Headspace Measurement of Evaporation Rates of Perfumes Applied onto Skin: Application to Rose Essential Oils and Their Principal Components †

By C. Vuilleumier, I. Flament and P. Sauvegrain, Firmenich SA, Geneva, Switzerland

Throughout the past decade, technologically important progress has been realized concerning the isolation, separation, identification and quantification of the volatile components released by odoriferous substances such as flowers or fruits, without in any way altering the living matter. This mild and convenient method, termed dynamic headspace chromatography, allows enrichment of the most delicate products prior to their injection into a gas chromatograph.

Since the advent of gas chromatography, at the beginning of the 1960s, the technique of static headspace, which implies direct injection of a vapor in equilibrium with a liquid or a solid sample kept in a confined space, has been widely employed. But this technique has low sensitivity, which limits its application to the analysis of only the major volatile components. Dynamic sampling is much more effective by permitting efficient enrichment of volatile, medium-volatile, and even sparingly volatile minor constituents. This method involves the adsorption of components from the gas phase surrounding the sample onto a solid, porous material, using the same principle as filtration through gas-mask cartridges. Evidently, static and dynamic headspace samplings will give different results. Activated carbon was mainly used until the end of the 1970s, but later discontinued when it was shown that it generated numerous artifacts, especially during thermal desorption. More successful were porous synthetic polymers, such as Porapak* and Tenax,** especially in environmental research.

At present, the headspace analysis of volatile products has reached such perfection and sensitivity that it has become the ideal tool for measuring the behavior of volatile odorants applied onto human skin or hair. Thus, it is now possible by chromatographic analysis to continually monitor the emanations from such a living and delicate surface. Whereas the technique is simple in principle, it is very sophisticated and much more complicated in practice, where efficiency, reproducibility and sensitivity during sampling, injection, separation, detection and quantification of the individual perfumery ingredients are crucially important.

A number of cursory investigations have already been published. Measurements have been made in vivo to assess the diffusion characteristics of a variety of odorants on different skins, but related results were not published despite the fact that they showed "that the subjects form distinct groups, indicating that skin type has a key role to play."[1,2] Reviews on the behavior of fragrance chemicals note the importance of measurable parameters such as vapor pressure, odor threshold, odor value, water solubility and matrix factors.[3,4]

A more recent article[5] evokes the lack of rigorous scientific data to explain differences between individuals. The target of this particular research was twofold: first, to understand how a perfume behaves on different skins, and, second, to understand how people in the immediate vicinity perceive the olfactive impact. Headspace measurements were complemented by olfactometric determination of perception thresholds in order to calculate the "odor value," defined as the ratio of the concentration of an odorant in the

* Proapak is a registered trade name of Millipore Corp., Bedford, Massachusetts

** Tenax (poly-diphenyl phenylene oxide) is a registered trade name of Tenax Corp., Indianapolis, Indiana

† This article is adapted from a speech presented in Grasse (France) at the 25th International Symposium on Essential Oils, September 5-7, 1994.

0272-2666/95/0002-0001$04.00/0—

gas phase and its perception threshold. It should also be noted that, up to now, very little research has been effected on the diffusion of fragrance materials from different types of hair.[6]

Purpose of the Study

The goal of this research was to study, by headspace analysis, the diffusion of the major components of essential rose oils applied onto skin. The rose oils examined—a rose absolute (Grasse quality) and a Bulgarian rose essence—were studied as 10% solutions in ethanol. Our results concern the following components:

- linalool
- 2-phenylethanol
- citronellol
- geraniol
- methyleugenol.

These compounds were also studied individually by applying a 1% alcoholic solution onto skin in order to compare their evaporation behaviors either alone in a solvent or in a complex mixture.

General Considerations

Invariably, application of the headspace analysis technique requires special attention as regards the experimental protocol. Because of the special nature of skin, it is of course essential to take into consideration the diversity of skin types together with their external characteristics. Thus, our study began with an analysis of these characteristics using a panel of 80 people, mainly Caucasians. The measured parameters were the degree of hydration, the lipid concentration, the pH and the temperature of the skin surface.

For the first two parameters, Table I shows the measurement limits generally accepted by dermatologists using the same instrumentation (Courage + Khazaka Instruments). Our 80 panelists showed values which were quite well distributed among these limits (Table II). In our panel, the 40 females

Table I. Measurement limits for sebum and hydration levels

Test area	Sebum (µg/cm²)			Hydration (relative unit)		
	Low	Normal	High	Low	Normal	High
Neck	0-65	65-110	>110	10-60	60-80	>80
Forehead	0-100	100-220	>220	10-60	60-80	>80
Cheeks	0-65	65-180	>180	10-60	60-80	>80
Nose	0-100	100-220	>220	10-60	60-80	>80
Chin	0-100	100-220	>220	10-60	60-80	>80
Forearm I (inner surface)	no characterization			10-60	60-80	>80
Forearm II (outer surface)	no characterization			10-60	60-80	>80

Experimental devices: Courage + Khazaka Instruments
- Sebumeter SM 810
- Corneometer CM 820
- pH meter PM 900

Table II. Distribution of sebum and hydration levels for the tested panel (80 panellists)

Test area	Sebum (µg/cm²)			Hydration (relative unit)		
	Low	Normal	High	Low	Normal	High
Neck	61.1%	30.6%	8.3%	16.7%	65.3%	18.0%
Forehead	26.4	65.3	8.3	4.2	69.4	26.4
Cheeks	68.1	27.8	4.1	4.2	58.3	37.5
Nose	22.2	54.2	23.6	87.5	11.1	1.4
Chin	27.8	63.9	8.3	18.1	55.6	26.3
Forearm I (inner surface)	no characterization			33.1	65.9	1.0
Forearm II (outer surface)	no characterization			93.2	6.8	0.0

Experimental devices: Courage + Khazaka Instruments
- Sebumeter SM 810
- Corneometer CM 820
- pH meter PM 900

Table III. Skin characteristics of the selected panelist

Test area	Sebum (µg/cm²)	Hydration (relative unit)	pH
Neck	72	65	5.53
Forehead	164	80	4.68
Cheeks	37	74	5.65
Nose	100	42	4.65
Chin	130	68	5.10
Forearm I	-	57	5.35
Forearm II	-	58	5.55

Perfumer & Flavorist **(ISSN 0272-266)** is published bi-monthly by Allured Publishing Corporation, 362 S. Schmale Road, Carol Stream, IL 60188-2787. **Subscriptions:** USA and Canada US$105.00 one year; all other countries US$145.00 one year shipped by air. Second Class postage paid at Carol Stream, Illinois and at additional mailing offices. **Postmaster:** Send address changes to ***Perfumer & Flavorist***, 362 S. Schmale Road, Carol Stream, IL 60188-2787, USA.

had an average age of 35.1 (standard deviation of 11.6), and the 40 males had an average age of 39.4 (standard deviation of 10.6).

For the present study, a Caucasian female, with an "average" skin type was selected (Table III). She was 32 years old. The temperature on the surface of her forearm was 30°C. Twelve hours before the characteristics were measured, the skin on her arm was washed with soap; nothing further was applied to the arm until after the measurements were taken.

Headspace Sampling

The application of headspace technology also requires a precise definition of the experimental protocol which describes the type of sampling used (static or dynamic), the air volume involved, the geometry of the vessel surrounding the matrix and the type of adsorbent used.

- ***Type of sampling:*** dynamic. For our study, a total of eight successive samplings were made. The first one lasted 15 minutes (15 minutes is the evaporation time for alcohol) and was followed by seven other samplings each of which lasted one hour (Table IV).
- ***Sampling system:*** glass gas mantle placed around the forearm, as illustrated in Figure 1. This system allows a constant circulation of air regulated by the sampling pump.
- ***Calibration of the sampling pump:*** 5 litres of air per hour representing the air-flow inside the glass gas mantle.
- ***Adsorbent:*** Tenax—Granulometry: 35-60 mesh.

Choice of the Samples and Experimental Protocol

The compounds whose diffusion from skin was examined are present in rose absolute and Bulgarian rose oil in the amounts indicated in Table V. Their structures are shown in Figure 2.

Using a microsyringe, 10 microlitres of each of the

Table IV. Headspace sampling timing

Sampling	Corresponding time (minutes)
1	15
2	60
3	60
4	60
5	60
6	60
7	60
8	60

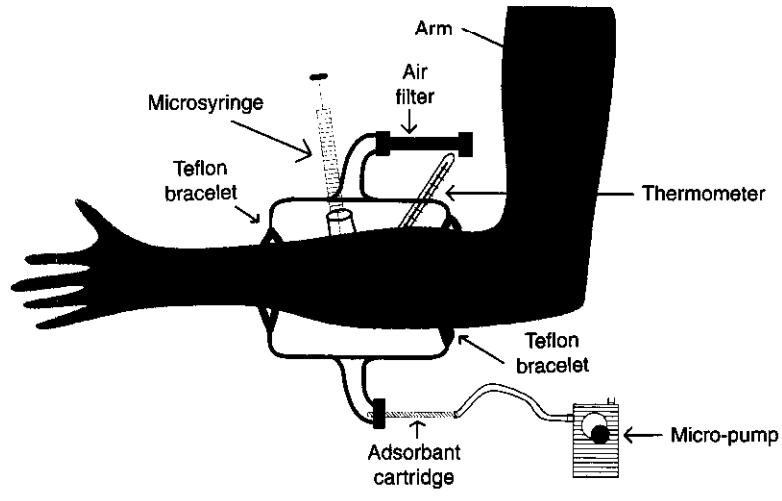

Figure 1. The headspace sampling system postioned on the forearm

Table V. Weight (by percent) of five selected compounds in two essential oils

Rose absolute (Grasse)	% (weight)
linalool	1.13
2-phenylethanol	65.54
citronellol	9.82
geraniol	4.20
methyleugenol	0.75
total	**81.44**
Bulgarian rose oil	**% (weight)**
linalool	1.33
2-phenylethanol	1.71
citronellol	49.21
geraniol	14.33
methyleugenol	2.75
total	**69.33**

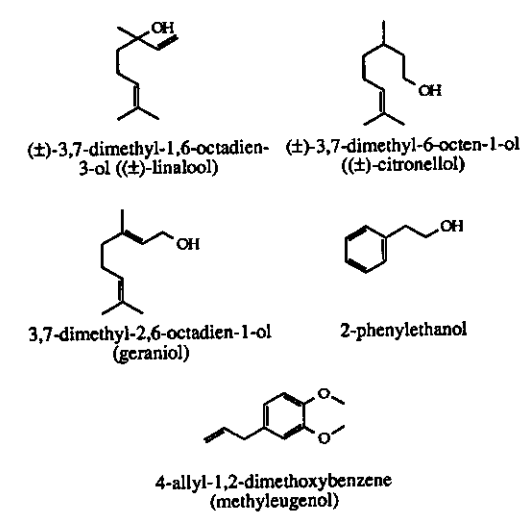

Figure 2. Structures of the five selected compounds

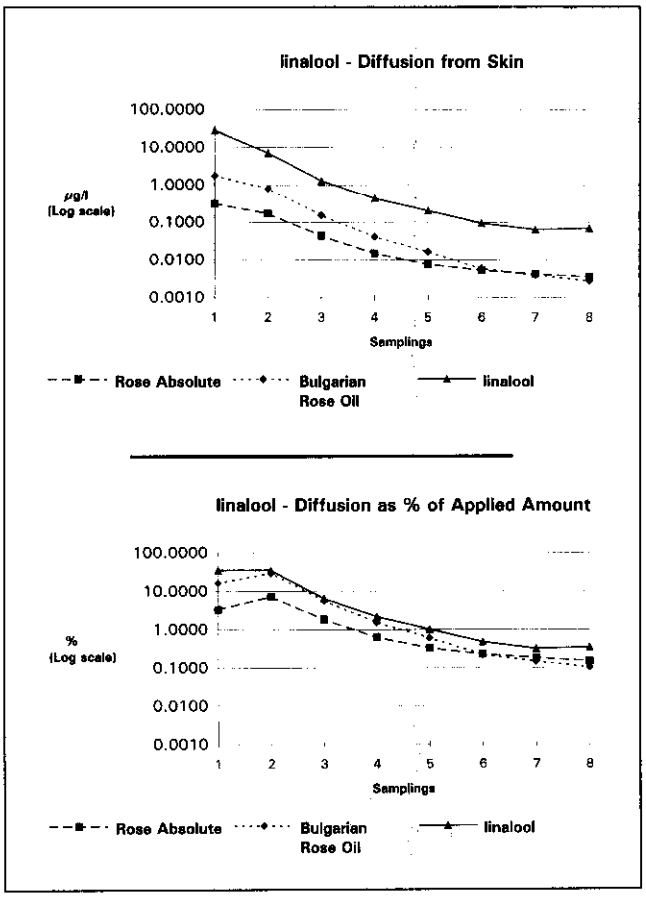

Figure 3. Kinetics of evaporation of linalool pure, in rose absolute and in Bulgarian rose oil

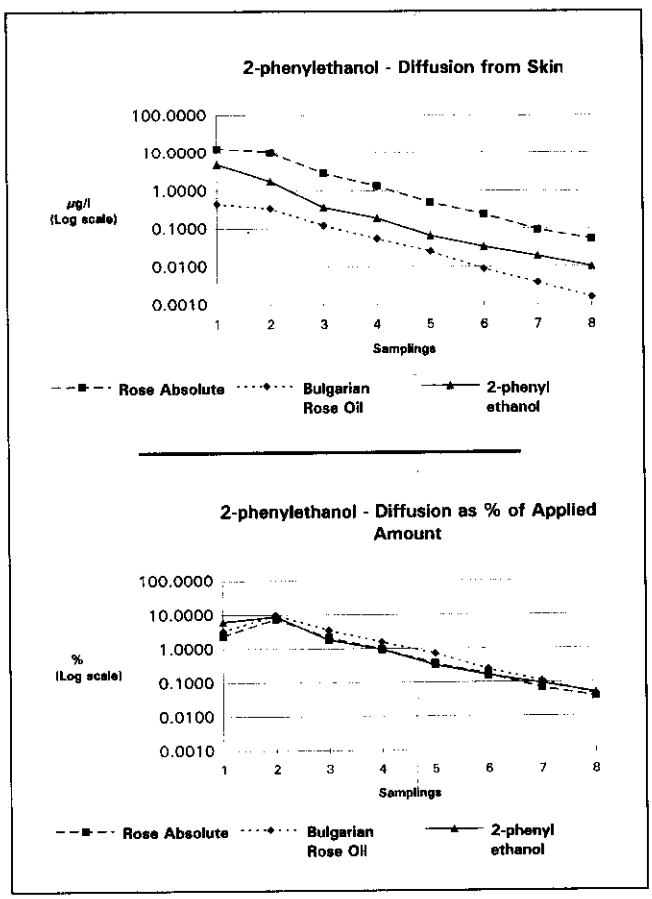

Figure 4. Kinetics of evaporation of 2-phenylethanol pure, in rose absolute and in Bulgarian rose oil

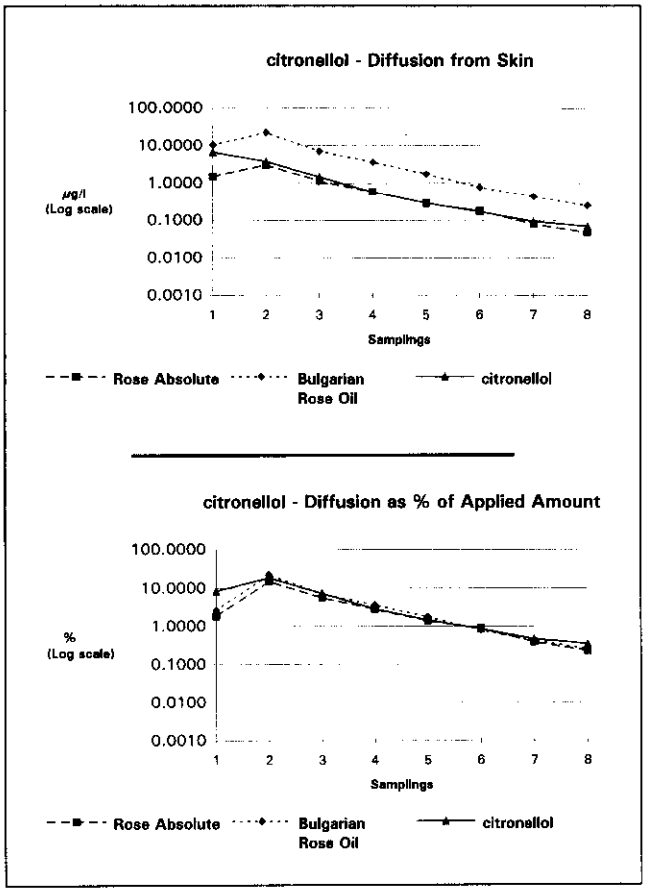

Figure 5. Kinetics of evaporation of citronellol pure, in rose absolute and in Bulgarian rose oil

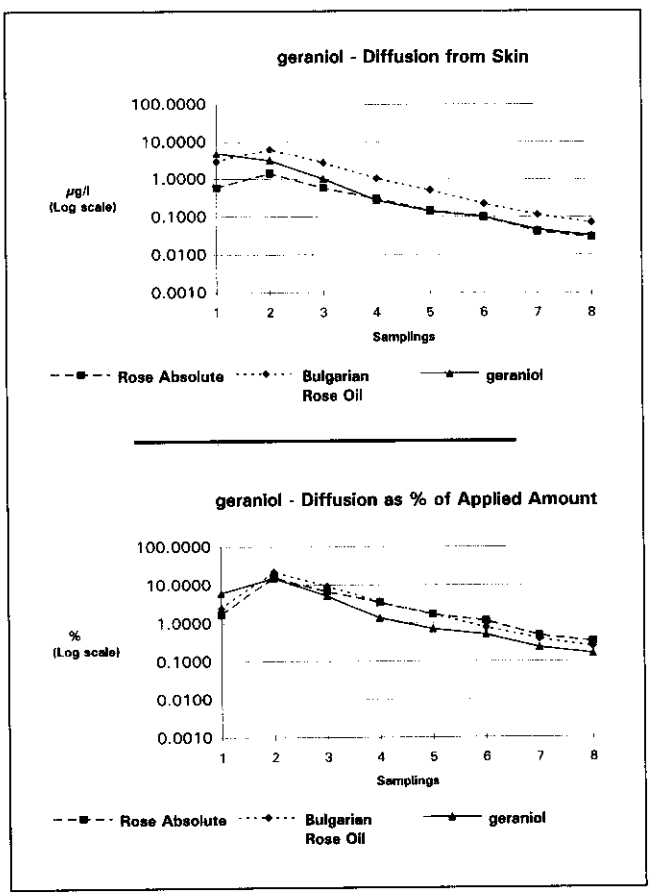

Figure 6. Kinetics of evaporation of geraniol pure, in rose absolute and in Bulgarian rose oil

10% or 1% ethanolic solutions previously mentioned were applied onto the panelist's inner forearm (washed beforehand with an unperfumed soap based on the sodium salts of fatty acids) as a thick line, 3 cm long and 0.2 cm wide. It is important to mention that the application of the product was effected after the installation of the glass gas mantle.

The eight samples were collected successively during one seven-hour and fifteen-minute period. The first sampling started immediately after the application of the product. It should be mentioned that each experiment was effected in duplicate in order to verify the reproducibility of the results.

Headspace Analysis and Determination of Diffusion Kinetics

After the samplings were made, cartridges containing the adsorbent were introduced into the automatic loader of the desorption system (Thermal Desorption Auto-Sampler TDAS, Fisons Instruments). The headspace was thermally separated from the adsorbent, and was directed into a cold trap where the vapors were condensed. This trap was then rapidly heated so as to inject simultaneously the total headspace into the chromatography column. The constituents thus separated were analyzed by a FID detector. The chromatographical analysis conditions were determined beforehand (choice of the column, temperature program, etc.).

Quantification of the data was effected by applying what is called the "external standard technique." This method takes into account the relative affinity between Tenax and the different compounds analyzed, and also corrects the response variation of the FID detector.

Results and Discussion

Figures 3 through 7 present the kinetics of evaporation for the compounds studied, resulting from the various applications onto skin. These figures are plotted logarithmically (log 10). On each graph three curves are represented: one corresponds to the averaged kinetics of the pure product applied as a 1% solution in ethanol; the two others correspond to the averaged kinetics of the same product either in rose absolute or Bulgarian rose oil, each diluted at 10% in ethanol. The results are also expressed in percentages representing the ratio of the amount evaporated with respect to the amount applied onto the skin in 10 microlitres of composition; in this manner, the results are more easily compared.

From our results, it is clear that there is little change

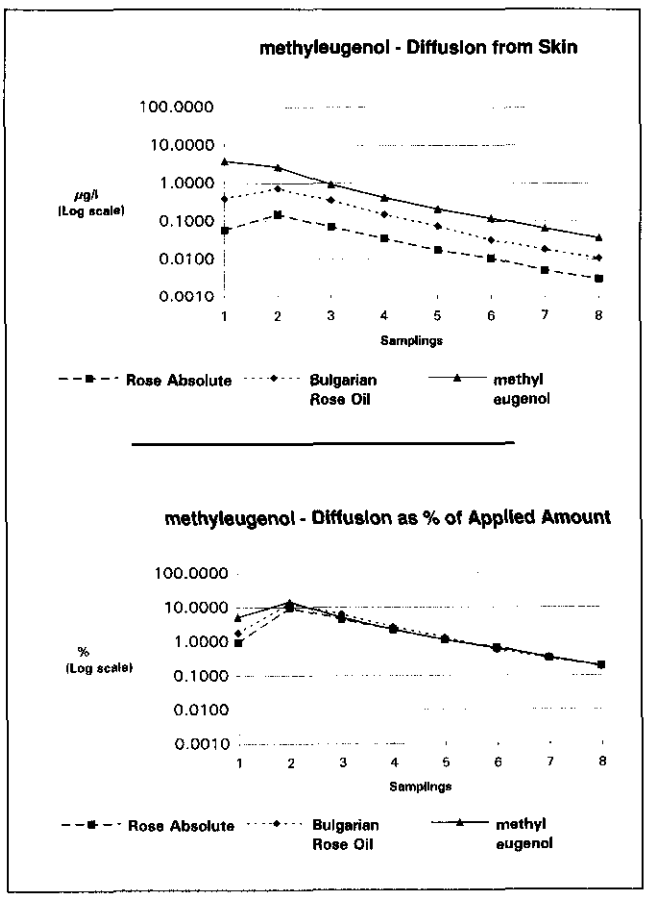

Figure 7. Kinetics of evaporation of methyleugenol pure, in rose absolute and in Bulgarian rose oil

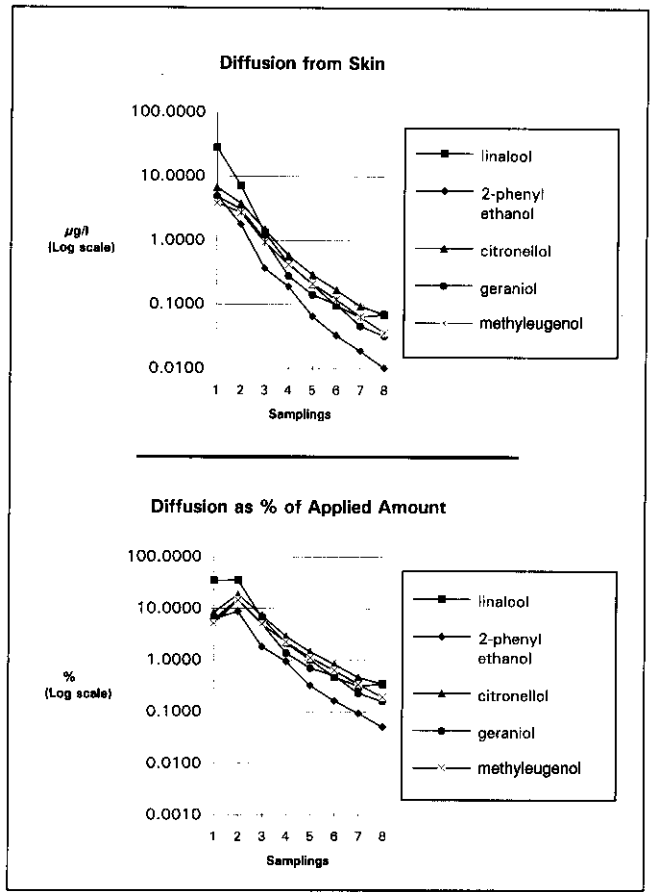

Figure 8. Composite kinetics of evaporation of select compounds pure

in the evaporation behavior of a compound on skin when it is applied as an alcoholic solution either pure or in a complex mixture; thus, the proportion of compound evaporated stays essentially the same, even if there are somewhat larger differences observed for linalool. Indeed, it seems that the evaporation of linalool in rose absolute is partially suppressed. In this case, it should be noted that linalool is the most volatile compound in the series and thus the effect of the other major components in the mixture becomes more important. Noteworthy is the behavior of the relatively abundant 2-phenylethanol, whose hydrophilic character results in a reduced evaporation with respect to other components such as citronellol and geraniol, which are also present in the oils examined and whose hydrophilicities are markedly lower than that of 2-phenylethanol, despite their similar volatilities. In addition, due to its possible retention by water present on the surface of the skin, 2-phenylethanol partially suppresses the evaporation of the other components, a phenomenon which is observed at the very beginning of each sampling process. This effect is even more noticeable in the case of linalool, whose vapor pressure is ten times higher than that of 2-phenylethanol and thus suffers the consequences of the hydrophilicity of the latter compound to a proportionally greater extent. It is also interesting to note that the first sample, corresponding to the first 15 minutes, a period where ethanol is evaporating, affords systematically higher results for the experiment in which the pure compound is employed. Due to headspace technology and rigorous application of the aforementioned method, these interesting phenomena can now be experimentally quantified in terms of the evaporation kinetics of each odorant.

Figure 8 presents the amounts evaporated in µg/l and also as a percentage of the applied amounts for the alcoholic solutions of the pure compounds. The classification of the compounds is proportional to their vapor pressures with the exception of 2-phenylethanol whose aforementioned hydrophilicity results in its retention in the water present on the surface of the skin. As a consequence, its evaporation is reduced in comparison to the other components.

Concerning the log-linear evaporation function shown in Figures 3 through 8, it should be mentioned that the range of vapor pressures for the different ingredients which have been studied is quite short and corresponds to a relatively high volatility. That explains why the evaporation process is so rapid. On the other hand, when we studied less

volatile constituents, we observed that the evaporation decreased less rapidly or increased with time. This implies that an absorption by skin is certainly not the major explanation for this process.

Finally, we have to differentiate the evaporation process which has been mentioned previously from a partitioning which could occur between the skin surface and air. This partitioning will result in a possible partial retention of the constituents, but is not especially linked to an absorption process.

Summary and Conclusion

We have demonstrated that headspace analysis allows the measurement of the evaporation of the individual components of rose essential oils from skin.

Using a special collection system applied on the inner surface of the forearm and allowing the adsorption of diffusing organic vapors from skin on to Tenax with a controlled air flow rate, we studied various rose essential oils with respect to their principal components. The diffusion rates of these components were measured by determining the concentration of each in the gas phrase versus time. Conversely, the same experiment was effected by application of an alcoholic solution of each individual component. In this manner, the relative diffusion from skin of the components alone or as a part of the essential oils was compared using the same experimental technique. It should be noted that these results were obtained on an "average type skin" which had been previously characterized by measuring surface parameters including pH, hydration and lipid content.

Regarding the most important points of our study, we observed:

- A slight difference in the evaporation of the constituents that were first studied alone (in alcoholic solution) and then in compositions (still in alcoholic solution).
- The unusual evaporation behavior of 2-phenylethanol, the hydrophilic character of which is more important than those of other constituents. This fact explains a slight retention effect of the other constituents in the mixture.
- A rather large homogeneity of the results, which could be explained by the fact that the constituents studied have comparable volatilities.

The development of this method is an important step for a realistic study of the performance of perfumery ingredients. It can be used for raw materials, essential oils, fine fragrances, as well as for perfumed cosmetic applications such as soaps, creams or shampoos, in order to characterize diffusion and air/skin or air/hair partitioning.

The present results represent only a small part of a comprehensive investigation in which the behavior of a large number of odorants applied onto skin is examined. Another part of this study was presented at the 18th International IFCCC Congress (Venice, Italy, October 3-6, 1994) and will be published in the *International Journal of Cosmetic Science*. However, it is important to note that, for completeness, this approach requires a precise measurement of the olfactive contribution of each of the components at the same concentration as that present in the headspace. It would then be possible to precisely interpret the effect of each odorant in a given cosmetic application.

References

Address correspondence to Dr Christine Vuilleumier, Firmenich SA, Research Laboratories, PO Box 239, CH-1211 Geneva 8, Switzerland.

1. R Ansari and S Bodicoat, Head to head, *Soap, Perfumery & Cosmetics* **66**(7) 34-35 (1993)
2. BJ Willis, Flavors and fragrances as functional ingredients, *Perf & Flav* **18**(4) 1-10 (1993)
3. N Neuner-Jehle and F Etzweiler, The measuring of odors, in *Art, Science & Technology*, PM Müller and D Lamparsky, eds, New York: Elsevier Applied Science (1991) pp 153-212
4. PM Müller, N Neuner-Jehle and F Etzweiler, What makes a fragrance substantive, *Perf & Flav* **18**(4) 45-49 (1993)
5. Y Guiserix, Le parfum et la peau: le mystère s'éclaircit, *Cosmetica* **37** 34-35 (Mar 1994)
6. AE Baydar, B Bouret and A Charles, Substantivity of fragrance materials on different hair types, *CTMS* **8**(5) 25-27 (1994)