NOTE

In Vivo Evaporation Rate of Benzyl Alcohol from Human Skin

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Received 30 June 2003; revised 11 September 2003; accepted 15 September 2003

ABSTRACT: The evaporation rate of benzyl alcohol from the human volar forearm under controlled conditions *in vivo* shows a similar dependence on airflow and time to that seen in earlier *in vitro* studies. After an initial time lag associated with the apparatus, evaporation rate over a 2-h time period post-dose was satisfactorily described by a single exponential decay with a rate constant proportional to airflow over the skin, ν. The cumulative percentage of dose evaporated after 2 h ranged from 16% at $\nu = 20$ mL min^{-1} to 52% at $\nu = 100$ mL min^{-1}. The absorption rate constant determined by an analysis of the *in vivo* data was equivalent to that determined *in vitro*, whereas the evaporation rate constants were related by the inverse ratio of the headspace volumes. The latter finding suggests that a simple laminar flow model can satisfactorily describe evaporation in both systems over the range of airflows used. J Pharm Sci 93:515–520, 2004

Keywords: benzyl alcohol; evaporation; *in vivo*; fragrance; mathematical model; skin

INTRODUCTION

We recently proposed a set of compartmental pharmacokinetic models to estimate the absorbed and evaporated fractions of potentially volatile compounds applied to skin.[1,2] The models were based on known or readily estimated properties including vapor pressure, molecular weight, and skin lipid solubility, in combination with skin temperature and surface airflow. Human *in vitro* skin penetration studies were conducted using excised human skin and diffusion cells specially modified for volatiles collection, in order to test the airflow dependence of evaporation under controlled conditions.[2] This report presents the results of a study showing a close relationship between the *in vitro* results and a controlled *in vivo* exposure. The study involved the use of dynamic headspace technology to measure the evaporation of benzyl alcohol from human skin, using a custom-built volatiles collection system similar to that of Vuilleumier et al.[3] The effect of airflow on evaporation rate was determined and compared with that in the *in vitro* apparatus.

THEORY

A simple model for disposition of volatiles on skin, in which the stratum corneum is treated as a single, well-stirred compartment, yields[1,2]

$$\%_{\text{evap}}(t) = 100\left[\frac{k_1}{(k_1 + k_2)}\right]\left[1 - e^{-(k_1+k_2)t}\right]. \quad (1)$$

where $\%_{\text{evap}}(t)$ is the percentage of applied dose evaporated at time t, k_1 is the evaporation rate constant, and k_2 is the absorption rate constant. The postulated physical properties dependencies of the rate constants in this model are[1]

$$k_1 = k_1^{\nu} \cdot P_{\text{vpr}}/(K_{\text{oct}} \cdot S_{\text{w}})_r \quad (2)$$

and

$$k_2 = k_2^T \cdot \text{MW}_r^{-2.7} \quad (3)$$

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Journal of Pharmaceutical Sciences, Vol. 93, 515–520 (2004)

where P_{vpr} = vapor pressure (torr)/1 torr, K_{oct} = octanol/water partition coefficient, S_w = water solubility (g L^{-1}), and MW_r = molecular weight/100 Da. The subscript r on the product $K_{oct}S_w$ means the value is to be divided by 1000 g L^{-1}. The parameters k_1^{ν} and k_2^T are factors shared by all permeants. They are dependent on airflow and temperature, respectively. Using regressed values of k_1^{ν} and k_2^T, eqs. 1–3 were shown to account for 52% and 74% of the variance in cumulative evaporation extrapolated to long times ($t \rightarrow \infty$) of fragrance mixtures containing 11 or 12 ingredients from human volar forearm *in vivo*.[1] In Ref. 2, it was shown that a suitable form for the airflow dependence of evaporation in the *in vitro* apparatus was

$$k_1 = k_1' \cdot \nu \quad (4)$$

where ν is the volume flow rate of air in mL min^{-1} and k_1' is a proportionality constant that implicitly contains the physical constants ratio in eq. 2. Equation 4 was found to be valid for flow rates in the range 10–100 mL min^{-1}. This relationship is further tested in the present study.

EXPERIMENTAL

Chemicals

Benzyl alcohol, 99% [gas chromatography (GC) assay] and GC-grade hexane were purchased from Sigma-Aldrich (St. Louis, MO). Nonanol was obtained from Fisher Scientific (Pittsburgh, PA).

Sampling Equipment

The sampling system (Fig. 1) consisted of a 10-cm^2 demountable glass cell with fittings for two Velcro® straps. The system was custom-made by Dana Enterprises (West Chester, OH). A Tenax TA® cartridge (Supelco, St. Louis, MO) was connected to a cell via an Omnifit® large variable connector for 4-11 mm tubing (Alltech Associates, Deerfield, IL). A PAS-500 micro air sampling pump (Spectrex, Redwood City, CA) was connected to the top of the adsorbent tube via silicone tubing to regulate the airflow through the cell. Room air was constantly drawn into the inlet of the trapping apparatus, over the skin surface, and through the adsorbent cartridge.

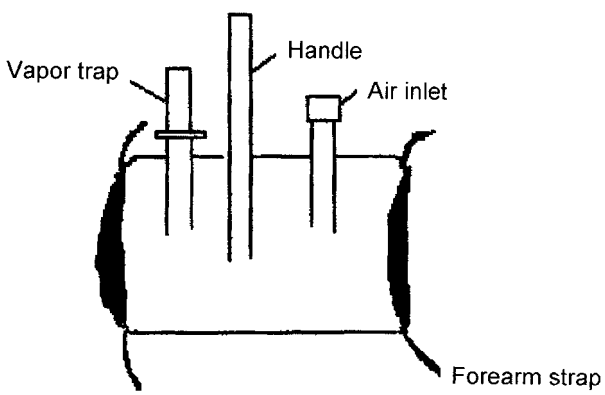

Figure 1. *In vivo* volatiles trapping apparatus.

Test Procedure

A single subject, a 23-year-old Indian woman, was enrolled in the study. Before each experiment, the subject was asked to wash her left forearm with a mild, unfragranced detergent (Oil of Olay Sensitive Skin Foaming Face Wash®) and then to dry it thoroughly. The subject then waited in the room for 30 min with the sleeve rolled up on her left arm. After equilibration, 100 μL of a 1% w/v benzyl alcohol solution in ethanol was applied to an 8-cm^2 area of the forearm using a disposable pipette, yielding an applied dose of 125 μg/cm^2. The collection cell was immediately placed over the dose site and secured with the Velcro® straps. The exact time of application was noted and designated as time zero for the experiment. The vapor evaporating from the skin surface was entrained in the air and collected continuously in the cartridge. The adsorbent tubes were exchanged at 0.08, 0.17, 0.25, 0.5, 1, and 2 h after application. Attempts to quantify benzyl alcohol evaporation at times >2 h were unsuccessful because of low signal level.

Sample Desorption

Each cartridge collected during the study was thermally desorbed into a glass wool-packed capillary tube mounted in a dry ice trap, into which the vapor was condensed. The desorption was performed at 180°C for approximately 15 min. During the desorption, ultra pure nitrogen gas at 20 mL min^{-1} was purged through the cartridges in the direction opposite to that of sample collection. After the desorption, one end of the capillary tube was sealed with a flame, and 20 μL of 0.1% w/v nonanol in hexane was added to extract benzyl alcohol from the glass wool. The other end of the capillary tube was then sealed. The sample was centrifuged at 4°C, 100*g* for 3 min and kept in the refrigerator before the analysis.

A collection efficiency of 80–90% was estimated by comparing radiolabel[2] and GC results. The desorbed cartridges were reconditioned by passage of 40 mL min^{-1} ultra pure nitrogen gas and heating at 10°–20°C above the desorption temperature for 20 min.

GC Analysis

Sample analysis was performed on a Varian 3300 gas chromatograph (Varian Associates, Palo Alto, CA) fitted with 15 m × 0.53 mm ID Supelcowax 10® capillary column. The flame ionization detector and the injection port were kept at 280°C. The injection was performed in the splitless mode. The column temperature was held at 110°C for 1 min, then raised to 180°C at a rate of 20°C min^{-1} and held at 180°C for 1 min. The flow rate of the nitrogen carrier gas was 20 mL min^{-1}. The samples (1 μL) were injected quickly into the injection zone using a Hamilton syringe (Hamilton Co., Reno, NV). A Varian 4270 integrator was used to measure the eluted peak areas. Nonanol (0.1% in hexane) was used as an internal standard. Further details are available from the authors.[4]

Data Analysis

The evaporated percentages of benzyl alcohol at each time interval were determined from the chromatograms. Average evaporation rates over each time interval were calculated from these data. The parameters k_1 and k_2 were optimized by fitting eq. 1 to the cumulative evaporation data using a computer program of our own design. The value of k_1' was then calculated from eq. 4. The 0.08 h data values were not included in the regression, for reasons described later. The sum of squared residuals $SSE = \Sigma[y_i(obs) - y_i(fit)]^2$ was minimized by means of a parabolic extrapolation algorithm.[5] Reduced χ^2 values, $\chi_v^2 = SSE/(n-p)$, where n is the number of observations and p is the number of adjustable parameters, were used to indicate the goodness of fit of the proposed models to experimental data. The optimum value of k_1' was determined by fitting the integrated rate equations to each data set, then averaging the results. Values for k_2 were redetermined using the optimum k_1', then averaged across data sets to give the final reported value. The regression statistics s, r^2, and χ_v^2 were calculated by using the average parameters k_2 and k_1' in eqs. 1 and 4 to calculate fitted values for each data point from 0.17 to 2 h, then comparing these results with the observed values.

RESULTS

Table 1 shows the percent of topically applied benzyl alcohol evaporated from the skin at each sampling time and airflow. The cumulative percent evaporated after 2 h is shown in Table 2. Higher airflows led to more rapid evaporation and a corresponding increase in cumulative evaporation, which ranged from 16% at 20 mL min^{-1} to 52% at 100 mL min^{-1}. Evaporation rates estimated from these data are shown in Figure 2. The peak rate occurred during the first collection interval (0–0.08 h) for the 100 mL min^{-1} experiment and at increasing times for lower airflows. The most delayed peak occurred between 0.25 and 0.5 h post-dose at 20 mL min^{-1}. These time lags, which are not described by eq. 1 or its first derivative with respect to time, are discussed later.

Results of the regression analysis for fits of eqs. 1 and 4 to the cumulative evaporation data are shown in Table 3. The rms deviation of the data from the fitted curves (s) was <3%, and the fraction of the variance explained by the model (r^2) exceeded 0.99. Representative plots of the

Table 1. Evaporation of Benzyl Alcohol from Human Skin *In Vivo*

	Percentage of Dose					
Airflow (mL min^{-1})	0.08 h	0.17 h	0.25 h	0.5 h	1 h	2 h
20	0.03	0.04	1.51	7.59	3.96	2.73
40	0.67	6.69	6.93	9.14	7.20	3.67
60	8.08	9.75	6.54	6.51	4.84	1.69
80	8.96	13.48	6.10	8.08	8.58	3.68
100	11.60	12.60	6.95	10.82	3.93	5.67

Values are expressed as the percentage of the applied dose (125 μg cm^{-2}) that evaporated during each time interval.

Table 2. Cumulative Percent of Dose Evaporated 2 h after Application of Benzyl Alcohol *In Vivo* and *In Vitro*

Airflow (mL min^{-1})	*In Vivo*	*In Vitro*[a] (Mean ± SD)	Ratio (*In Vivo*/*In Vitro*)
20	16	59 ± 3	0.27
40	34	76 ± 7	0.45
60[b]	37	79 ± 6	0.47
80	49	84 ± 4	0.58
100	52	86.1 ± 0.2	0.60

[a]Data from Ref. 2; $t = 2.25$ h, normalized values, $n = 2$.
[b]65 mL min^{-1} for *in vitro* experiments.

observed and predicted cumulative percent evaporated (linear scale) and evaporation rate (log scale) versus time are shown in Figure 3. With one exception (100 mL min^{-1}), the semilog evaporation rate plots were found to be linear following the initial time lag.

DISCUSSION

To the extent the simple model represented by eq. 1 can represent the complex process of evaporation and absorption from skin, analysis of the evaporation of volatile ingredients after topical application allows the estimation of dermal absorption rates. This follows because the slope of the semilog plot of evaporation rate versus time (cf. Fig. 3b) is equal to $-(k_1 + k_2)$ and the total percent evaporated after a long time is equal to $k_1/(k_1 + k_2)$. Practically, this estimation method is limited to cases in which k_1 and k_2 differ by no more than, say, a factor of 10. This condition is evidently met for benzyl alcohol, because the estimated ratio k_1/k_2 ranges from 0.21 to 1.06 over the range of airflows used. The physical properties leading to this circumstance (cf. eqs. 2 and 3) are discussed elsewhere.[1]

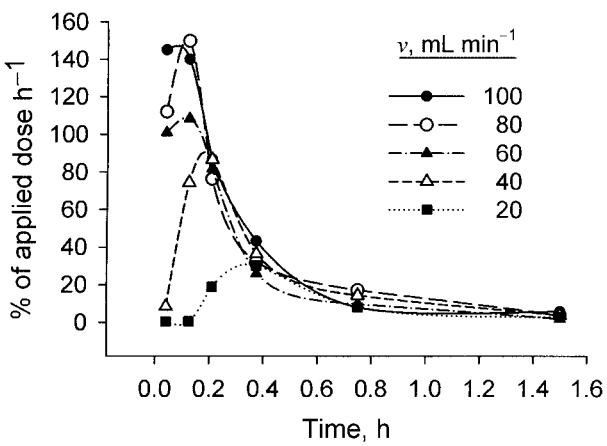

Figure 2. Observed evaporation rates of benzyl alcohol from human volar forearm *in vivo*, based on the data in Table 1. The values have been plotted at the midpoint of each collection interval.

The primary purpose of the present study was to provide a link between an *in vitro* experimental system allowing detailed observations of evaporation and absorption rates from skin[2] and *in vivo* absorption. Admittedly, the link reported here is only partial because the *in vivo* conditions were carefully controlled and did not reflect real-world exposures. Nevertheless, a number of observations may be made that support the use of both laboratory systems in estimating absorption from everyday exposures.

As shown in Table 3, independent analyses of the evaporation rate data for benzyl alcohol *in vitro* and *in vivo* led to consistent estimates of the absorption rate constant, k_2. This supports our premise that the cadaver skin model studied *in vitro* adequately represents the barrier properties of skin *in vivo*. However, the evaporation rate constant multiplier k_1' obtained by fitting the model to *in vivo* evaporation data was significantly lower (7.4-fold) than that obtained by fitting it to *in vitro* data. This ratio is very close to the *in vivo*/*in vitro* dose cell volume ratio, 32 mL/4 mL = 8.0. To a first approximation, this is the expected relationship, because the headspace air moved on average eight-fold more slowly in the *in vivo* study and the airflow dependence of k_1 in both cells has been found to be approximately linear in v (cf. eq. 4 and Ref. 2). The different cell geometries and potentially nonlinear airflow dependencies could have led to a more complicated relationship between *in vivo* and *in vitro* cell parameters. However, it seems that the inverse dose cell volume ratio is a good starting point for relating the properties of these two systems.

Table 3. Regression Parameters (Mean ± SD) for Fit of One-Compartment Kinetic Model (Eqs. 1 and 4) to Benzyl Alcohol Evaporation Data in Table 1

Parameters	Units	*In Vivo*[a]	*In Vitro*[b]
k_1'	h^{-1} (min mL^{-1})[c]	0.019 ± 0.004	0.141 ± 0.051
k_2	h^{-1}	1.8 ± 0.3	1.6 ± 0.8
n		25	120
s	% of dose	2.9	4.7
r^2		0.9916	0.9792
χ^2_ν	(% of dose)^2	8.5	22.2

[a]This study.
[b]Data from Ref. 2.
[c]Yields h^{-1} when multiplied by ν in mL min^{-1}.

Consistent with the differences in k_1', the amounts of benzyl alcohol evaporated *in vivo* from 0–2 h were substantially lower than those evaporated *in vitro* at comparable airflows (Table 2). For the same area-adjusted dose, the *in vivo* values ranged from 27% (20 mL min^{-1}) to 60% (100 mL min^{-1}) of the *in vitro* values. The time lag between dosing and peak observed evaporation rate (Fig. 2) was also more pronounced *in vivo* than *in vitro*. It seems logical to attribute this time lag to the accumulation of vapor in the headspace between the skin surface and the vapor trap.[2] We developed a modified analysis that explicitly considered this headspace (volume V_h) to be a separate, well-stirred compartment between the skin and the trap. The analysis led to a second exponential function, with a time constant equal to V_h/ν, in the *observed* evaporation rate measured at the trap. The time lag so introduced provided a qualitative explanation for the delays evident in Figure 2; however, the quantitative agreement was poor. The time lags at the lower values of ν were greater than could be accounted for by accumulation in a well-stirred headspace. Thus, it seems that the details of the evaporation process in the *in vivo* dose cell are not yet fully understood, at least at low airflows.

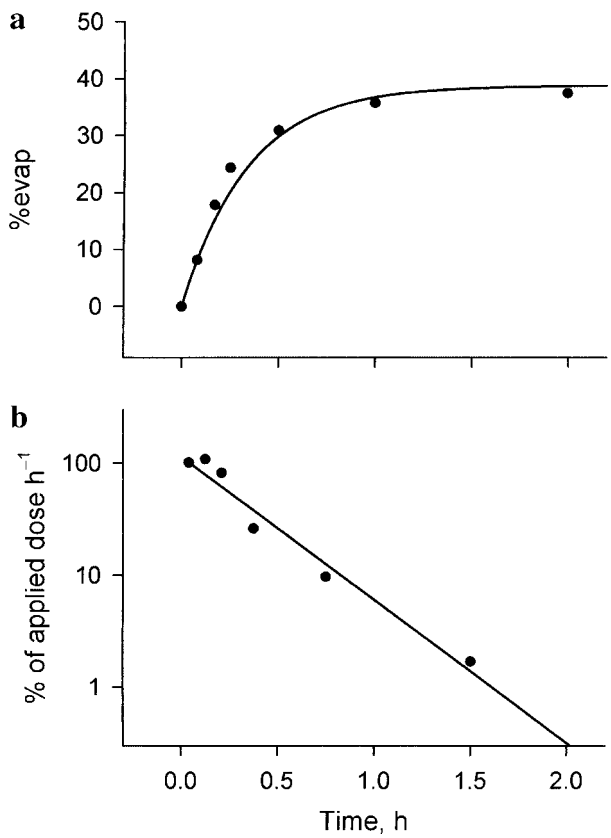

Figure 3. *In vivo* evaporation data for benzyl alcohol at 60 mL min^{-1}. (a) Cumulative percent evaporated, and b) evaporation rate, plotted at the midpoint as in Figure 2. The theoretical curves are based on eqs. 1 and 4, using the parameters in Table 3.

Because the evaporation data were obtained only during the first 2 h post-dose, we could not accurately assess curvature of the log(evaporation rate) versus time plots as in the *in vitro* experiments. Thus, we did not fit two-compartment models (vehicle layer + skin layer, cf. Ref. 2) to these data. Equations 1 and 4 were found to adequately describe both the cumulative percent of dose evaporated and evaporation rate data after the initial time lag, as already discussed.

The current methodology still requires calibration with materials whose evaporation under less-controlled conditions is known. This would close the loop between the *in vitro* studies, controlled *in vivo* studies, and common *in vivo* exposure conditions. Experiments of this nature are underway in our laboratory.

CONCLUSIONS

Evaporation of benzyl alcohol from human skin *in vivo* under controlled conditions appears to follow the same (nearly first-order) kinetics as evaporation *in vitro*. A calibration factor relating the two experimental systems is suggested. Further work is required to relate these values to evaporation rates under conditions experienced by fragrance products consumers.

ACKNOWLEDGMENTS

The authors thank Dr. Pedro Rodriguez for advice on the vapor trapping methodology. P. S. thanks the University of Cincinnati for a Distinguished Dissertation fellowship. The work was supported by the Procter and Gamble Company's International Program for Animal Alternatives, the University of Cincinnati, and NIOSH grant RO1 OH007529-01.

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