talline hydrophobic structural network of the petroleum jelly. These findings are in agreement with those of Buckwalter and Dickison (14), who found that small particles of procaine penicillin G suspended in oily vehicles gelled with aluminum stearate were superior to large particles in delaying absorption. This finding was attributed to the reduction in rate of solution of the suspended solid from the thixotropic vehicle.

The effect of variation of fusion temperature of petrolatum on the diffusion of salicylic acid is illustrated in Fig. 2. It appears that white petrolatum fused at 50° before incorporation of the drug gave a higher rate of drug diffusion than that demonstrated when petrolatum was fused at 70 or 90°. This may be explained on the basis that, on heating petrolatum at temperatures higher than 50°, it became a more flowable and less viscous liquid that would more effectively wet, strongly adhere to, and coat the suspended drug particles. Such difference in diffusion rate could not be attributed to any changes in the crystalline structure of petrolatum produced as a result of the fusion process. This is based on the fact that heating petrolatum to its melting point permits a rearrangement of the crystal network, so that the product, after cooling, exhibits its initial thixotropic condition (13).

Ointments prepared by fusion at 50°, however, demonstrated higher rates of diffusion than those of the ointments prepared by mechanical incorporation at room temperature. The reason might be that the less viscous base maintained at 50° could have enhanced aggregation of salicylic acid particles. This would be expected to increase the diffusion rate due to an increase of particle size (Fig. 1). This effect, however, was not exhibited by the ointments prepared at the higher temperatures of 70 and 90°, probably due to better dispersion of the drug particles caused by the greatly reduced viscosity of the base. The slower release rates demonstrated by ointments made by fusion at 70°, as compared with those prepared by mechanical incorporation at room temperature, confirm the results previously reported for the effect of small-scale preparation techniques of ointments on the release of salicylic acid (8). It appears, therefore, that the optimum temperature of fusion should be considered in the preparation of ointments by fusion.

Salicylic acid was found to sublime and collect on the bowl cover as tiny crystalline needles in the case of ointments prepared at 90°. No sublimation was noted for ointments prepared at 50 or 70°. The loss of salicylic acid due to sublimation at 90° was 1.5%.

Figure 3 shows the effect of homogenization of salicylic acid ointment on its diffusion from the various bases. It is evident that milling enhanced the rate of drug diffusion from the emulsion bases, but no apparent difference in diffusion rate was noted for petrolatum base. This could be due to an increase in the degree of dispersion and/or solubility of salicylic acid in the emulsion phases as a result of homogenization.

It can be also seen that the emulsion-type ointments were superior to white petrolatum in salicylic acid release. The oil-in-water type base gave a better release than the water-in-oil type. These results are in agreement with those already published (3).

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ACKNOWLEDGMENTS AND ADDRESSES

Received May 6, 1974, from the *School of Pharmacy, University of Georgia, Athens, GA 30602*

Accepted for publication June 18, 1974.

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Fluorocarbon Aerosol Propellants III: Effect of Water Vapor on Sensitivity of Electron-Capture Detector during GC Analysis

JAUE-HUEY HSIAO and WIN L. CHIOU ˣ

Abstract □ The quantitative depressive effects of the presence of various amounts of water in samples injected onto a GC column on the detector response to three fluorocarbon aerosol propellants were investigated.

Keyphrases □ Propellants (fluorocarbon aerosol)—effect of water vapor on sensitivity of electron-capture detector, GC analysis □ Aerosols—fluorocarbon propellants, effect of water vapor on sensitivity of electron-capture detector, GC analysis □ Fluorocarbon aerosol propellants—effect of water vapor on sensitivity of electron-capture detector, GC analysis □ GC—effect of water vapor on sensitivity of electron-capture detector during analysis of fluorocarbon aerosol propellants

The presence of water vapor has been known to affect adversely the performance of ionization detectors in GC analysis. Lovelock (1) stated that the contamination of water in the carrier gas was objectionable. Although its presence could not be immediately detected, it could lead to a serious reduction in the detector sensitivity. It was shown (2) that the sensitivity of a macro-argon detector would be reduced 10-fold by a change in water vapor concentration from 30 to 1000 ppm (v/v). It was also shown (3) that

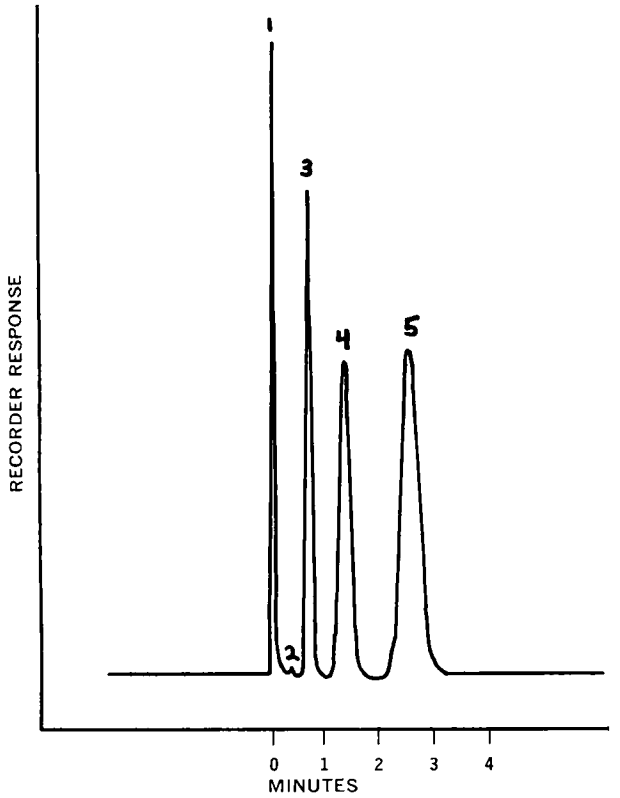

Figure 1—*Typical gas chromatogram of a mixture of three propellants and a negligible amount of water vapor. Key: 1, air; 2, water vapor; 3, dichlorodifluoromethane; 4, dichlorotetrafluoroethane; and 5, trichloromonofluoromethane.*

a slight increase in water vapor concentration from 8 to 10 ppm resulted in a 20% reduction in the response of the same type of detector to ether.

Aerosol products have been widely used for household, cosmetic, pharmaceutical, and other purposes. The possible toxicity of the most commonly used aerosol propellants, fluorinated hydrocarbons, has been a subject of intensive research and controversy (4–8). Quantitation of these fluorocarbon propellants in biological samples could be most sensitively performed by the GC method using an electron-capture detector (8–13). Although the technique of direct injection of blood samples onto GC columns has been used (9, 10, 12), it can result in serious contamination of the column and therefore require frequent changes of the column. Whether the presence of a large amount of water in blood or other aqueous samples could affect the sensitivity of electron-capture detectors toward these fluorocarbon propellants has not been reported. A quantitative study was undertaken to determine the extent of such an effect. The results of the present investigation can also be applied to other types of compounds using this kind of detector.

EXPERIMENTAL

Materials—Only the three most commonly used fluorocarbon aerosol propellants were used: trichloromonofluoromethane[1] (bp 23.7°), dichlorodifluoromethane[1] (bp −29.8°), and dichlorotetrafluoroethane[1] (bp 4.1°). Common stock solutions of the three propellants in distilled water and cyclohexane were prepared according to the method described previously (12).

Procedure—Ten microliters of the propellant stock solution in distilled water was injected into a 15-ml serum bottle[2] sealed with a flange-type, lacquer-coated rubber stopper[3] and aluminum cap. The bottle was shaken in a vortex mixer[4] for 30 min. Fifty microliters of the equilibrated air sample was then taken from the bottle, using a 100-μl gas-tight syringe[5], and injected onto the column of the gas chromatograph. Duplicate samples and injections were made and constant readings were obtained.

To measure the reduction in response of the electron-capture detector toward propellants in the presence of different amounts of water, 50 μl of the air sample from the equilibrated bottle was transferred to a 100-μl gas-tight syringe followed by drawing a certain amount of distilled water (usually less than 1 μl) into the same syringe, and the mixture was injected onto the column immediately. This process was repeated several times when the normal detector response was recovered. Different peak heights corresponding to different amounts of water vapor and three propellants were obtained on each injection. The peak height of the 1-μl water vapor response at the same GC condition was obtained by direct injection of exactly 1 μl of distilled water, using a 1-μl syringe[5], onto the GC column.

The duration of the water vapor effect on the reduction of detector response toward the three propellants was determined by direct injection of 0.5 μl of distilled water onto the column of the gas chromatograph immediately followed by the injection of 50 μl of air sample taken from the bottle containing equilibrated propellants. Injection of air samples obtained at various times was car-

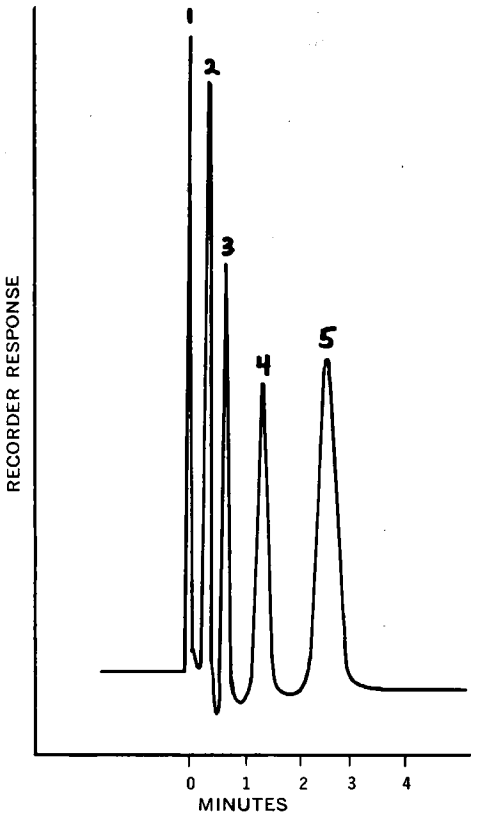

Figure 2—*Typical gas chromatogram of a mixture of three propellants and a significant amount of water vapor. Key: 1, air; 2, water vapor; 3, dichlorodifluoromethane; 4, dichlorotetrafluoroethane; and 5, trichloromonofluoromethane.*

[1] Supplied by DuPont de Nemours and Co., Wilmington, Del.

[2] Wheaton Scientific, Millville, N.J.

[3] West Co., Phoenixville, Pa.

[4] Vortex-Genie mixer, Cat. No. 12-812-V1, Fisher Scientific Co., Springfield, Mass.

[5] Hamilton.

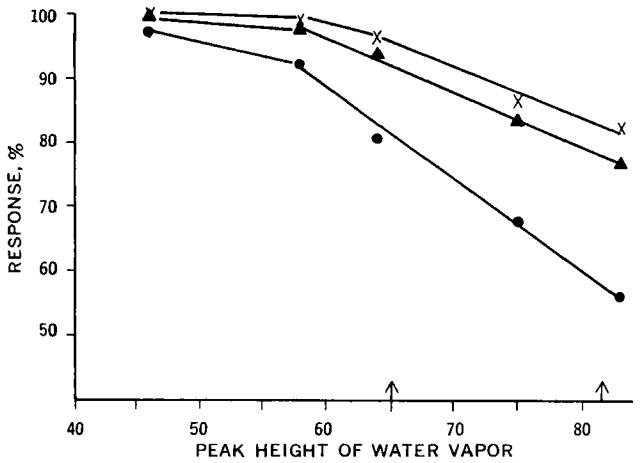

Figure 3—*Percent of maximum response for three propellants in the presence of different amounts of water vapor. Key: ×, trichloromonofluoromethane; ●, dichlorodifluoromethane; and ▲, dichlorotetrafluoroethane. Two arrows on the abscissa correspond to 0.5 and 1.0 μl of water injected.*

ried out until constant peak heights were obtained (about 90 min). In a separate study, no significant loss of the three propellants from the bottle in 2 hr was found.

Instrumentation—The GC unit[6] was equipped with a tritium-foil (150 μCi) electron-capture detector and a 1.8-m (6-ft) U-shaped glass column (4 mm i.d.) packed with Porapak Q[7]. The conditions of the GC analyses were: attenuation, 64×10^{-10}; column temperature, 170°; injection port temperature, 175°; detector temperature, 200°; and carrier gas (nitrogen) flow rate, 85 ml/min.

RESULTS AND DISCUSSION

A typical chromatogram for the three propellants in the presence of a negligible amount of water vapor (10 μl of aqueous propellant solution added to a 5-ml sealed empty serum bottle) obtained at the GC conditions described previously is shown in Fig. 1. The retention times for water, dichlorodifluoromethane, dichlorotetrafluoroethane, and trichloromonofluoromethane were 0.38, 0.72, 1.40, and 2.58 min, respectively. The retention times of the three propellants were not changed by the presence of a large amount of water. However, the baseline of the chromatogram was shifted toward the negative direction and then returned back gradually to the original position (Fig. 2). The lowering of the baseline is much more pronounced if the chromatograph is run at a higher sensitivity or lower attenuation. Sometimes a sharp overshooting in the baseline between the peaks of water and of dichlorodifluoromethane was observed, and deflection appeared at the point where the signal of dichlorodifluoromethane started. In such a case, the deflection point was used to measure the peak height of the propellant, although the peak height measurement is generally used to quantitate the concentration of the propellant. If the injection of the sample is not completed rapidly, the broad water peak could also overlap the signal of dichlorodifluoromethane, resulting in poor resolution and inaccurate estimate of the propellant concentration.

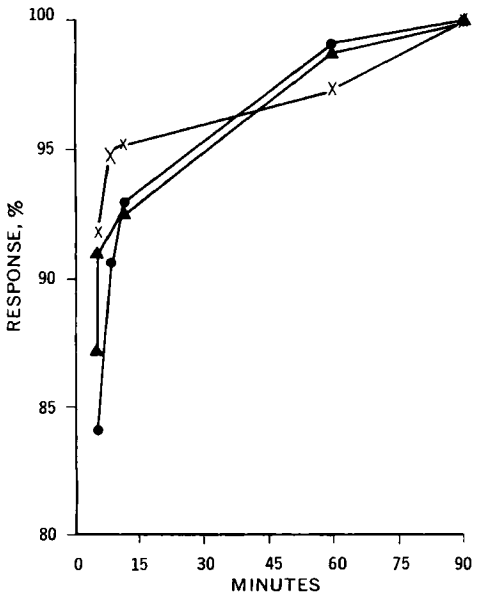

Figure 4—*Recovery of detector response toward three propellants as a function of time after an injection of 0.5 μl of distilled water. Key: ×, trichloromonofluoromethane; ●, dichlorodifluoromethane; and ▲, dichlorotetrafluoroethane.*

The presence of a large quantity of water injected into the column can also decrease the detector sensitivity toward the propellants. Figure 3 shows the relationship between the percentages of the maximum response for the three propellants and the amounts of water present in samples, expressed in terms of its peak height on the chromatogram. One unit of peak height corresponds to 0.254 cm (0.1 in.) of peak height on the 25.4-cm (10-in.) chart paper under the GC conditions described previously. The peak heights of water from normal atmospheric air samples were negligible, and injections of 0.5 and 1.0 μl would give rise to 65 and 81.5 units of peak height, respectively, indicating the existence of nonlinear response. The relative responses in the presence of 1 μl of water were 58.2, 78.5, and 83.4% for dichlorodifluoromethane, dichlorotetrafluoroethane, and trichloromonofluoromethane, respectively, compared to their responses in the presence of a negligible amount of water. These data indicate that the magnitude of reduction in detector sensitivity is inversely proportional to the length of retention time. The data in Fig. 3 also show that the reduction in detector sensitivity would not be pronounced in the presence of only a small amount of water.

Figure 4 shows the recovery of the detector response toward the three propellants as a function of time after an injection of 0.5 μl of distilled water. The recovery rates were found to be higher in the first 15 min, and total recovery occurred in 90 min. Such a time- and water content-dependent detector response as observed in this study should be of importance to researchers using direct injection onto GC columns of biological or aqueous samples. The mechanisms for these findings are not known. It is thought that adsorption of water molecules on the surface of tritium foil may cause a temporary inhibition of β-emission and, hence, the sensitivity of the electron-capture detector, because the response of the detector is directly proportional to the amount of radioactivity (β-emission).

In a recent study (13) from this laboratory on the partition coefficients of the three propellants between distilled water or normal saline and air, the propellant concentrations in both aqueous and air phases at the equilibrium state were measured by injecting the samples directly onto the column. The partition coefficients (water–air or normal saline–air) obtained were 0.284, 0.06, and 0.026 for trichloromonofluoromethane, dichlorodifluoromethane, and dichlorotetrafluoroethane, respectively. Since 1 μl of aqueous sample was injected, an underestimate of the propellant concentration is expected in light of the results from the present study. After correction for the reduced detector sensitivity, these partition coefficients should be 0.340, 0.103, and 0.031. To confirm these results, propellants in the aqueous phase were extracted into cyclohexane, and the concentrations in cyclohexane were analyzed by direct injection onto the column. The partition coefficients obtained by this extraction method were 0.346, 0.104, and 0.032 for the three propellants; these values are in excellent agreement with the data after correction.

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ACKNOWLEDGMENTS AND ADDRESSES

Received March 15, 1974, from the *Department of Pharmacy, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612*

Accepted for publication May 13, 1974.

Supported in part by Grant 1 R01 FD-00574-01 from the Food and Drug Administration.

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Influence of Drug Concentration on *In Vitro* Release of Salicylic Acid from Ointment Bases

F. BOTTARI, G. DI COLO, E. NANNIPIERI, M. F. SAETTONE [x], and M. F. SERAFINI

Abstract □ The effect of drug concentration on *in vitro* release of salicylic acid from a series of ointment bases was investigated. The bases were commercially available vehicles containing lanolin and/or lanolin derivatives and formed stable water-in-oil or oil-in-water emulsions. Release tests were performed both with the anhydrous and with the emulsion forms of the bases, at varying salicylic acid concentrations (0.5–5.0% w/v), and involved the use of silicone rubber membranes. The release of salicylic acid from the bases was in agreement with a reported diffusional model. A linear relationship between release rate, $q/\sqrt{t}$, and drug concentration in ointments existed when the drug was completely dissolved in the vehicles. The method reported is of potential utility for the determination of drug solubility in ointments and for the evaluation of the optimal drug concentration in topical vehicles. The relationships among type of vehicle, drug concentration, drug solubility, and release rate are discussed.

Keyphrases □ Vehicles (lanolin and lanolin derivatives)—*in vitro* release of salicylic acid, influence of drug concentration □ Ointment bases—influence of drug concentration on *in vitro* release of salicylic acid □ Drug concentration—influence on *in vitro* release of salicylic acid from ointment bases □ Lanolin and derivatives as ointment vehicles—influence of drug concentration on *in vitro* release of salicylic acid

It is generally recognized that a topical vehicle or base may affect drug penetration by modifying the permeability of the skin barrier phase and by releasing the drug to the skin in adequate amounts at a sufficient rate (1–3). A series of physicochemical factors, both pertaining to the drug and to the vehicle, appear to be involved in the latter process (4–6). Although several investigations have been directed toward determining these factors and elucidating their role in release, many unexplored points still exist whose study might prove profitable. The present paper is concerned with an *in vitro* study of the influence of drug concentration in different topical vehicles on release.

THEORETICAL

In the case of a membrane separating the donor and receptor phases, the release process may obey two different kinetic laws, depending on the resistance offered by the membrane to drug penetration. The relevant mathematical relationships have been developed and mainly investigated by T. Higuchi, W. I. Higuchi, and their coworkers (7–11). When the membrane offers little resistance to drug penetration (as may occur with injured skin or with some artificial membranes), large concentration gradients develop in the donor phase, and diffusional migration of the drug within the vehicle constitutes the slowest step in the release process. The following equations, derived from Fick's law, have been found to describe adequately the rate of release of drugs from ointment bases under these conditions (7, 10). The first equation refers to uniform solutions of drugs in ointments:

$$Q = q/A = 2C\sqrt{Dt/\pi} \qquad \text{(Eq. 1)}$$

where Q is the amount of drug (q) released to the sink at time t per unit area (A) of contact, D is the diffusion coefficient of drug in the vehicle, and C is the initial concentration of drug in the vehicle, expressed in units per milliliter. The second equation refers to suspension-type ointments:

$$Q = q/A = \sqrt{Dt(2C - Cs)Cs} \qquad \text{(Eq. 2)}$$

where C is the total drug concentration, and Cs is the solubility of drug in ointment; both values are expressed in units per milliliter. Equations 1 and 2 predict that plots of the amounts of drug released with $\sqrt{t}$ will give straight lines passing through the origin. The origin as intercept may not be observed in some cases because of the lag time phenomenon (12).

The preceding model is based on a series of simplifying assumptions: *(a)* only a single drug species is important in the base; *(b)* the diffusion coefficient is constant with respect to both time and position in the base; *(c)* the drug alone is allowed to diffuse out of the base; *(d)* the drug is rapidly removed upon reaching the base–membrane interface, and the receiving phase is a "perfect sink"; *(e)* the percent drug released is not too large (<30%) in the case of solutions; and *(f)* C is substantially greater than Cs in the case of suspensions.

The assumption that D must be constant with respect to both time and position is a serious limitation, because in many situa-