studied in detail, it would seem that the decrease in the dissolution rate could occur due to excessive turbulence of the dissolution medium inside the confined space of the chamber and/or the compression of the tablet against the upper filter. So ultimately the concentration of drug appearing in solution at the slower flow rates exceeds the correction made for rate of flow in calculation of the instantaneous dissolution rate. This behavior is substantiated by the fact that if the dissolution of a tablet is allowed to run to completion, the tablet being dissolved at a slower flow rate returns to the baseline before that being dissolved at the higher rate.

It was also observed that the dependence of the dissolution rate on volume flow rate varied with hardness, strength, and formulation of the tablet. With each different type of tablet, the flow rate had to be empirically determined to provide the most revealing dissolution rate. In addition to the nature of the tablet, the limitations of the analytical module must be considered in selecting the volume flow rate. The concentration of the dissolving drug must be maintained within the linear calibration range of the sodium-ion electrode or spectrophotometer.

The ability of the dissolution apparatus to monitor common variables is shown in Figs. 5 and 6. In Fig. 5, the dissolution profile for a sodium butabarbital tablet is compared to the dissolution profile for a tablet from the same batch that had been crushed to a powder before placement in the dissolution apparatus. As expected, the initial dissolution rate was greatly increased when the disintegration phase was eliminated. This observation is in agreement with the data by Tingstad and Riegelman (2). Figure 6 illustrates the ability of the apparatus to differentiate between tablets of exactly the same formulation but of different hardnesses. The sodium salicylate tablets used were prepared in these laboratories and, as expected, a rate of dissolution increasing in rank order of decreasing tablet hardness was seen.

The versatility of this apparatus is further illustrated with the dissolution of sodium warfarin tablets. A flow rate of 0.75 ml./min. allows the instrument to differentiate among 5-, 10-, and 25-mg.

tablets. The dissolution of sodium bicarbonate tablets was also followed by adjustment of the flow rate to 3.5 ml./min.

The use of a flowing stream dissolution apparatus in conjunction with either the sodium-ion electrode or spectrophotometric module provides an automated means of following tablet dissolution quickly and accurately. The apparatus differentiates between the common tablet parameters of hardness and drug potency and provides a variability adjustment through flow rate. In conclusion, this apparatus provides a quick and accurate means of analyzing *in vitro* tablet dissolution.

REFERENCES

(1) F. Langenbucher, *J. Pharm. Sci.*, **58**, 1265(1969).
(2) J. E. Tingstad and S. Riegelman, *ibid.*, **59**, 692(1970).
(3) "Remington's Pharmaceutical Sciences," 14th ed., J. E. Hoover, Ed., Mack Publishing Co., Easton, Pa., 1970, p. 284.
(4) H. Jacobson, *Anal. Chem.*, **38**, 1951(1966).
(5) W. D. Mason, T. E. Needham, and J. C. Price, *J. Pharm. Sci.*, **60**, 1756(1971).
(6) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970.
(7) G. Levy and B. A. Hayes, *N. Engl. J. Med.*, **262**, 1053(1960).
(8) G. Levy and B. Sahli, *J. Pharm. Sci.*, **51**, 58(1962).
(9) R. E. Shepherd, J. C. Price, and L. A. Luzzi, *ibid.*, **61**, 1152 (1972).
(10) T. E. Needham, L. A. Luzzi, and R. E. Shepherd, *ibid.*, **62**, 470(1973).

Effect of Adverse Storage Conditions on Vacuum-Holding Ability of Large-Volume Parenteral Containers

MARTIN B. PINCKNEY, Jr.*, LOUIS A. LUZZI▲, and JAMES C. PRICE

Abstract ☐ The ability of large-volume vacuum-packed parenteral containers to maintain vacuum under adverse conditions of temperature and agitation was examined. It was the intent of the study to examine the possible effects of accelerated aging during travel and storage. Containers were stressed by being subjected to alternating high and low temperatures for 30 days (24-hr. intervals) and by shaking for 30 days at temperatures up to 50°. Preliminary tests were carried out to establish a statistically significant number of experiments. It was found, in all cases, that the vacuum seal was maintained under these conditions. It is concluded that such factors

as decomposition of ingredients and faulty glassware should be suspected if vacuum loss is found with these types of containers.

Keyphrases ☐ Parenteral containers, large volume—effects of temperature and agitation on vacuum-holding ability ☐ Large-volume parenteral containers—effects of temperature and agitation on vacuum-holding ability ☐ Vacuum retention—effects of temperature and agitation on large-volume parenteral containers ☐ Containers, large-volume parenteral—effects of temperature and agitation on vacuum-holding ability

In the last decade, there has been a surge of interest regarding the safety of parenteral preparations. Recent reports have been concerned with particulate matter (1–4), sterility control (5, 6), and contamination during opening, preparation, and use (7–9).

That serious problems can still occur with this type of preparation is indicated by recent references to

septicemias caused by microbial contamination (10–12). Some of these infections have been attributed to improper use or handling of the parenteral product itself (10). Other problems have been caused by introduction of contaminants during administration (11, 12).

One possible source of contamination is the failure of the closure during storage. A means of testing for

Table I—Effects of Temperature Fluctuation on the Vacuum-Holding Ability of Vacuum-Packed Parenterals

Temperature Range	Type and Volume (ml.) of Container	Number Each of Test and Control Containers	Test Containers, avg. ml. Water to Relieve Vacuum	Control Containers, avg. ml. Water to Relieve Vacuum
25-40°	Aᵃ, 250	24	36.9	36.8
25-40°	Bᵇ, 250	24	73.2	73.3
5-25°	A, 250	24	35.9	36.8
5-25°	B, 250	24	74.5	73.3
5-40°	A, 250	24	37.8	36.8
5-40°	B, 250	24	74.8	73.3
5-50°	A, 250	24	38.9	37.6
5-50°	B, 250	24	71.4	70.8
25-40°	Aᶜ, 500	22	82.3	80.4
25-40°	Bᵈ, 500	21	76.7	74.5
5-25°	A, 500	24	90.3	90.0
5-25°	B, 500	26	77.0	74.5
5-40°	A, 500	26	85.7	90.0
5-40°	B, 500	27	75.0	74.5

ᵃ Type A containers of 250 ml. from Lot N20N3. ᵇ Type B containers of 250 ml. from Lot TU9790A. ᶜ Type A containers of 500 ml. from Lot N20F3. ᵈ Type B containers of 500 ml. from Lot TU9617C.

closure failure has not been reported in the literature. In vacuum-packed parenterals, this possibility can be examined by measuring the vacuum retained under various stressing storage conditions. This report describes the vacuum-holding ability of large-volume parenterals under storage conditions somewhat more severe than would be encountered during normal use.

EXPERIMENTAL

Description of Systems Tested[1]—The systems examined were: Type A, a vacuum-packed nonfiltered air system; and Type B, a vacuum-packed filtered air system (9). Both systems contained lactated Ringer's solution in 250- and 500-ml. sizes, and only one lot number was used for each size. Headspace and fill volume in both types of container were found to vary less than 1% of the total container volume for each size container reported.

Temperature Fluctuation Tests--Each described system was exposed to four temperature ranges, which were chosen as extreme test conditions and were designed to approximate or exceed the maximum and minimum conditions encountered during transit or storage. Temperature ranges employed were 25-40°, 5-40°, 5-25°, and 5-50°.

The filled containers were initially maintained in a constant-temperature circulating bath at the lower temperature for 24 hr. and then transferred to another constant-temperature circulating bath at the higher temperature for 24 hr. Each set of containers was alternated at the indicated temperatures for 8 days. At the end of the tests, the containers were adjusted to room temperature[2] to establish equilibrium and were examined for vacuum retention.

Agitation Tests—Shaking at constant elevated temperatures was performed to study the effects of agitation on vacuum loss. The filled containers were placed in a temperature-controlled shaker[3] and run at the highest shaking speed [approximately 380 oscillations/min. with an oscillatory amplitude of 2.54 cm. (1 in.)]. The size of the container holders built into the shaker necessitated the use of 500-ml. containers for the agitation tests (Tables I and II). Sets of con-

[1] Systems included are those marketed by Cutter Laboratories, Berkeley, CA 94710, and by Baxter Laboratories, Inc., Morton Grove, IL 60053
[2] Accomplished by placing the bottles in a 25° water bath for 24 hr. and then passing room temperature air (via an electric fan) over the bottles for 2-4 days; room temperature for these tests was 23.5-24.5°.
[3] Gyrorotary model G-25, New Brunswick Scientific Co., New Brunswick, N. J.

Table II—Effect of Agitation for 30 Days on Vacuum-Holding Ability of 500-ml. Vacuum-Packed Parenterals

Agitation Temperature	Type of Container	Number Each of Test and Control Containers	Test Containers, avg. ml. Water to Relieve Vacuum	Control Containers, avg. ml. Water to Relieve Vacuum
30°	Aᵃ	21	88.9	88.5
30°	Bᵇ	23	75.9	75.0
40°	A	12	78.6	78.0
40°	B	12	77.5	78.6
50°	A	12	74.2	74.3
50°	B	12	73.3	75.0

ᵃ Type A containers from Lot N20F3. ᵇ Type B containers from Lot TU9617C.

tainers were agitated at 30, 40, and 50° for 30 days; the bottles were then removed from the shaker, adjusted to room temperature, and tested for vacuum.

Controls—Containers from the same lot were stored at room temperature and were examined at the same time as the test containers in both the temperature fluctuation and agitation studies to assure that "aging" of the bottles would not be a variable factor in the studies. Pairing controls with test containers in each lot eliminated the possibility of lot-to-lot variation.

Vacuum Measuring Apparatus--The apparatus consisted of a 250-ml. buret equipped with attached tubing terminating with an administration needle; the buret was equipped with an automatic refilling device (Fig. 1).

Container vacuum was measured by filling the 250-ml. buret with water and inserting the administration needle through the recommended puncture site of the parenteral container. Water from the buret was allowed to pass into the inverted intravenous container until the partial vacuum had been satisfied. Liquid levels in the container and buret were maintained at the same level by adjusting the container height. Water drawn from the buret into the vacuum was accurately read to 1.0 ml. and estimated to 0.5 ml. The larger the quantity of water drawn into the bottle, the greater was the vacuum that had existed prior to puncture. As a control, at least 25 containers of both types were examined for void space and fill volume.
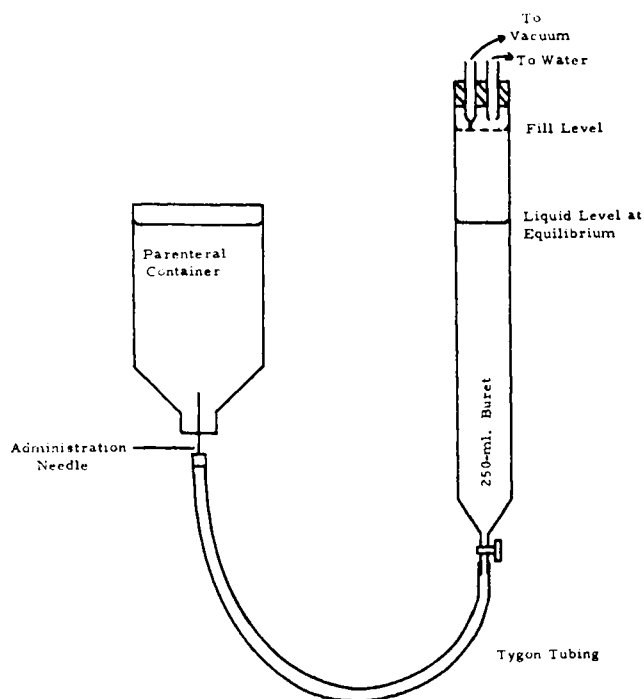

Figure 1--Apparatus used to examine vacuum.

Use of the water-filled buret technique eliminated the possibility of errors due to air leakage about the site of cannulation. If air entered, it would pass by the needle, go up through the liquid in the inverted container, and become visible as bubbles.

RESULTS AND DISCUSSION

The results from the experiments are shown in Tables I and II. Statistical evaluation of these data yields evidence that temperature fluctuation and agitation treatments have no detrimental effect on vacuum seal.

The greatest difference detected between average vacuums for test and control groups was found in the temperature fluctuation experiments. Table I shows that the 5–40°, 500-ml. Type A group had average vacuums of 85.75 ml. water for the test bottles and of 88.99 ml. water for the control bottles. A standard "pooled" or two-sample t test on this test–control pair of samples indicated that the differences were insignificant since the calculated t value is 2.0 or less than 2.413 at the 1% level.

The natural variation in vacuum from bottle to bottle among the Type A and Type B control samples is given by their standard deviations: 4.54 ml. water for Type A and 4.37 ml. water for Type B. When using the F test, it was found that there was no significant difference in the vacuum variance between the two types of control samples.

It is apparent from analysis that the conditions imposed upon the test containers did not affect the vacuum in these systems. The test conditions were strenuous to the point where they exceeded the normally expected environments to which the containers would be exposed. It may be concluded that normally expected amounts of agitation experienced in transport or fluctuations in temperature as in transport or storage are not primarily responsible for a loss of vacuum in these types of containers. When loss of vacuum is suspected, other factors such as decomposition of ingredients and faulty glassware or stoppers should be considered.

REFERENCES

(1) N. M. Davis, S. Turco, and E. Sively, *Bull. Parenteral Drug Ass.*, **24**, 257(1970).
(2) N. M. Davis, S. Turco, and E. Sively, *Amer. J. Hosp. Pharm.*, **27**, 822(1970).
(3) N. M. Davis and S. Turco, *ibid.*, **28**, 620(1971).
(4) A. Das, *Mfg. Chem. Aerosol News*, **June 1972**, 21.
(5) T. J. Macek, *Bull. Parenteral Drug Ass.*, **26**, 18(1972).
(6) J. T. Mayernik, *ibid.*, **26**, 205(1972).
(7) T. R. Arnold and C. D. Hepler, *Amer. J. Hosp. Pharm.*, **28**, 614(1971).
(8) E. N. Deeb and G. A. Natsios, *ibid.*, **28**, 764(1971).
(9) M. B. Pinckney, Jr., L. A. Luzzi, and T. E. Needham, Jr., *J. Pharm. Sci.*, **62**, 80(1973).
(10) R. J. Duma, J. F. Warner, and H. P. Dalton, *N. Engl. J. Med.*, **284**, 257(1971).
(11) K. W. Ashcraft and L. L. Leape, *J. Amer. Med. Ass.*, **212**, 454(1970).
(12) B. McGovern, *Mil. Med.*, **135**, 1137(1970).

# Semiautomated Spectrophotofluorometric Determination of Trimethoprim in Biological Fluids

S. A. KAPLAN▲, R. E. WEINFELD, and T. L. LEE

Abstract ☐ A semiautomated spectrophotofluorometric method for the determination of trimethoprim in blood, urine, and tissue is described. The initial extraction procedures are performed manually. The subsequent alkaline permanganate oxidation and the chloroform extraction of the fluorescent trimethoxybenzoic acid are performed by the automated system at a rate of 30 specimens/hr. The fluorescence is measured by a microflow cell in a spectrophotofluorometer. The method exhibits the same specificity and precision as the manual procedure, with a sensitivity limit in blood of 0.2 mcg./ml.

Keyphrases ☐ Trimethoprim in blood, urine, and tissue—semiautomated spectrophotofluorometric analysis ☐ Spectrophotofluorometry—analysis, semiautomated, trimethoprim in biological fluids

Trimethoprim[1] [2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine, I] is an inhibitor of dihydrofolate reductase which potentiates the activity of sulfonamides against a wide variety of bacterial species (1, 2). The previously reported manual method for determining I in blood and urine (3) met the requirements for specificity, sensitivity, accuracy, and precision. However, the generation of a large number of specimens required
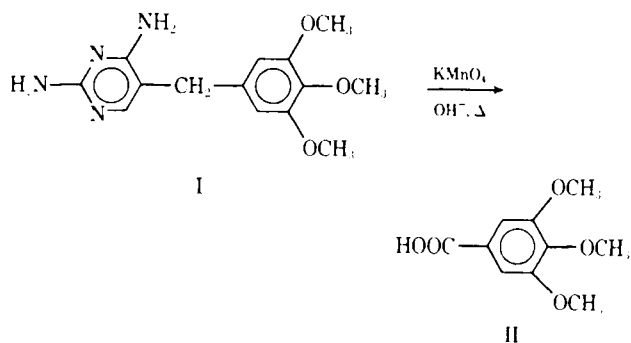
Scheme I

[1] Trimethoprim is an active ingredient in Bactrim, F. Hoffmann–La Roche and Co., Basle, Switzerland.