Table III—Stability Constants of (Pyrrolidone-5-hydroxamato)iron(III)[a]

Index	K	log K	β	log β
I	3.09×10^1	1.49	3.09×10^1	1.49
II	1.15×10^0	0.06	3.55×10^1	1.55
III	4.57×10^{-2}	−1.34	1.62×10^0	0.41
1	1.38×10^{10}	10.14	1.38×10^{10}	10.14
2	5.13×10^8	8.71	7.08×10^{18}	18.85
3	2.04×10^7	7.31	1.44×10^{26}	26.16

[a] Chelates were calculated from Eqs. 5a–5c, 6a–6c, 7a–7c, and Table II and are expressed as K_I, K_{II}, K_{III}, K_1, K_2, K_3, β_I, β_{II}, β_{III}, β_1, β_2, and β_3.

M and 2.168×10^{-4} M, respectively. The agreement is significantly improved by rejecting one extreme value of Fe_T, which gives the calculated average value of 2.075×10^{-4} M. The precision decreases in the order $\beta_I > \beta_{II} > \beta_{III}$ (Table II). The value of log β_I (= log K_I) = 1.49 is in excellent agreement with the spectrophotometrically obtained value of log β_I = 1.52.

Overall stability constants of related iron(III) hydroxamates are listed in Table IV. The decrease in stability when going from benzohydroxamic to pyrrolidone-5-hydroxamic acid chelates of iron(III) is larger than may be expected from the increase in acidity in the same direction. Some other factors must be involved such as the interference by chelating iron(III) *via* carbonyl and imine groups of the pyrrolidone ring.

The stability of (pyrrolidone-5-hydroxamato)iron(III) chelates is important for the behavior of these complexes in living systems. There is an indication that hydroxamic acid-containing compounds are constituents of siderophilin, the iron-binding β-pseudoglobulin of human plasma (17), and of ferritin (18). It is obvious that the higher the stability of hydroxamic acid–iron(III) chelates, the easier is iron transport and the better is iron resorption at various biological pH's. Iron-containing hydroxamic acids have been patented as metalotherapeutics (5). (Pyrrolidone-5-hydroxamato)iron(III) chelates may be considered as possible metalotherapeutics in view of their stability and the presence of the pyrrolidone ring, which, like povidone, is used as a substitute for blood plasma (7) and also shows a mitostimulating effect (19).

Table IV—Comparison of Stabilities of Iron(III) Hydroxamines

Acid	pKa	log β_3
Acetohydroxamic	9.37[a]	28.30[a]
Benzohydroxamic	8.79[a]	27.83[a]
Pyrrolidone-5-hydroxamic	8.65[b]	26.36[c]

[a] From Ref. 16. [b] From Ref. 12. [c] This work.

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ACKNOWLEDGMENTS AND ADDRESSES

Received July 9, 1975, from the *Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Yugoslavia.*

Accepted for publication April 23, 1976.

Supported by the Republic Foundation for Scientific Work of the Croatia.

The authors are grateful to the Alexander von Humboldt Foundation for the Opton PMQ II spectrophotometer.

M. Pečar was the recipient of a fellowship from the Foundation at the Max Planck Institute for Medical Research, Heidelberg.

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Methods for Evaluating Hermetic Closures for Screw-Capped Bottles

R. D. CILENTO

Abstract □ With the described method, a large number of sealed bottles can be tested to determine their effectiveness, integrity, and reliability in preserving an inert atmosphere. The method consists of placing milligram quantities of dry ice into the bottles, capping them, and periodically weighing them on an analytical balance; leaky bottles are detected by a loss in weight. This method can be applied to test a large number of bottles with a minimum of effort and manpower and without complicated instrumentation. The data presented are highly reproducible and correlate well with data from other methods and with the physical defects of the bottles.

Keyphrases □ Seals, hermetic—testing method developed for large numbers of screw-capped bottles □ Bottles, screw capped—method of testing hermetic seals developed for large numbers □ Technology, pharmaceutical—method of testing hermetic seals of large numbers of screw-capped bottles developed

By definition, hermetic containers are impervious to air and any other gas under ordinary conditions of handling. For the primary container of a drug to qualify as hermetic, it must be leakproof; *i.e.*, no gases or vapors must be capable of entering it or escaping from it.

A product is hermetically sealed to protect it from con-

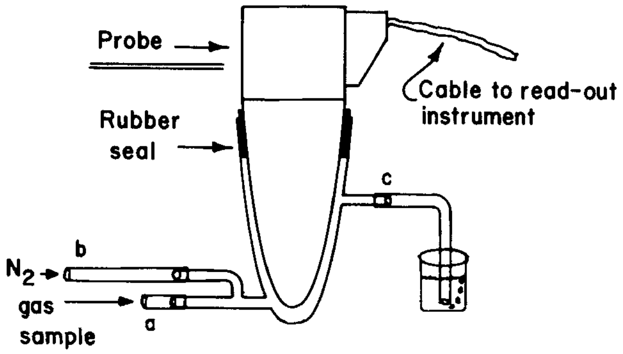

Figure 1—*Oxygen analyzer glass cell.*

tact with components of the atmosphere that may cause it to deteriorate, *e.g.*, oxygen or water vapor, or to prevent its volatile components from escaping. The need for this practice is well documented (1–17).

A review of the work done in the field of inert-gas flushing of containers has revealed an extensive effort dedicated to the development of procedures for purging primary containers (13, 15, 18) and to measurement of the effectiveness of these procedures (14, 15, 19–22). What seems to be lacking, however, is information on methods for studying the effectiveness of screw-capped bottles in preserving an inert atmosphere within them throughout their shelflife. Such methods could be subdivided into two categories: (*a*) testing the efficacy of components (in particular, seals and seal materials), and (*b*) testing the impermeability of the assembled system.

Much published work exists for the first category (3, 5, 9, 23–28). In the second category, methods have been reported for testing plastic bottles (29), single-dose pouches (28), and ampuls; but no effective, simple, fast, precise method has been reported for testing screw-capped glass bottles. It is not enough to demonstrate that seal materials are impervious to moisture or oxygen; their effectiveness must be demonstrated after they have been applied as finished closures.

This paper presents a method by which a large number of sealed bottles can be tested for determination of the most effective seal and for the cause of malfunctioning of a container, *e.g.*, the bottle, the seal, the cap, or the assembling operation. The results should provide guidance in developing necessary controls or precautions.

EXPERIMENTAL

Equipment—A vacuum chamber (20 × 20 × 30 cm), an analytical balance[1], a spring torque tester[2], a pressure vessel (a cylindrical 110-liter tank), and an oxygen analyzer[3] were used.

Materials Tested—The bottle[4] size was 100 ml with beaded glass lips, 38 mm. Standard metal screw caps matched the bottle size and finish. The liners were polyvinyl chloride, 0.7-mm thick solid disks.

Test Methods—The following test methods were developed to study the efficacy and reliability of closure systems.

Test 1: Vacuum Chamber—A number of bottles are capped at a specified torque and placed into the vacuum chamber for 4 hr. The vacuum is quickly broken, and each bottle is immediately placed, cap down, in a tray containing enough water to keep the cap entirely submerged. The bottle is vented by holding the cap with a pair of pliers or similar device and twisting the bottom of the bottle. Any bottle that had been evacuated through a leaky closure will be partially or totally filled with water.

Test 2: Dry Ice—Immediately after a chip or two of crushed dry ice has been placed into a tared bottle, the bottle is capped at a specified torque and then weighed to the nearest milligram on the analytical balance. The amount of dry ice placed in each bottle should produce enough gaseous carbon dioxide to give a pressure of 40.000–70.000 Nm^{-2} (6–10 psi). The amount varies with bottle size, *i.e.*, about 50 mg for a 25-ml bottle, 150 mg for a 100-ml bottle, and 250 mg for a 200-ml bottle.

A leak-free bottle prepared in this way maintains its weight indefinitely. A leaky bottle starts losing weight immediately and, depending on the magnitude of the defect, loses 70–80% of the carbon dioxide gas within minutes, hours, or days; the remainder of the gas diffuses out more slowly. If a perfectly sealed bottle is opened months later, the hissing sound of the escaping gas under pressure is still audible. A skilled technician can prepare more than 30 sample bottles/hr.

Test 3: Oxygen Diffusion—Each bottle is flooded with nitrogen and capped at a specified torque. The bottles are held for 1–4 days in a pressure vessel that has been flooded with oxygen. Oxygen is maintained in the vessel under a positive pressure, being permitted to flow in continuously and to bubble out through a vent tube. At the end of the storage period, the bottles are tested for oxygen content.

Nitrogen flooding of the bottles is accomplished in either of two ways.

1. The bottles are first evacuated in the vacuum chamber, and then the vacuum is broken with nitrogen. While nitrogen is still flowing, the cap is screwed on each bottle fingertip tight. A specified torque is applied to the cap as soon as the bottle has been removed from the chamber. Precautions must be taken to avoid introducing air into the bottle during capping.

2. The procedure of blowing nitrogen into open bottles is not as effective as is the use of the vacuum chamber, but it is much faster and, if properly controlled, can yield very reproducible results. The four critical factors in the control of this procedure are the nitrogen velocity, time, position of the nitrogen-delivery tube with respect to the neck of the bottle, and mode of application of the cap. The optimum conditions for each size and shape of the bottle must be determined experimentally.

Procedure 1 can produce bottles containing less than 2% oxygen, whereas Procedure 2 produces bottles containing 2–5% oxygen.

The oxygen content of the bottles is determined by use of the oxygen analyzer, which can be adapted to receive a gas sample by mounting a specially built glass cell on its probe (Fig. 1). The cell is purged with a stream of nitrogen through intake line b; as soon as the instrument reads zero, this line is closed and a 5-ml gas sample is introduced *via* syringe through line a. The instrument response is practically immediate. The instrument is calibrated by introducing samples of known oxygen composition; a straight-line relationship exists between sample composition and meter readings, with the curve passing through the zero point. The method can be used to measure accurately oxygen concentrations of 0–60 ± 0.5%. Concurrent testing of several samples proved this method to be as accurate and reproducible as a GC assay.

To obtain a gas sample, a hole is made in the cap of the bottle with an awl; a 5-ml sample of gas is withdrawn into a syringe through a hypodermic needle fitted with a rubber flange at its base. The gas is then immediately injected into the cell of the instrument. The main advantage of this method is the speed with which a large number of samples can be tested (a skilled technician can test more than 30 samples/hr) with results immediately determined from a standardized graph; its main disadvantage is the large sample of gas (5 ml) needed.

RESULTS AND DISCUSSION

The described test methods were used to determine the most effective closure system for screw-capped glass bottles. The three methods were developed concurrently, but only the dry ice method was used extensively in the screening and concluding phases of the study. The oxygen-diffusion method was used only to confirm the results of systems that had passed the dry ice test once the validity of the dry ice method had been demonstrated by good correlation between these two methods. The vacuum chamber method, developed early in the program, produced results that correlated with those of the other methods, but its use was discontinued after the superiority of the dry ice method had been demonstrated.

The data in Table I show that a good correlation exists between results obtained by the dry ice and oxygen-diffusion tests. Leakage of Bottles 1, 4, 7, 12, 15, 16, 18, and 19 was demonstrated by the dry ice test and confirmed by the oxygen-diffusion test. The data in Table II confirm the

[1] Sartorius type 2472, 200-g range, 0.0001-g sensitivity.
[2] Owens-Illinois Glass Co.
[3] Model 300, Weston & Stack, Inc.
[4] GCMI glass finish No. 400.

Table I—Determination of Leakage of Bottles[a] by Dry Ice and Oxygen Diffusion Tests

	Dry Ice Test, Weight Loss, %				
	First Run		Second Run		Oxygen Diffusion Test, Oxygen Content, %
Bottle	18 hr	42 hr	20 hr	44 hr	
1	54	66	77	76	7.0
2	0	6	0	0	3.0
3	0	2	0	0	2.5
4	58	67	46	57	4.5
5	0	0	0	0	2.5
6	5	8	0	0	2.0
7	27	40	46	55	9.0
8	0	2	0	0	2.5
9	12	24	7	10	2.5
10	0	0	0	0	2.0
11	0	0	0	0	2.0
12	68	69	78	76	15.5
13	0	5	0	0	2.5
14	22	34	3	7	2.5
15	77	72	81	82	13.5
16	79	75	72	74	15.5
17	0	3	0	0	2.0
18	35	54	57	68	7.5
19	27	39	11	22	>60
20	2	5	0	0	2.0
21	24	39	7	11	2.0
22	0	0	0	0	2.0
23	28	42	5	7	2.0
24	0	0	0	0	2.5
25	0	0	0	0	2.5

[a] Bottles were picked at random from the same lot of the supplier. In this experiment, the caps were applied with torques of 2.2 ± 0.2 mN.

results shown in Table I and also show that leakage could be corrected by increasing the torque applied to the caps. In this experiment, carried out with the same set of bottles used earlier (Table I), the effect of torque on the efficacy of the closure system was studied. The caps were applied at torques of 1.65–1.85, 2.50–2.75, and >2.75 mN. For the low and intermediate levels of torque, the caps were twisted to the prescribed torque and quickly released; for the high torque level, the caps were twisted to the limit of the torque tester and held so for 5 sec. For the three levels of torque, 50, 20, and 0% of the bottles had lost weight after 4, 24, and 96 hr, respectively. The complete integrity of the seal for bottles in the third group must be attributed both to the magnitude of the torque (>2.75 mN) and to the duration of its application.

The results of the first two experiments demonstrated that the set of bottles tested included highly defective, moderately defective, and perfect specimens. The third experiment sought to discover the nature of the defect. The initial assumption of the cause of leakage was a lack of flatness of the bottle lip. To test this hypothesis, a vacuum device (Fig. 2) to measure the flatness of the bottle lip was built. The testing procedure consisted of connecting the device to a source of vacuum, placing the Plexiglas plate on the bottle, and reading the gauge. The results were highly reproducible and are assumed to be an accurate measure of the degree of flatness of the lip of the bottle, the highest reading being obtained for a bottle with a perfectly flat lip (Table III).

The results of this experiment, however, showed no correlation with the results of the first two experiments. For example, Bottles 15 and 16, which were among the worst leakers, showed the highest readings; on the other hand, Bottles 11 and 22, which did not leak, showed very low readings. It was concluded that the lack of flatness of the bottle lip was not responsible for the leak. At this point, the bottles were closely inspected with a magnifying glass and a stereoscopic microscope; most of them showed thin, smooth grooves across the lip (Fig. 3). All grooves were

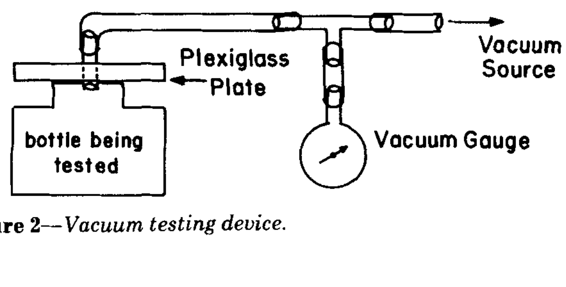

Figure 2—*Vacuum testing device.*

Table II—Relationship between Leakage[a] and Put on Torque (P.O.T.) Applied to Caps

	P.O.T., mN, Weight Loss, %		
Bottle	1.65–1.85[b]	2.50–2.75[c]	>2.75[d]
1	5.5	0	0
2	0	0	0
3	0	0	0
4	61	59	0
5	0	0	0
6	0	0	0
7	66	0	0
8	0	0	0
9	9	0	0
10	0	0	0
11	0	0	0
12	77	15	0
13	0	0	0
14	19	0	0
15	76	76	0
16	84	85	0
17	19	0	0
18	18	0	0
19	69	41	0
20	0	0	0
21	39	0	0
22	0	0	0
23	48	0	0
24	0	0	0
25	0	0	0

[a] As measured by the dry ice test. [b] Determined at 4 hr from initial dry ice charge. [c] Determined at 24 hr. [d] Determined at 96 hr.

ranked on an arbitrary scale (from 0 = no grooves to 10 = widest).

The results showed that bottles lacking grooves or having grooves of minimal width had not leaked under any of the conditions shown in Table II. The five bottles that leaked after the caps had been applied with a torque of 2.50–2.75 mN showed grooves rated between 4 and 7. One bottle (26) that had, in another experiment, leaked even after it had been capped with a maximum torque showed a groove much larger than any other found. Grooves ranked 3, 6, 7, and 10 on this scale were later shown, by microscopic comparison with hairs of known diameter, to have widths of 50, 75, 100, and 200 μm, respectively.

Table III—Determination of Defects of Bottle Lip

		Groove Size Estimate	
Bottle	Vacuum Device[a], Nm^{-2}	Rank Order[b]	Microscope[c]
1	53.2	4	—
2	62.2	1	—
3	48.6	1	—
4	30.5	4	—
5	66.0	2	—
6	64.2	2	—
7	56.5	4	—
8	56.8	0	—
9	60.8	3	—
10	50.0	0	—
11	36.2	0	—
12	26.4	7	100
13	66.8	0	—
14	67.8	3	—
15	75.5	6	75
16	75.5	7	—
17	53.2	3	50
18	73.7	3	—
19	60.0	5	—
20	54.0	1	—
21	48.4	3	—
22	48.6	1	—
23	59.2	3	—
24	49.0	1	—
25	56.8	1	—
26	39.0	10	200

[a] Average of two measurements taken 90° apart. [b] Estimates by three persons, averaged to closest whole number. [c] By matching against hairs of known diameters.

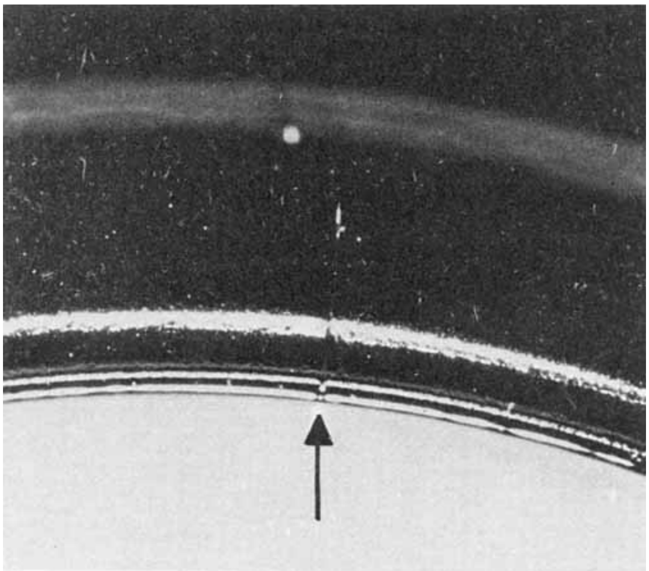

Figure 3—*Groove defect (at approximately 10×) (photograph courtesy of R. Walton)*

The dry ice test method was also used to evaluate a variety of liner seals. To compensate for the groove defects in bottle lips, polyvinyl chloride foam liners required an application torque as great as that required for polyvinyl chloride solid liners. Moreover, wax-coated laminates were inferior to polyvinyl chloride, and pressure-sensitive seals[5], applied with an acceptable torque, were more effective than the other liner seals.

SUMMARY AND CONCLUSIONS

A dry ice method has proved very useful for testing the effectiveness of screw-capped bottles in preserving an inert atmosphere within them. The method permits the testing of a large number of samples with a minimum of effort; it utilizes uncomplicated equipment and is simple to apply. The results are highly reproducible and correlate well with those of the oxygen-diffusion method and with the physical defects of the system.

This method is specifically designed to test the assembled package, but it can be used to test components or procedures. By its use, various seals were evaluated. Polyvinyl chloride proved to be an excellent sealing material which compensates very well for out-of-flat defects, but it requires too great a torque to be useful in compensating for the groove defects.

The method can be used by packaging engineers to develop and evaluate closure systems and procedures and by quality-control units for routine spot checks of container materials or for extensive tests of questionable shipments.

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ACKNOWLEDGMENTS AND ADDRESSES

Received December 17, 1975, from the *Department of Pharmaceutical Research & Development, Squibb Institute for Medical Research, New Brunswick, NJ 08903.*

Accepted for publication May 5, 1976.

Presented at the APhA Academy of Pharmaceutical Sciences, Atlanta meeting, November 1975.