Fluorocarbon Aerosol Propellants II: Loss in Containers and Its Implications for Quantitative Analysis

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Abstract □ Loss of the three most commonly used fluorocarbon aerosol propellants from various types of containers with different stoppers as a function of time, temperature, and initial concentration was investigated. The loss process was found to continue for at least 3 days. The decreasing order of loss from serum bottles with three types of stoppers was usually: lacquer-coated rubber stopper < regular (plain) rubber stopper < silicone stopper. Significant loss was also with storage in commercially available vacuum tubes. Such mechanisms for loss as adsorption, absorption, complexation, and permeation are discussed.

Keyphrases □ Propellants (fluorocarbon aerosol)—loss from various containers as a function of time, temperature, and initial concentration, relationship to analysis □ Aerosols—loss of three fluorocarbon propellants from various containers, effects of time, temperature, and concentration □ Fluorocarbon propellants, aerosol—loss from various types of containers, effects of time, temperature, and concentration, mechanisms

Fluorocarbon aerosol propellants such as trichloromonofluoromethane, dichlorodifluoromethane, and dichlorotetrafluoroethane are widely used in aerosol products. Their potential toxicity to humans and animals has been a subject of intensive study in the past few years (1–18). Concern over the potential acute toxicity to susceptible asthmatic patients after inhalation of bronchodilator aerosol products was recently raised (19).

The quantitative determination of concentrations of these propellants in air and biological samples is important in pharmacological and toxicological studies. In a previous study (16) concentrations of the propellants stored in serum bottles sealed with plain rubber or lacquer-coated rubber stopper and aluminum cap could decrease after storage. Since various types of sealed containers such as serum bottles (16, 18, 20, 21) and vacuum containers[1] (22, 23) are generally required to keep propellant samples during and before assay, and since any loss of propellants from the containers would result in an underestimation of the concentrations, it was decided to perform a systematic and quantitative study of the loss of the propellants stored in various types of containers. Effects of storage temperature and propellant concentration on the extent of loss were also explored.

EXPERIMENTAL

Materials—Only the most commonly used fluorocarbon aerosol propellants, trichloromonofluoromethane[2], dichlorodifluoromethane[2], and dichlorotetrafluoroethane[2], were investigated. Their common stock solution in distilled water was prepared according to the method described previously (16).

[1] Vacutainers, 2, 3, 5, and two 10-ml draw, Catalog No. 4850 3272, 4851 3273, 4880 3275, 4707 3200 TGS, and 4716 3200 KA, Becton Dickinson and Co., Rutherford, N.J.
[2] Supplied by E.I. duPont de Nemours and Co., Wilmington, Del.

Table I—Average Percent Loss of Trichloromonofluoromethane Stored in Various Containers at Room Temperature as a Function of Time

Type of Container	Percent of Loss at Various Times 2 hr	6 hr	24 hr	48 hr	72 hr
5-ml serum bottle with regular rubber stopper	14.0	24.0	43.0	49.0	59.0
5-ml serum bottle with lacquer-coated stopper	8.0	21.5	26.0	36.4	49.0
5-ml serum bottle with silicone stopper	16.0	40.2	87.5	93.0	99.0
15-ml serum bottle with lacquer-coated stopper	5.2	8.3	15.5	33.0	38.0
50-ml serum bottle with lacquer-coated stopper	1.5	1.7	3.2	6.1	7.8
2-ml vacuum container	10.1	23.0	38.5	51.5	59.6
3-ml vacuum container	8.6	18.6	31.4	43.6	52.0
5-ml vacuum container	1.4	15.0	23.6	37.7	40.0
10-ml vacuum container	3.1	21.5	29.2	35.6	37.3
10-ml vacuum container without silicone coating	5.3	27.3	38.0	42.7	47.0

Three sizes of serum bottles[3], 5, 15, and 50 ml, were used. The diameter of the internal opening of the 5-ml bottle is 13 mm, and that of the 15- and 50-ml bottles is 20 mm. Three types of stoppers were used: *(a)* regular, uncoated rubber stopper[4], *(b)* lacquer-coated, gray rubber stopper[4], and *(c)* silicone stopper[4]. The serum bottle was sealed with a stopper and an aluminum cap[5]. Four different sizes, 2, 3, 5, and 10 ml, of plain vacuum containers[1] with interior silicone coating were studied. A 10-ml vacuum container[1] without interior silicone coating was also studied for comparison.

Propellant Loss Studies at Room Temperature—An appropriate amount of propellant solution in distilled water was introduced into each sealed container through the stopper using a 10-μl syringe[6]. The containers were then usually shaken in a vortex mixer[7] for 5–10 min. The 50-ml bottles were shaken for 30 min to reach an equilibrium for propellant distribution in the bottles. Since the propellants studied exist in the gaseous state at 25° and their partition coefficients between the aqueous phase and the air phase are low (18), essentially all of the propellants added would distribute homogeneously in the container. The concentrations of the propellants in the container were determined by drawing certain volumes of the gas sample, with a 50- or 100-μl gastight syringe[6] equipped with an adaptor[8], which were then injected directly onto the GLC column. The concentrations determined immediately after shaking were assumed to represent the initial concentrations of the propellants in the container and were used to estimate the percent of the propellants lost during storage.

Several samples were obtained and analyzed in duplicate by the GLC method at appropriate times (usually at 2, 6, 24, 48, and 72 hr) after the beginning of the study. They were highly reproducible (within 2% from the mean). Three containers were used for study on each type of container. Intercontainer variation in percent of propellant lost at various times was generally insignificant. Since it was found that the extent of propellant loss might depend on the

[3] Wheaton Scientific Co., Millville, N.J.
[4] Regular uncoated rubber stopper, V-32; lacquer-coated rubber stopper, V-32, 324, Y-40; and silicone stopper, V-32, X-9711; West Co., Phoenixville, Pa.
[5] Catalog No. 224182 and 224183, Wheaton Scientific Co., Millville, N.J.
[6] Hamilton.
[7] Vortex-Genie mixer, Cat. No. 12-812-V1, Fisher Scientific Co., Springfield, Mass.
[8] Chaney.

Table II—Average Percent Loss of Dichlorodifluoromethane Stored in Various Containers at Room Temperature as a Function of Time

Type of Container	Percent of Loss at Various Times				
	2 hr	6 hr	24 hr	48 hr	72 hr
5-ml serum bottle with regular rubber stopper	5.1	12.5	17.2	28.8	38.9
5-ml serum bottle with lacquer-coated stopper	5.3	10.9	14.5	23.8	48.0
5-ml serum bottle with silicone stopper	10.0	22.4	57.0	84.4	93.0
15-ml serum bottle with lacquer-coated stopper	0.5	1.0	2.0	3.1	4.0
50-ml serum bottle with lacquer-coated stopper	0.4	0.5	0.8	1.0	1.2
2-ml vacuum container	6.9	16.8	19.3	23.2	25.7
3-ml vacuum container	5.5	12.0	14.0	20.5	23.6
5-ml vacuum container	0.4	10.5	18.2	28.9	33.4
10-ml vacuum container	0.7	15.0	19.1	23.9	26.5
10-ml vacuum container without silicone coating	1.7	10.1	18.0	27.7	31.4

initial concentration of the propellant in the container, unless otherwise indicated, the initial concentration for each propellant used in all studies was approximately the same: around 0.2 μg/ml for trichloromonofluoromethane, 0.3 μg/ml for dichlorodifluoromethane, and 1.4 μg/ml for dichlorotetrafluoroethane. All of these studies were conducted at room temperature (25 ± 1.0°).

Effect of Concentration on Loss—Since the lacquer-coated rubber stoppers have been routinely used in this laboratory (16, 18), the effect of different concentrations of the propellants on the extent of loss was only studied in 5-ml serum bottles sealed with that type of stopper and regular aluminum cap. Two concentrations of each propellant, differing by a factor of 10, were studied in six bottles. The lower concentrations of the three propellants used were similar to those described in the room temperature studies. Samples were analyzed for propellant concentrations at various times.

Effect of Temperature on Loss—Only the 5-ml serum bottles sealed with lacquer-coated rubber stopper were used. Approximately the same amounts of the three propellants as described for the other variables were injected into six bottles. Three bottles were stored at room temperature and the remaining three bottles were stored at 5° in a refrigerator. To prevent possible contamination of the propellants in the refrigerator (refrigerants generally used are fluorocarbons), the serum bottles were kept in a sealed 240-ml (8-oz) ointment jar. Prior to sampling for propellant analysis, the container was warmed in a 37° water bath for 5 min and then shaken in a vortex mixer for 5 min.

Permeation Study—This study was designed to determine whether permeation through the stopper could contribute to the loss of the propellants from the container. Three empty 5-ml serum bottles, each sealed with a different type of stopper, were stored in a 240-ml (8-oz) ointment jar containing initially about 1.8 μg/ml (w/v) of trichloromonofluoromethane, 3.9 μg/ml of dichlorodifluoromethane, and 15.3 μg/ml of dichlorotetrafluoroethane. After 24 hr of storage at room temperature, air samples inside the sealed serum bottles were obtained and analyzed for propellant contents. This study was performed in duplicate.

GLC Analysis—The instrument and conditions for the GLC analysis were essentially the same as those reported previously (16), except that the detector temperature was raised to 200°. This change in detector temperature increased the detector sensitivity for the three propellants by approximately 50% and reduced the sensitivity for the cyclohexane used to prepare the standard solution of propellants. The propellant standard solution in cyclohexane stored in a 0.94-liter (1-qt) amber glass bottle with screw-cap was stable for at least several months at room temperature.

Table III—Average Percent Loss of Dichlorotetrafluoroethane Stored in Various Containers at Room Temperature as a Function of Time

Type of Container	Percent of Loss at Various Times				
	2 hr	6 hr	24 hr	48 hr	72 hr
5-ml serum bottle with regular rubber stopper	5.7	13.0	18.1	23.0	31.9
5-ml serum bottle with lacquer-coated stopper	4.5	10.4	13.3	28.9	38.0
5-ml serum bottle with silicone stopper	9.4	23.7	56.0	82.2	91.0
15-ml serum bottle with lacquer-coated stopper	0.6	1.1	2.5	4.2	5.3
50-ml serum bottle with lacquer-coated stopper	0.5	0.6	1.0	1.1	1.2
2-ml vacuum container	5.5	17.5	20.7	28.7	31.8
3-ml vacuum container	5.3	13.8	17.6	22.4	27.3
5-ml vacuum container	1.8	12.0	24.2	29.7	32.2
10-ml vacuum container	1.9	6.0	15.6	19.5	20.7
10-ml vacuum container without silicone coating	2.9	14.1	20.9	22.1	31.5

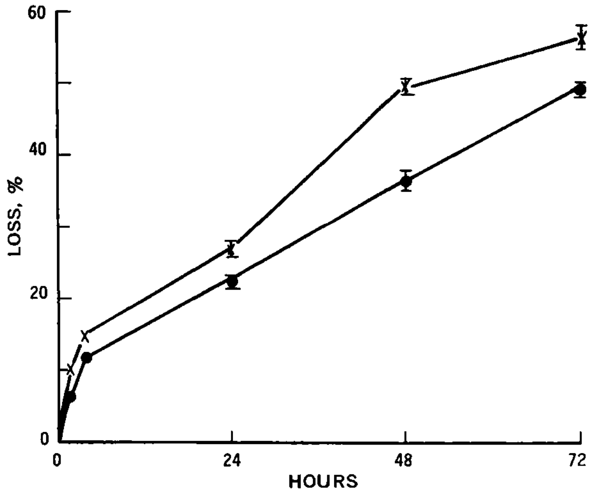

Figure 1—*Effect of concentration on the percent loss of trichloromonofluoromethane from 5-ml serum bottle sealed with lacquer-coated stopper. Key:* ×, *57.3 ng/ml; and* ●, *5.3 ng/ml.*

RESULTS AND DISCUSSION

The results of the average cumulative percentage of loss at various times of the three propellants from 10 different types of containers at room temperature are summarized in Tables I–III.

The loss of all three propellants from most of the containers continued for at least 3 days. The only exception was the 50-ml serum bottle sealed with a lacquer-coated rubber stopper, from which only a negligible loss could be detected for dichlorodifluoromethane and dichlorotetrafluoroethane after 3 days of storage. The loss of trichloromonofluoromethane from this container (6% in 3 days) was lower than from all of the other types of containers. These data indicate that this type of container should be used to store propellant samples for long periods. The rates of loss during the first few hours of study were generally greater than at later times.

With the 5-ml serum bottles, the use of both regular rubber and lacquer-coated rubber stoppers resulted in almost the same extent of loss of dichlorodifluoromethane and dichlorotetrafluoroethane within the first 24 hr. At 48 and 72 hr, the lacquer-coated rubber stopper appeared to permit a more extensive loss of both propellants. The silicone-type stopper permitted a most extensive loss of all three propellants. For trichloromonofluoromethane, the rate of loss followed first-order kinetics with a half-life of 8.8 hr. The kinetics for dichlorodifluoromethane and dichlorotetrafluoroethane, however, seem to be complex and are beyond the scope of this investigation. The vacuum containers coated with silicone appeared to permit less loss than did those not coated with silicone. These findings are in agreement with the general observation that interactions (adsorption, absorption, and complexation) of most chemicals with glass and polymers such as plastics and rubber will be retarded by coating with a silicone layer.

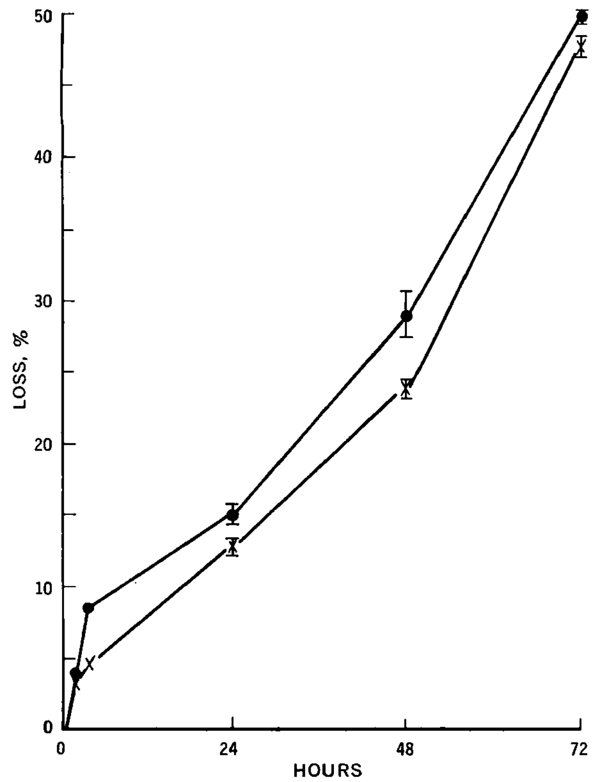

Figure 2—*Effect of concentration on the percent loss of dichlorodifluoromethane from 5-ml serum bottle sealed with lacquer-coated stopper. Key:* ×, *319.8 ng/ml; and* ●, *32.8 ng/ml.*

Trichloromonofluoromethane appears to be lost more readily than the other two propellants from all 10 types of containers. Approximately 93% was lost in 3 days from 5-ml serum bottles sealed with silicone stoppers. Generally, the larger the size of container, the smaller was the percentage loss of the propellants. This may be partly due to the fact that, on the average, the propellant molecules in the larger size container will take a longer time to travel to contact the surface of the stopper. It may be also attributed to the smaller surface area of stopper exposed to the propellants per unit volume of container in a larger size. For example, one can estimate that the relative surface area per unit of volume for a 50-ml serum bottle is only about 23.6% of that for a 5-ml serum bottle.

Several possible mechanisms can be speculated to explain the extensive and prolonged loss of propellants from the containers. They include: *(a)* adsorption onto the surface of the stopper; *(b)* absorption into the matrix of the stopper; *(c)* complexation between propellants and some ingredients of the stopper; *(d)* permeation to the outside of the container through the matrix space of the stopper, which is generally made of polymeric materials; and *(e)* permeation through the holes made after repeated puncturing by a syringe for sampling.

The loss of propellant from the holes that resulted from repeated puncturing can be ruled out because stoppers initially punctured 10 times with a 22-gauge needle, which is larger than the needle used in the studies, also produced the same extent of loss of the three propellants. The possible loss of propellant due to permeation through the matrix space of stoppers was suggested in the permeation study where considerable amounts of the three propellants could be detected in the sealed empty serum bottles. The bottles sealed with silicone stoppers contained higher concentrations of the propellants than did the ones sealed with other types of stopper. This finding is in agreement with the loss study in which the bottles sealed with silicone stoppers had the highest percent of loss. Since air is essentially not permeable through the rubber stopper of a vacuum container, as demonstrated by its vacuum state over a period of years, and since the molecular weights of the propellants are much greater than those of the constituents of air, it is concluded that permeation was not a cause of the loss from that type of container in the present study.

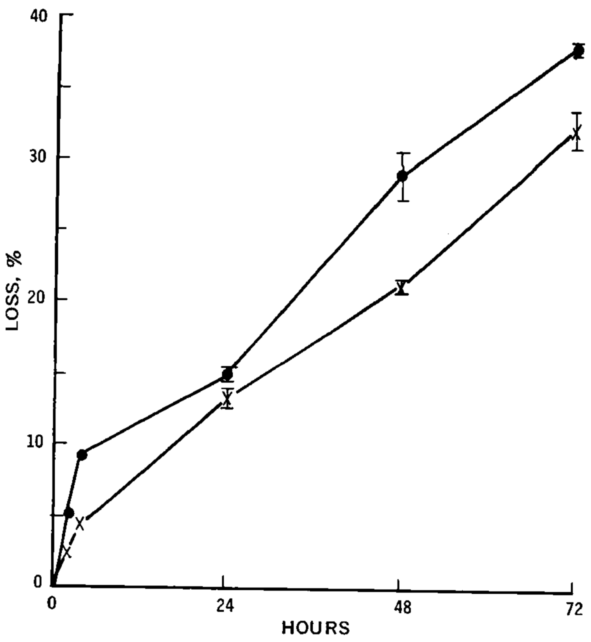

Figure 3—*Effect of concentration on the percent loss of dichlorotetrafluoromethane from 5-ml serum bottle sealed with lacquer-coated stopper. Key:* ×, *692.7 ng/ml; and* ●, *75.0 ng/ml.*

The adsorption phenomenon is generally a very fast or instantaneous process (24, 25). The observed loss of the propellants from the containers is, however, a slow process. For example, the extent of loss of trichloromonofluoromethane at 72 hr from 5-ml serum bottles sealed with lacquer-coated stoppers was about 4.5 times higher than that found at 2 hr. The loss of dichlorotetrafluoroethane from a 5-ml vacuum container at 72 hr was about 30 times higher than that at 2 hr. These results indicate that adsorption is not a major cause. Since the absorption and complexation

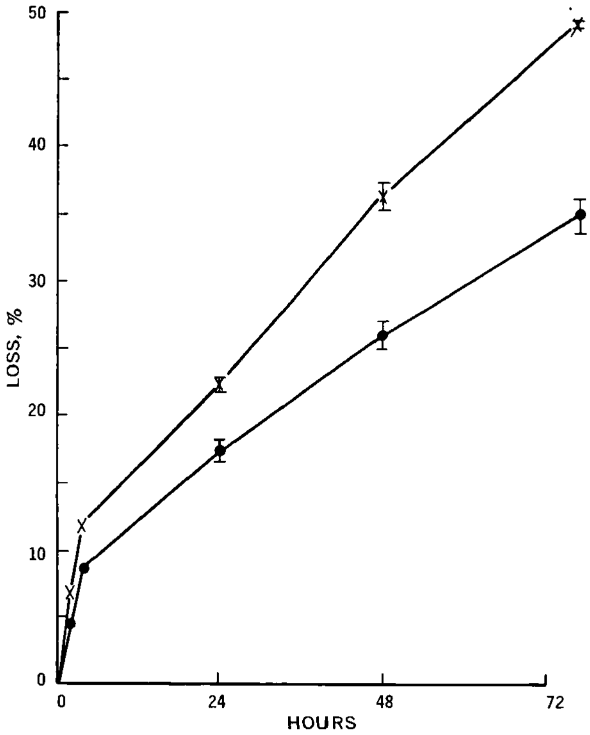

Figure 4—*Effect of temperature on percent loss of trichloromonofluoromethane from 5-ml serum bottle sealed with lacquer-coated stopper. Key:* ×, *at 25 ± 1°; and* ●, *at 5 ± 0.5°.*

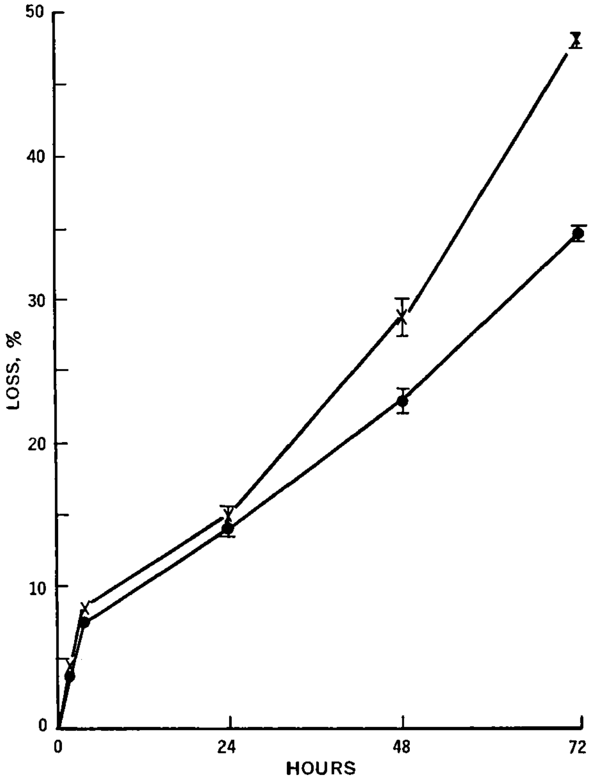

Figure 5—*Effect of temperature on percent loss of dichlorodifluoromethane from 5-ml serum bottle sealed with lacquer-coated stopper. Key:* ×, *at 25 ± 1°; and* ●, *at 5 ± 0.5°.*

are usually slow processes, rate limited by the diffusion rate of molecules in the matrix (25), it is believed that they are the major factors causing prolonged loss of the propellants from various containers. The possibility of physicochemical interaction between stoppers and propellants is further substantiated by the profound swelling of stoppers when a large amount of pure liquid trichloromonofluoromethane was stored in various containers. Many common fluorocarbons, including the three studied here, have been

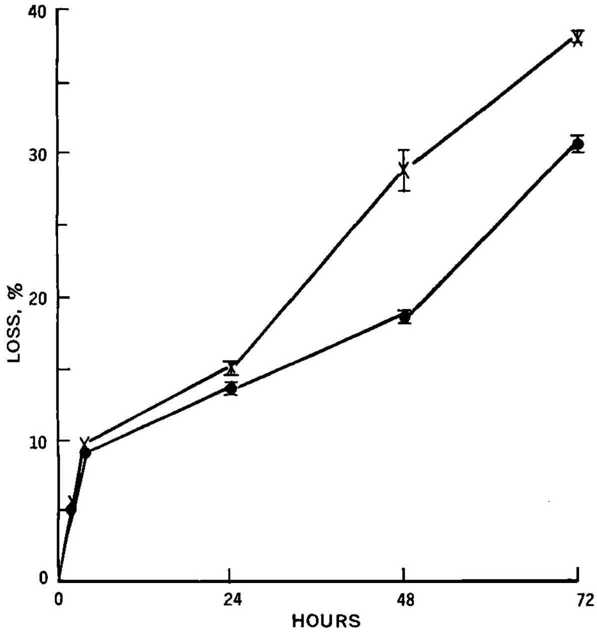

Figure 6—*Effect of temperature on percent loss of dichlorotetrafluoroethane from 5-ml serum bottle sealed with lacquer-coated stopper. Key:* ×, *at 25 ± 1°; and* ●, *at 5 ± 0.5°.*

shown to cause swelling of several elastomers such as natural rubber (26). As much as 300 ml of halothane, a fluorocarbon anesthetic gas, could be absorbed into the rubber tubing in the anesthetic breathing circuit during anesthesia and transferred subsequently and unintentionally to a patient administered a different general anesthetic through the same circuit (27).

The effects of concentration of the propellants on the extent of loss from 5-ml serum bottles sealed with lacquer-coated rubber stoppers are shown in Figs. 1–3. For dichlorodifluoromethane and dichlorotetrafluoroethane, the cumulative percents of loss from higher concentrations at 48 hr are significantly lower than those from lower concentrations ($p < 0.025$ using the Student t test). At 72 hr, however, the difference is not as significant ($p > 0.10$ for dichlorodifluoromethane; $p > 0.15$ for dichlorotetrafluoroethane). Such a concentration-dependent effect is consistent with the saturable absorption or interaction phenomenon as observed with drug–plastic interactions (25). On the contrary, the higher concentration of trichloromonofluoromethane gave rise to a higher percent of loss compared to the lower concentration ($p < 0.0005$), probably due to a strong initial chemical interaction between the propellant and the rubber resulting in a state more favorable for further interaction. A similar effect was also found at 5°.

The effects of storage temperature on the extent of loss are shown in Figs. 4–6. At 48 and 72 hr, the loss for three propellants was smaller ($p < 0.025$) when stored at 5°, primarily because of the lower diffusion rate of the molecules at lower temperatures. Again these data are consistent with absorption or complexation theory (25). The cumulative loss at 72 hr was between 30 and 35% for the three propellants. A higher percentage of loss would be anticipated with longer storage. The extent of loss observed in the present lower temperature study is quite surprising since the temperature was well below the boiling point (bp 23.7°) for trichloromonofluoromethane and just slightly above the boiling point (4.1°) for dichlorotetrafluoroethane. It is doubtful that the loss can be completely avoided even with storage at a much lower temperature.

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Effect of Small-Scale Preparation Techniques on Diffusion of Salicylic Acid from Various Ointment Bases

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Abstract □ The effect of small-scale preparation techniques on the diffusion of salicylic acid from ointment bases was studied. The method of mechanical incorporation of the drug in cold petrolatum base, using a spatula, appeared to result in higher rates of salicylic acid diffusion than those encountered with ointments prepared by fusion, regardless of drug concentration and the presence or absence of a surfactant. A similar effect was produced in the case of a 10% salicylic acid ointment made with a water-in-oil emulsion base.

Keyphrases □ Ointments—effect of small-scale preparation techniques on salicylic acid diffusion, petrolatum bases with and without surfactant and water-in-oil emulsion base □ Salicylic acid—diffusion from bases, effect of small-scale ointment preparation techniques □ Diffusion—salicylic acid from ointment bases prepared by small-scale techniques

The effect of various liquids on the diffusion of salicylic acid from ointments was reported previously (1). A literature review revealed that several reports have been published on the influence of numerous additives on the diffusion and absorption of drugs from ointments (2–7). However, it appears that little or no attention has been paid to the effect of preparation methods of ointments on the diffusion of the incorporated drug.

The aim of this investigation was to study the effect of small-scale preparation techniques of ointments on the diffusion of salicylic acid from some ointment bases. Mechanical incorporation of the drug using a spatula and the fusion process were investigated.

EXPERIMENTAL

Materials—Salicylic acid[1] USP, white petrolatum[2] USP, and sorbitan monooleate[3] were used as supplied. Other ingredients were official or analytical grades.

Preparation of Ointments—The bases used were white petrolatum, white petrolatum containing 2% sorbitan monooleate, white petrolatum containing 5% sorbitan monooleate, and a water-in-oil base consisting of 64% white petrolatum, 6% sorbitan monooleate, and 30% distilled water.

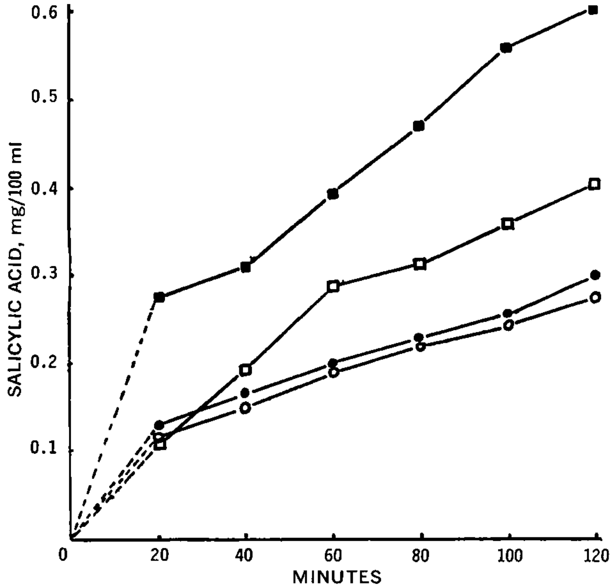

Figure 1—*Diffusion of salicylic acid from white petrolatum. Key:* ■, *10% prepared by mechanical incorporation;* □, *10% prepared by fusion;* ●, *5% prepared by mechanical incorporation; and* ○, *5% prepared by fusion.*

Mechanical Incorporation—The salicylic acid as supplied in fine powder was triturated with 150 g of the base on a glass slab using a spatula until a smooth homogeneous ointment was obtained.

Fusion—Each oleaginous base was first melted on a water bath at 70°. Salicylic acid was then gradually added to the molten material and stirred constantly until the ointment cooled to room temperature. In case of the ointments prepared with the water-in-oil base, white petrolatum and sorbitan monooleate were mixed and melted on a water bath at 70°. Salicylic acid was then added and dispersed in this fatty base at 70°. The distilled water, previously heated to 70°, was incorporated while stirring was maintained until the ointment cooled to room temperature.

Diffusion—The techniques used were the same as described previously (1). The amount of salicylic acid diffused into 100 ml of distilled water was determined by measuring the absorbance on a spectrophotometer[4] at 297 nm.

[1] Fisher Scientific Co.
[2] Oils Inc., Patterson, N.J.
[3] Span 80, Atlas Chemical Industries, Wilmington, Del.

[4] Cary model 118 recording spectrophotometer.