could be penetrated and the head space sampled for its oxygen content. This combination of modified head-space sampler and oxygen analyzer was used to evaluate the efficiency of various systems. Confidence in this sampling method was based on its ability to correlate with data obtained by vapor-phase chromatography. One of the first important observations made with the oxygen analyzer was that, although the oxygen content of a vessel is kept at a low level by continuous nitrogen layering, once the nitrogen supply is discontinued, replacement of the nitrogen with air is extremely rapid. It was therefore felt that the best approach to the problem of retaining an inert atmosphere in the head space was to allow the nitrogen layering to occur in an environment consisting almost entirely of nitrogen. While it was recognized that the capping should likewise occur in such an environment, the existing filling-equipment design precluded such a modification during this study.

The controlled-environment system as conceived consisted of a plastic hood which completely enclosed the vials during the filling and layering operations. The hood was form fitting around the filling apparatus, and the point of exit of the vials was as close as possible to the plugging site. Because of the necessity of inlets for filling tubes, layering needles, oxygen electrode, gas atmosphere, and vials, the system could not be completely airtight. Nevertheless, the oxygen level in the hood could be monitored with the oxygen analyzer and the nitrogen-inlet flow rate adjusted for optimal conditions.

The overall theory of layering under nitrogen involves recognition of the fact that layered nitrogen in the head space of a vial will, as indicated by the second law of thermodynamics, equilibrate with the gases surrounding the vials. Therefore, if air is the surrounding medium, then air will ultimately comprise the vial's head space, the rate of exchange being dependent on relative densities, the vial's neck size, turbulence, and other aerodynamic considerations. However, in a closed system, if nitrogen constitutes the surrounding atmosphere, then in effect there will be no loss of layered nitrogen. One other condition necessary for retention of the nitrogen is, as previously suggested, that after leaving the controlled environment, the vial is plugged rapidly.

Studies with Hooded Systems—In a preliminary study the nitrogen layering occurred under a simple plastic hood into which nitrogen was admitted through an orifice at the top. Presparging of the empty vials with nitrogen and the filling operation were both performed outside the hood. Accelerated temperature studies (Table II) on the vials filled under these conditions indicated better product protection than that obtained by the conventional method.

Table I—Accelerated Stability Data on Vials Layered with Nitrogen *via* Different Methods

Methods	Active Ingredient (% of Initial) Days at 42° 13	22	41
No nitrogen layering	48	38	43
Conventional method	83	77	70
Manual nitrogen layering	104	99	93

Table II—Accelerated Stability of a System Layered with Nitrogen and Filled Under a Hood

Line Speed (Vials/min.)	Active Ingredient (% of Initial) Days at 42° 13	19	31	96	Gas Content VPC O_2	VPC N_2	Head-space Sampler O_2
38	98	76	85	48	1.45	93.3	—
54	94	92	88	40	1.42	94.3	1.35
100	94	95	89	61	1.35	95.1	2.80
100 (Control, no nitrogen)	77	68	57	35	13.3	82.9	15.9

From the relative stabilities and chromatographic nitrogen determination, it appeared that higher line speeds produced better nitrogen levels in the vials. This could represent the shortened time period between vials leaving the hood and their subsequent plugging. In this study it was found that the oxygen content in the head space of the controls filled without nitrogen, as determined by gas chromatography, was 15.9% O_2 rather than the expected 20.9% value indicating oxygen uptake by the solution during elapsed time before assay. This point will be discussed later in more detail. However, the implicit amounts of oxygen in each vial (determined by subtracting the nitrogen values from 100%) are in accord with the general stability pattern observed.

Considering the fact that in the experiment described above nitrogen was essentially "dumped" into the hood *via* the entrance port, one realizes that the resulting harsh flow patterns produced inside the semiclosed hood may not have permitted adequate purging of the already present gases (air). If, however, the nitrogen were to enter the hood through a baffle system which provides a smooth, predetermined, gentle flow, it should be possible to obtain an atmosphere more closely approximating that of pure nitrogen. It also seemed logical to nitrogen sparge, fill, and nitrogen layer the vials in such a hood. In the next series of experiments these steps were performed in a larger hood which was modified to include a baffle system. Schematic diagrams depicting both end and side views of this hood are shown in Figs. 2 and 3. In Fig. 2, the area to the left of the baffle plate is the filling chamber. In Fig. 3, the baffle plate has been partially omitted and lowered so that the filling chamber may be seen. The label side chamber refers to the area to the rear of the baffle plate, which is labeled N_2 manifold in Fig. 2. With the hood in operation, the inside oxygen levels were found to be less than 0.5%. This was a marked improvement over the values of 2–4% obtained using the nonbaffled hood, especially when one considers the additional operations taking place within the hood and their effect on gas turbulences. It was felt that this improvement probably reflected both reduced turbulence and greater homogeneity within the hood due to the presence of the baffle.

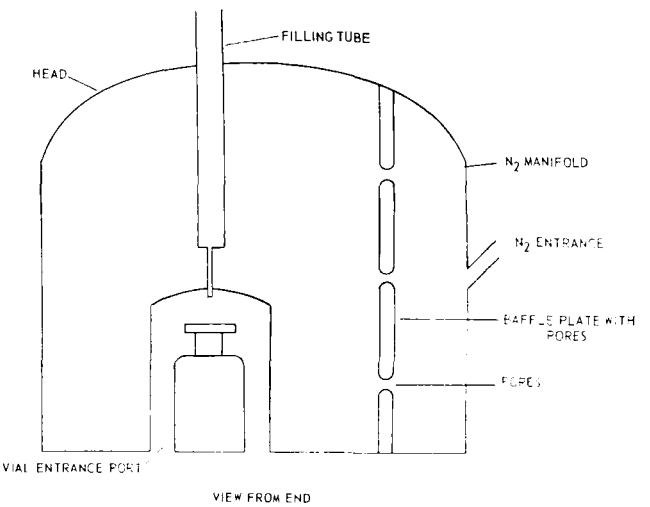

Figure 2—*Schematic end view of controlled-atmosphere hood.*

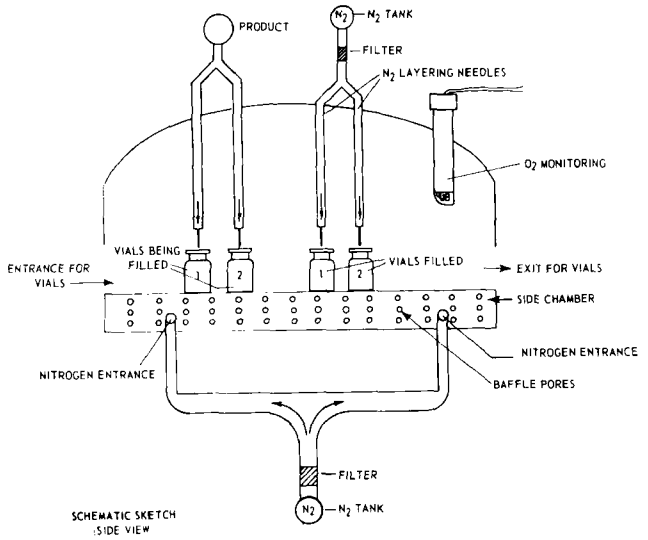

Figure 3—*Schematic side view of controlled-atmosphere hood.*

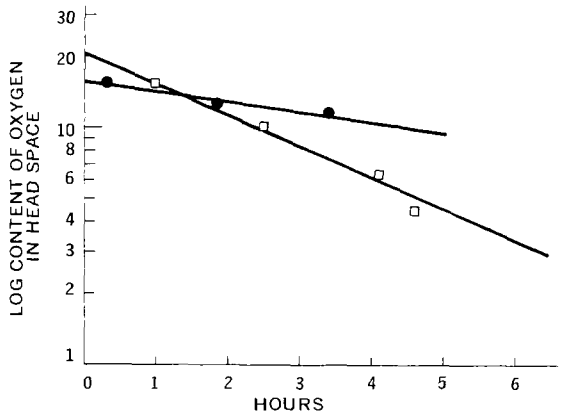

Figure 4—*Relationship between head-space oxygen content and time. Key:* ●, *system layered with nitrogen (extrapolation to zero time ~ 16% oxygen);* □, *system without nitrogen layering (extrapolation to zero time ~ 21% oxygen).*

To overcome the problems associated with a solution that consumed head-space oxygen with time, distilled water rather than product was filled into the vials. The variables measured in this study were: (*a*) line speed, (*b*) sparging of the empty vials with nitrogen before filling, (*c*) nitrogen layering of the filled vials, and (*d*) maintaining the nitrogen flow to the proposed hood. The oxygen levels in the sealed vials were determined and are shown in Table III. These data represent an average of several vials. Although the results were evaluated statistically, the data make it readily apparent as to which factors most influence oxygen levels. The oxygen level in the hood was also measured at different nitrogen flow rates, and the optimum values determined. It was observed that both higher and lower nitrogen flow rates elevated the hood oxygen level from a minimal value. In general, it was found that: (*a*) the faster the line speed the lower the oxygen level in the vials; (*b*) presparging of the empty 1-ml. vials had little effect; (*c*) flow of nitrogen to the hood was very important; and (*d*) layering of the filled vials was critical, as large vial-to-vial variation occurred when nitrogen layering was not performed. This latter result demonstrated the importance of layering the filled vials even in a nitrogen atmosphere. Thus the best conditions (that is, the lowest oxygen level in the vial) result from a higher line speed with nitrogen layering of the vial in a baffled hood receiving an independent supply of nitrogen. Possibly, line speed might not be as critical, if the equipment would also permit capping in the hood. As a result of this study the authors were able to specify the exact conditions for filling this product so as to result in a minimal oxygen level in the head space of the finished product and provide for a stable system.

Operation of Nitrogen Hooding and Treatment of Bulk Material—The hooding is so constructed that it forms a tight air seal over the area it covers on the vial filling machine. Entrance and exit openings for the vials are provided at both ends. The size of these openings is minimal, just permitting vials to be accommodated. This is important as larger openings will allow excessive escape of nitrogen from and entrance of air (oxygen) into the hood. In practice the importance of the size is reflected in ratio of hood volume to entrance or exit area.

A manifold, attached to the side of the filling chamber of the hood, receives the nitrogen directly from the nitrogen tank after it passes through sterile filters. The outlet side of this manifold consists of a number of small holes (pores) through which the nitrogen entering the filling chamber of the hood must pass. In this manner a diffusion of the gas into the filling chamber is established. Without such a designed intake manifold, higher levels of oxygen will remain in the hood. The nitrogen delivery to the vials is through stationary but adjustable needles, as it was found that the up-and-down movement of layering needles attached to the product-filling cam entrains air into the hood. Filters are also attached to these lines to provide sterile nitrogen delivery.

Table III—Oxygen Levels in Vials Filled and Layered with Nitrogen under Baffled Hood

Line Speed (Vials/ min.)	Nitrogen Rate to Empty Vials Layering Filled Vials Nitrogen to Hood	Oxygen in Head Space, %—High	Low	None	High	High
		Yes Yes	Yes Yes	Yes Yes	No Yes	Yes No
50		4.3	3.7	3.3	5.4	7.8
90		3.1	2.7	2.8	5.8	6.3
120		2.6	2.7	2.8	5.9	6.1

It was found that when the track system which carries the vials into the hood is in motion, there is a slightly lower oxygen content present in the hood. In addition, a full complement of vials in the hood also results in a slightly lower oxygen level. For this reason the first and last six vials to enter and leave the hood are discarded, as the level of oxygen during their filling is higher than with the other vials.

Flow meters are attached to all nitrogen tanks delivering into the system so that conditions may be duplicated from one run to another. The flow rate of nitrogen which is used to layer the vials is that which will not cause undesirable splattering in the vial. A visual check for dimpling of the liquid surface of the vials, as well as a knowledge of the volume of nitrogen delivered per unit per volume of head space, are adequate criteria. The quantity delivered should always be in excess to provide a margin of safety. The usual line speeds employed readily produce such a margin.

The critical step is the capping operation, which should occur as quickly as possible after a vial leaves the hood and arrives at the capping station. With the present hood, a short curtain of nitrogen surrounds the exit door's exterior and provides at least some measure of nitrogen protection between the hood and capping point. It is believed that higher line speeds favor better nitrogen protection, since they shorten this critical period.

To assure protection of the bulk material from manufacture through packaging, the contents of bulk bottles are simultaneously sparged and layered with nitrogen. In addition, this must be repeated when the caps of the bulk bottles are removed. At the time of filling, nitrogen rather than air is allowed to enter the bulk bottle through its vent tube as the liquid level is lowered during filling of vials. In this manner, it is possible to maintain the integrity of the protective nitrogen atmosphere from manufacture of bulk material through the final packaged product.

Effect of Time on Values Obtained from Head-Space Sampling—It is possible that several hours or longer could transpire between nitrogen layering of a product and measurement of the oxygen level in the head space. If protection by layering with nitrogen was inadequate, and the oxygen present in the head space of a vial were to react rapidly, a long time interval between layering and oxygen head-space analysis would produce results comparable to those obtained when layering with nitrogen was highly efficient. That is, both conditions would result in a low head-space oxygen

level. An indication that such a phenomenon occurs has been suggested in this paper when nonsparged vials assayed 16 rather than 21% oxygen. To demonstrate this further, vials of another parenteral product were filled using both the conventional nitrogen-layering technique and also with no attempt to incorporate nitrogen into the vial. The oxygen content of the head space was determined at various time intervals after filling. Because this product was able to react with oxygen, Fig. 4 shows that measurements performed as little as 1–2 hr. after filling gave erroneous results. At the end of 5–6 hours, the data obtained implied that the product was adequately protected. The true oxygen level in the head space was determined by extrapolation to zero time (time of filling). These results further emphasize the need for an awareness of the elapsed time when determining the efficiency of a nitrogen-protecting system.

Antioxidant and Nitrogen—Although an antioxidant could not be considered for inclusion in the system discussed, it may be felt by some that an antioxidant can take the place of an inert atmosphere in other systems. The authors believe that the inert atmosphere should serve to protect a product during its manufacture, filling, and storage prior to use. In this way the integrity of the antioxidant is maintained for the actual use-life of the product. An antioxidant consumed during the filling and manufacture operations may not be available for protection during a product's use-life, unless large amounts are employed. High concentrations of antioxidants should not be used to overcome less than adequate manufacturing techniques, and the maximum amount permitted might not be sufficient to protect the product during both its manufacture and use-life. Although this study has been concerned with a parenteral product, the same approach to filling other dosage forms is obvious. Therefore, in an oral liquid product, where taste is important, it is desirable to use minimum rather than maximum antioxidant concentrations.

Conclusions—As a result of this study, it is felt that anyone using a conventional method for layering nitrogen onto an oxygen-sensitive product should closely scrutinize the technique. A procedure similar to that described in this paper would be useful with any oxygen-sensitive product. Although these studies indicated good nitrogen protection in vials layered by this method, it must be remembered that it represented only a single product in a particular size vial. Therefore, the specific conditions for producing minimum oxygen concentration must be evaluated for each individual product and container.

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Dispensing Efficiency of Nonmetered Topical Spray Aerosols

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Abstract □ In spite of the often-quoted more efficient application advantage of pharmaceutical aerosol dosage form over a cream, an ointment, or a lotion topical preparation, the dispensing or pickup efficiencies of the majority of 10 commercially available nonmetered topical spray aerosols tested were found to be low. The pickup efficiency decreased with increasing target distance, increasing temperature of the aerosol, and tended to increase with increasing nonvolatile content.

Keyphrases □ Aerosols, nonmetered—dispensing efficiency □ Particle size—dispensing efficiency □ Pickup efficiency—nonmetered aerosols □ Temperature effect—pickup efficiency □ Target-aerosol distance—pickup efficiency

Active drugs must usually be dispensed in a formulated dosage form for ease of application. With the advent of pressurized packaging, pharmacists attempted to substitute aerosol dosage forms for the classical dosage forms with less success than anticipated (1). An explanation for the slow growth of the pharmaceutical industry into aerosols has been suggested by some (2) to be due to the poor quality of aerosol-filling services and aerosol components. In the case of drugs used topically, the classical medicated applications consist of creams, lotions, and ointments. Some of the claims (1, 3–7) usually made for topical spray aerosols are: (*a*) no waste or messiness associated with applicator or cotton swab; (*b*) efficient application.

Aerosol products may be broken into three categories (8): (*a*) space sprays; (*b*) surface coating; (*c*) aerated foams.

It is rather obvious that aerosol products intended for topical use can only be made by aerosols of Types *b* and *c*. Space sprays would leave very little deposit of medication on a body surface and thus would tend toward zero dispensing efficiency. At the other extreme, the aerated-foam aerosols would tend toward