Application of Radioisotopes to Absorption in Pharmaceutical Closures

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Moisture absorption by rubber stoppers was determined by means of tritiated water. Residual activity in the stoppers and ferrules was determined after storage at room temperature at 50° and observed to be 0.56 and 0.025 per cent, respectively, at maximum for time periods involved.

IN THE PACKAGING of biological and injectable pharmaceutical products, the absorption of packaged components into the package materials with a subsequent loss of product is a critical problem. Mattocks and Milosovich (1) reported on the absorption of water by rubber stoppers and on the effects of inorganic salts in solution. Lowry and Kohman (2) discussed the mechanism of transmission into the stoppers and the role of stopper constituents. Barry (3) described the criteria for judging rubber closures in a symposium on the subject. The absorption of bacteriostatic agents by stoppers was reported by Wing (4, 5) and Sykes (6). The measurement of transmission and product loss, with the evaluation of various packaging materials, has been determined essentially by three methods (7). (*a*) Trial and error, using packaging materials of different types and checking weight losses; (*b*) holding the container and contents (sealed) under a vacuum for an extended time and checking the weight loss; (*c*) the Karl Fisher titration method.

These methods are either approximations and establish a series of assumptions or are time-consuming and complex procedure-wise. The time involved in these tests usually extends over a period of several months for a reasonable evaluation. In addition, many variables are involved that are critical or difficult to evaluate.

In the vacuum test method, the weight losses observed are assumed to be due to solvent transmission through or around the stopper and seal. This procedure may accurately determine a loss but neither permits determination of the actual route of solvent transmission, nor allows detection of potential residual uptake of solvent in the stopper involved.

The Karl Fisher titration method has been widely employed for the determination of water loss and absorption; several modifications have been utilized. The complexity of the procedure and necessity of exact details in application to these problems makes it difficult to obtain reproducible data.

In view of these problems and because of the importance of the data to packaging of biologicals, the evaluation of a testing method utilizing radioisotopes was proposed. It was believed that the application of radioisotopes would permit the accurate determination of true absorption, transmission rates, and the actual routes of losses from the containers. The specificity of radioisotopes appeared to lend itself particularly well to these determinations. In conducting the preliminary test it was determined that 15 vials and stoppers, sealed by conventional methods, would serve to check both the theory and the method.

EXPERIMENTAL

Counting Method.—All samples were counted by means of liquid scintillation counting.[1] The instruments were operated at settings determined to permit an efficiency of 40% for tritiated water. All samples were counted for 10 minutes and corrected for background.

Procedure.—Fifteen glass vials[2] of 9-ml. capacity were divided into three groups of five vials each. A 2.5-ml. quantity of tritiated water[3] with a total measured activity of 1.59×10^6 c.p.m. was added to each group. A 20-mm. rubber stopper,[4] preconditioned by autoclave sterilization and siliconed as in production processing, was added to each vial. A 20-mm. aluminum ferrule[5] was placed into position; the vials were sealed with a Firmpress capping device.[6]

One group of five vials was maintained at room temperature for 2 weeks. A second group was maintained at 50° for 1 week in a constant temperature oven, and the third group maintained at 50° for 2 weeks. These periods at 50° are assumed equivalent to 3 months and 6 months at room temperature, respectively (8). At the conclusion of the designated times, the vials were cooled to room temperature, and the ferrules removed. Each ring

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[1] Ekco Liquid Scintillation Detector, model 6641, Ekco Spectrometer and Scaler model N610A, American Tradair Corp., Long Island City, N. Y.
[2] Wheaton Glass Vials 2-104E.
[3] Tritiated Water Reference Standard, Tracer Laboratories Co.
[4] Gillon Stoppers No. A-46.
[5] West Seals No. 20-1.
[6] Firmpress HO-207, Wheaton Co., Millville, N. J.

TABLE I.—PER CENT RESIDUAL ACTIVITY

Vial	Room Temp., 2 wks.		50° C., 1 wk.		50°C., 2 wks.	
	Stopper	Rings	Stopper	Rings	Stopper	Rings
1	0.32	0.019	0.35	0.012	0.53	0.023
2	0.29	0.034	0.41	0.030	0.58	0.009
3	0.36	0.024	0.43	0.026	0.56	0.025
4	0.22	0.007	0.44	0.020	0.50	0.020
5	0.28	0.014	0.33	0.019	0.56	0.025
$\bar{X}$	0.29	0.019	0.39	0.021	0.55	0.020
S_x	0.051	0.001	0.047	0.0068	0.030	0.0067

was placed into a glass counting vial,[7] 1 ml. of absolute ethanol added, and 10 ml. of liquid phosphor introduced. (PPO[8] 4.00%, POPOP[9] 0.05%, and toluene to 1 L.) The vials were labeled by group and numbered.

The rubber stoppers were withdrawn with forceps, dusted with talc, and brushed clean to remove any external adhering water. Each stopper was placed into a glass vial and 10 ml. of ethanol was added. The vial was sealed with a screw cap and incubated at 50° for 48 hours to leach residual water. The 48-hour period was observed to achieve complete extraction of the tritium from the stoppers on the basis of a constant specific activity of the alcohol extract. At the end of this time, the vials were cooled to room temperature, opened, and 1-ml. aliquots measured accurately and added to individual glass counting vials containing 10 ml. of the liquid phosphor solution. Five samples of ferrules and identically treated rubber stoppers were prepared for controls and background measurements.

The activity in c.p.m. for each ferrule and stopper was determined as described and compared to the total activity placed originally into the Wheaton vial to determine the per cent of residual activity. These values are tabulated in Table I and plotted for the stoppers in Fig. 1.

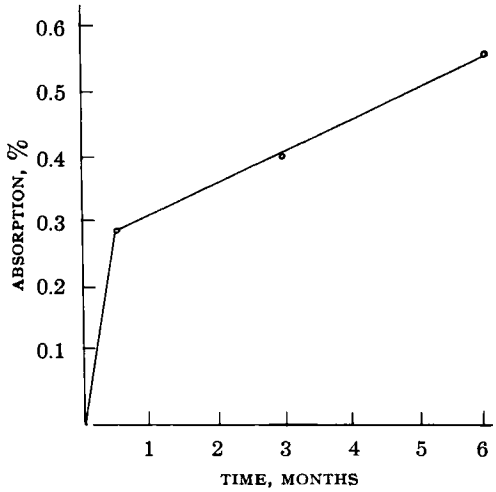

Fig. 1.—The per cent absorption in rubber stoppers *vs.* time in months based on 50°C. incubation periods.

DISCUSSION

The per cent of residual activity detected on both the ferrules and in the stoppers was not the total water uptake or transmission, but only the residue at the conclusion of the incubation period. The rather constant values for the ferrules would indicate that an actual exit passage may occur for the water in the vials, or that this is a saturation level—and once reached, no additional uptake occurs. The uptake of the stoppers, as seen in Table I and Fig. 1, occurs very rapidly and probably commences immediately after sealing the vials. The process is noted to increase in a near linear proportion, reaching the observed peak of 0.55% at the assimilated 6-month period. Whether transmission occurred was not determined, but weighing the sealed vials prior to and at the completion of the incubation periods would serve to measure this factor. This preliminary approach has clearly indicated that uptake into the rubber stoppers does occur, and that a potential exit from the sealed vials may occur around the metal rings. The method is efficient, accurate, and most specific. Any radioactivity detected in the stoppers and on the metal rings could have come only from the tritiated water added to the vials. It should be noted also, that these residues represent a loss of available volume contained within the sealed vial itself, and when an evaluation of solvent loss is made by weighing, this loss would not be detected as it would contribute to total weight.

[7] Counting Vials, American Tradair Corp., Long Island City, N. Y.
[8] PPO-2,5-Diphenyloxazole.
[9] POPOP—*p*-bis [2-(Phenyloxazol 1)]1-benzene.

SUMMARY

The results of the preliminary study indicate that the use of radioisotopes in evaluating vial closures and stoppers and sealing efficiency offers a very specific and rapid method to obtain information with considerable importance to the packaging of biological products.

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