Inadequacies in Leakage Test Procedures for Flame-Sealed Ampuls

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Abstract □ Leakage tests for flame-sealed ampuls currently in general use were demonstrated to be inadequate for determining whether passageways to the outside exist in finished ampuls. Ampuls containing passageways may or may not pass existing leakage tests, depending upon the severity of the test.

Keyphrases □ Leakage procedures for flame-sealed ampuls—inadequacies demonstrated □ Ampuls, flame sealed—inadequacies of leakage test procedures demonstrated

USP XVII (1) defines a single-dose container (containers for injections) as: "... a hermetic container holding a quantity of sterile drug intended for parenteral administration as a single dose, and which when opened cannot be re-sealed with assurance that sterility has been maintained." It further states: "Containers are closed by fusion, or by application of suitable closures, in such manner as to prevent contamination or loss of contents." NF XII (2) contains similar statements.

Although the official compendia propose no leakage tests for flame-sealed ampuls, *Remington's Pharmaceutical Sciences* (3) proposes a test "to determine whether or not a passageway remains to the outside," the intimation being that passage of such a test assures that the container is, in fact, a hermetic container. General descriptions are offered of modifications of a method intended to produce negative pressure in an incompletely sealed ampul submerged in a deeply colored dye solution. Conditions described may be obtained by applying a vacuum to ampuls immersed in a dye solution or by autoclaving ampuls and then submerging them in a dye solution immediately after autoclaving. A 1% methylene blue solution is suggested for the test.

Test methods are more definitively outlined in D.P.S.C. Standards (4). These Standards are used as a basis for qualifying drug manufacturers and packagers in accordance with the requirements of the Armed Services Procurement Regulations and form a basis for acceptance or rejection by a division of the U. S. Government.

These leakage tests for flame-sealed ampuls are described as follows:

> *"All flame-sealed ampuls shall be tested by the manufacturer for leakage in accordance with A, B, or C below:*
>
> *"A. Thoroughly wash all ampuls with a suitable detergent and then rinse. The ampuls are completely immersed in a hydroalcoholic solution (containing about 10 percent denatured alcohol) that is highly colored with a suitable dye or combination of dyes, yielding a color that is different from the injection. The solution containing the ampuls is heated between 105°F. and 110°F. for 10 minutes, and then allowed to cool to room temperature. The contents of each ampul is examined for color change or presence of dye. Ampuls showing color change or presence of dye are rejected.*
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> *"B. Thoroughly wash all ampuls with a suitable detergent and then rinse. The ampuls are completely immersed in an aqueous solution that is highly colored with a suitable dye or combination of dyes, yielding a color that is different from the injection. A vacuum of at least 25 inches is applied on the vessel containing the ampuls and dye solution, and the vacuum is maintained for at least 5 minutes. After releasing the vacuum, the contents of each ampul is examined for color change or presence of dye. Ampuls showing color change or presence of dye are rejected.*
>
> *"C. The ampuls are completely immersed in water that is highly colored with a suitable dye or combination of dyes, yielding a color that is different from the injection. Apply at least 10 inches of vacuum for at least 5 minutes and follow with 35 pounds air pressure for 15 minutes. After releasing the air pressure, the contents of each ampul is examined for color change or presence of dye. Ampuls showing color change or presence of dye are rejected."*

The merits of dye bath testing, as well as some limitations, were discussed by Stafficker (5) and Artz *et al.* (6). The need for more definitive test methods has been emphasized by the severe inadequacies that recently came to our attention. The purpose of this paper is to report the theoretical, as well as practical, limitations of the tests now generally used.

EXPERIMENTAL AND RESULTS

During a routine stability inspection of 1-ml. ampuls containing 50 mg. drug substance/ml., solid material was observed in areas adjacent to the seals in 12 of 100 ampuls. An example of this is shown in Fig. 1. These ampuls had been pull sealed[1].

The ampul that contained the largest amount of solid material (as judged visually) was selected. The solid material was carefully scraped off, weighed (4.9 mg.), and identified as active ingredient. The tip had the appearance of a "good seal" to several experienced observers. Examination of the ampul under a polarizing magnifier[2] (2× magnification) revealed no detectable opening in the tip area.

Further examination under a microscope[3] (40× and 100× magnification) with and without varying degrees of light polarization revealed a passageway near the center of the tip area. Because of the tortuous nature, size, and configuration of the passageway, it cannot be accurately defined by techniques used. It might best be described as an irregular conically shaped chip or bruise with an orifice approximately 36 μ in diameter at the external surface, narrowing to approximately 3 μ or less at the apex, in which area the penetration to the interior is assumed to occur. The passageway appeared birefringent.

Actual measurements in an area at, and adjacent to, the pull seal of the ampuls in question indicated an average thickness of the glass wall of 3.8×10^{-2} cm.

This ampul was leak tested according to Method B of D.P.S.C. Standards (4), with 0.1% D&C Red No. 19 dye in deionized water as the dye solution and 76.2 cm. (30 in.) (gauge) of vacuum. No evidence of color was detectable visually, nor could evidence of the dye be detected on reexamination of the tip area with the polarizing magnifier.

[1] Cozzoli automatic ampul filler and sealer, model FPS2.

[2] Polarizing magnifier: inspection machine, type 208, P. W. Allen and Co., London, England.

[3] Bausch & Lomb Dynazoom flat field photo binocular microscope with polarizer.

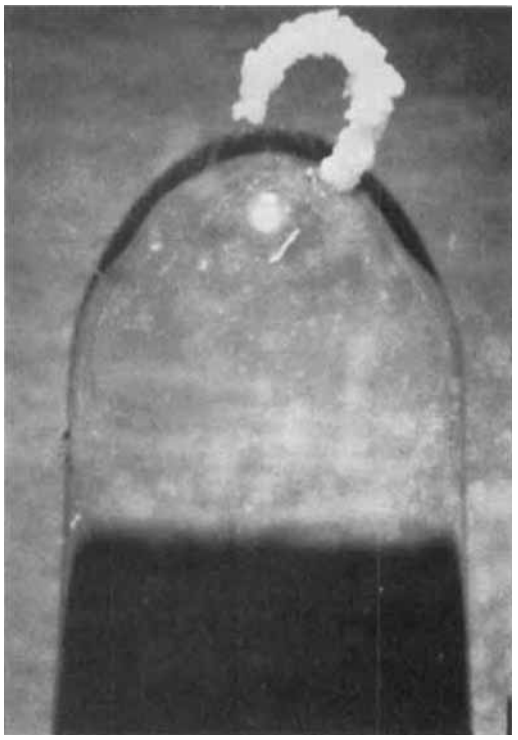

Figure 1—*Ampul with crystal growth because of imperfection. Magnification approximately 10×.*

The other 11 ampuls that contained dried material at their tips were also subjected to the Method B leakage test of D.P.S.C. Standards. Critical examination revealed only 1 of the 11 ampuls having a very faint pink tinge.

The 10 remaining ampuls were then subjected to Method C of the D.P.S.C. Standards. Two distinctly pink ampuls were detected by this test. Examination of the tips of these two ampuls with the polarizing magnifier in the manner previously described revealed no obvious defects.

All of the ampuls described had passed a leakage test immediately after manufacture, 7 months prior to the inspection that precipitated this investigation. This leakage test consisted of immersing ampuls immediately after autoclaving in a highly colored dye solution containing approximately 0.1% FD&C Red No. 2 dye, 0.5% phenol, and 0.15% trisodium phosphate and subjecting the containers to 43.2 cm. (17 in.) (gauge) of vacuum for 30 min., followed by rinsing and visual inspection.

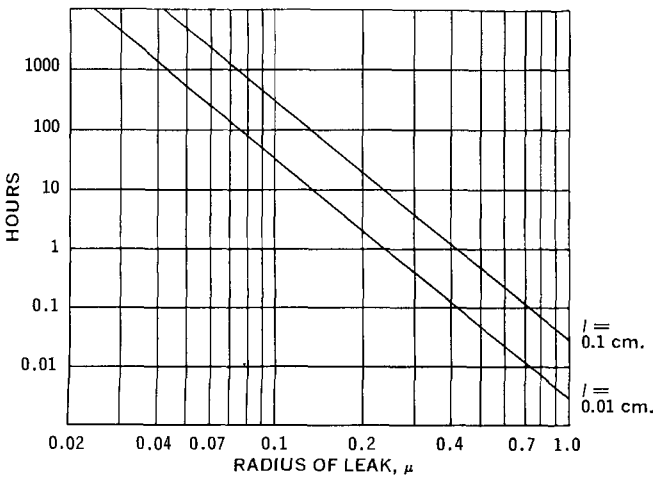

Figure 2—*Nomogram for length of time needed (in hours) for dye testing with a pressure differential of approximately 2 atm.; l denotes the length (in centimeters) of the capillary leak. It is assumed that the amount of dye solution that must penetrate in order to ascertain visual detection is 10^{-5} ml. and that the viscosity of the dye solution is 1 cps. The radius of the capillary leak, ρ, is expressed in microns.*

DISCUSSION

From a theoretical point of view, a leakage test depends on Poiseuille's law (7), which in transposed form may be written:

$$t = \frac{8 \times l \times V \times \eta}{\pi \times r^4 \times \Delta P} \qquad \text{(Eq. 1)}$$

This formula governs the (minimum) time t required for a volume V of a test solution of viscosity η to penetrate a capillary of length l and radius r, when the pressure differential is ΔP. C-g-s units (cm., cc., sec.) must be used; the viscosity, for instance, is expressed in poise (dyne-sec.-cm.$^{-2}$) not cps., and the pressure in dyne-cm.$^{-2}$ (not p.s.i., mm. Hg, or atm.).

The expression predicts that viscosity of the test medium may affect the testing time and, indeed, this was reported in qualitative fashion by Macintosh (8), Cope *et al.* (9), and Steinberg (10). Reducing the volume necessary to detect a leaker would decrease V and hence t. Fluorescent techniques were reported by DeForest (11) and Völcker (12). Artz *et al.* (6), however, showed that they have several drawbacks which prohibit their general use.

It was determined that 10^{-5} ml. of a 1% methylene blue solution[4] will color 1 ml. of water to a distinguishable level. If one then accepts that 10^{-5} ml. of test solution of viscosity 10^{-2} poise must penetrate an ampul for detection, and if one assumes ΔP to be 2×10^6 dynes-cm.$^{-2}$ (approximately 2 atm.), then:

$$t = \frac{8 \times 10^{-5} \times 10^{-2}}{\pi \times 2 \times 10^6} \frac{l}{r^4} \text{ sec.} = 1.27 \times 10^{-13} \times \frac{l}{r^4} \text{ sec.} \qquad \text{(Eq. 2)}$$

It was mentioned in the *Experimental* section that for the ampuls tested $l = 3.8 \times 10^{-2}$ cm. Since this may vary according to ampul size, t, of course, will also vary. Furthermore, l is a function of the pathway and is to be expected to be longer than the measured thickness. For the sake of practicality, r would usually be expressed in microns and t in hours. For this purpose, and with the values stated, Eq. 2 may be rewritten:

$$\tau = 0.355 \times \frac{l}{\rho^4} \text{ hr.} \qquad \text{(Eq. 3)}$$

or:

$$\log \tau = 0.547 - 1 - 4[\log \rho] + \log l \qquad \text{(Eq. 4)}$$

where τ is time in hours, and ρ is the radius in microns; l is still expressed in centimeters. The nomogram in Fig. 2 shows this relationship for values of l from 10^{-2} to 10^{-1} cm.

In the example ampul, which had a wall thickness of 3.8×10^{-2} cm., if the diameter of the opening was 0.1 μ, the time necessary for perceptible dye solution penetration at a pressure of 2 atm. would be approximately 2200 hr. If the time is reduced to a practical value, of say 16 hr., the test procedure would then detect ampuls having an opening of approximately 0.36 μ diameter. It should be noted that the smallest bacteria have a size of this magnitude.

The important fact from a safety point of view is that ampul solutions having a water vapor pressure (when saturated) less than that of the moisture content of the air space in the storage area will never appear as a questionable unit on storage. Since most saturated solutions and, in particular, multicomponent systems have quite low vapor pressures, most ampuls with imperfections of the described type will defy detection in the marketplace. Only in two component systems with relatively high saturation vapor pressure is there a greater possibility of crystal growth on the outside of the ampuls. In addition, the Kelvin equation, at low capillary radius, will dictate a smaller than normal vapor pressure; thus, defects of the kind mentioned may go unnoticed.

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Acute and Chronic Effects of 3,4-Dimethoxyphenylacetamide on Plasma Glucose and Cholesterol in Rats

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Abstract □ One hour after a single intraperitoneal dose of 50–200 mg./kg. of 3,4-dimethoxyphenylacetamide to satiated and 24-hr. fasted rats, plasma glucose was markedly increased but plasma cholesterol was reliably decreased only by the highest dose in one of two studies with satiated animals. At 24 hr. after the 10th daily oral drug administration, plasma cholesterol of rats was unaffected by 3,4-dimethoxyphenylacetamide (100 mg./kg.) but markedly decreased by a standard hypocholesterolemic agent (20,25-diazacholesterol). An acute, toxic effect of 3,4-dimethoxyphenylacetamide was indicated by a marked decrease in spontaneous motor activity and in body weight gain. Contrary to a previous suggestion, 3,4-dimethoxyphenylacetamide does not appear to be a promising hypocholesterolemic agent; observations of acute hyperglycemia are at least partly attributable to the stressful effects of the toxic doses given.

Keyphrases □ 3,4-Dimethoxyphenylacetamide—hypocholesterolemic and hyperglycemic activity, compared to 20,25-diazacholesterol, rats □ Hyperglycemic activity—3,4-dimethoxyphenylacetamide □ Hypocholesterolemic agents, potential—3,4-dimethoxyacetamide, compared to 20,25-diazacholesterol

A previous article from these laboratories (1) reported the pharmacological evaluation of 3,4-dimethoxyphenylacetamide. The crystalline compound was isolated from the leaves of *Catharantus lanceus* and later synthesized (2). A possible therapeutic use of this compound was suggested by structural similarities to phenylethylacetic acid, a compound shown to have hypocholesterolemic properties (3). Sofia *et al.* (1), in a test for this effect at 30 and 60 min. after administration of a single dose (100 mg./kg.) to rats fasted for 24 hr., reported a significant reduction of plasma cholesterol at the 60-min. postinjection time, with no change in plasma glucose levels. A test of spontaneous activity in mice showed marked CNS depression with doses as low as 6.25 mg./kg.

The first experiment reported in the present paper evaluates the acute effects of 3,4-dimethoxyphenylacetamide, repeating the previous studies on the effects of the compound on plasma glucose and cholesterol levels (1). In addition, fasted and satiated rats were compared because this variable may be important with oral administration of the drug. The effect of the compound on spontaneous activity was determined in the same animals, immediately preceding the test for plasma glucose and cholesterol.

Most references on hypocholesterolemic compounds indicate that chronic, oral administration is used for lowering the plasma cholesterol (4–8). Therefore, the present paper reports a second experiment comparing acute oral with intraperitoneal effects of 3,4-dimethoxyphenylacetamide and a third experiment comparing chronic oral effects of this compound with 20,25-diazacholesterol dihydrochloride[1], a standard hypocholesterolemic agent (8), both administered for 10 days prior to determination of plasma glucose and cholesterol. In this chronic study, hypercholesterolemia was induced in some animals by concurrent administration of propylthiouracil in the drinking fluid.

[1] SC-12937.