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# Retronasal Transport of Aroma Compounds

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A comparison was made between the amounts of volatiles in the headspace above a solution and the breath volatile content (exhaled from the nose or mouth) after consumption of the same solution. The amounts of volatiles in the breath were lower than those in the headspace, with breath exhaled via the mouth containing, on average, 8-fold more volatiles than breath exhaled via the nose. Dilution of the sample by saliva in-mouth did not appear to be a major factor affecting volatile delivery. Instead, the rate of in vivo equilibration (mass transfer) appeared to be the most significant factor, principally affecting volatile delivery from the solution to the gas phase. Thereafter, gas-phase dilution of the volatile as it passed through the upper airway resulted in a further decrease in volatile concentration. The final factor affecting the volatile concentration exhaled from the nose was absorption of volatiles to the nasal epithelia, which was greatest for those compounds with the lowest air/water partition coefficients.

**KEYWORDS: QSPR; APCI; API; aroma release**

## INTRODUCTION

Delivery of volatile aroma compounds to the olfactory epithelium can take place via the nostrils during inhalation (orthonasal route) or retronasally when food is eaten. In the latter case, volatile compounds pass from the pharynx, over the soft palate, and into the nasal cavity. Previous reports comparing the retronasal and orthonasal routes for volatile delivery have primarily taken a sensory rather than an analytical chemical approach. These have focused on the intensity of perception and stimulus recognition. Intensity studies typically rate the perception of a single probe compound, whereas recognition studies involve more complex systems and may be affected by differential delivery of compounds.

Many of the results are contradictory: Kuo and co-workers (*1*) found that the perceived intensity of a citral solution was less when sampled retronasally compared with orthonasally. Voirol and Daget (*2*) found the opposite, which they attributed to "the higher concentration of odorant molecules in the vapor phase and to the influence of nonolfactory stimulations". Other intensity studies have found no differences between orthonasal and retronasal perception (*3*, *4*).

Sensory analysis of retronasal and orthonasal perception focusing on stimulus recognition have reported differences between the two profiles (*5*, *6*). This could be due to differences in the efficiency of volatile delivery or discrimination between the two routes. Alternatively, the differences may be associated with nonolfactory stimulation such as trigeminal effects.

One further complicating factor can be the system used to deliver volatiles orthonasally and the instructions given to panelists (inhale or sniff). The use of sniffing vessels, which were opened just prior to inhalation, gave results different from those of olfactometers, presumably due to dilution of the gas phase from the sniff vessels during sensory evaluation (*7*). Vuilleumier and co-workers (*7*) went on to compare the perceived stimulus intensity with that actually delivered (measured analytically) by the orthonasal and retronasal routes. They found that the perceived intensity was the same, when the concentration delivered was the same, thereby eliminating experimental problems associated with dilution or chemical losses.

Vuilleumier and co-workers observed substantial differences between the equilibrium headspace concentration above the solutions they tested and the retronasal breath volatile concentration. The most extreme example was limonene which was 1000 times lower in retronasal breath than that expected on the basis of headspace analysis. Similar results were found by Doyen et al. (*8*) while studying flavor delivery from dilute emulsion systems; and Deibler et al. (*9*) reported 50 to 200-fold lower breath volatile concentrations than those obtained with the retronasal aroma simulator (RAS) for a range of foodstuffs.

The key question is, why are breath volatile concentrations so low in comparison with those of headspace? For solid foods, mass transport from the food to the saliva might be an explanation. However, the situation was found to be similar for aqueous systems, where the volatile had merely to partition into the gas phase in-mouth and then be exhaled through the nostrils. For some compounds, absorption on the nasal epithelium might reduce the volatile concentration leaving via the nostrils (*10*, *11*). This, however, would not account for the differences observed for limonene (*7*), which should show minimal interactions with the nasal epithelia (*11*).

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A series of analyses have been performed to investigate which factors (dilution, absorption, etc,) have the most significant effect on retronasal volatile delivery. The breath volatile content has been analyzed from the mouth (mouthspace) and nose (nosespace), following the consumption of a wide range of compounds in aqueous solutions. These results have been evaluated along with breath flow measurements, and a quantitative structure property relationship (QSPR) model has been generated to describe and predict the differences between headspace and nosespace volatile concentrations.

## MATERIALS AND METHODS

**Solution Preparation and Analysis.** Each volatile was dissolved in water and diluted until the headspace signal (measured using atmospheric pressure chemical ionization−mass spectrometry (APCI−MS)) was approximately 10% of full scale (0.1 to 5 ppm). Headspace analysis was performed on solutions (500 mL in 1-L flasks) equilibrated at 22 °C. A plug (4 mm o.d.) in the cap of the flasks was removed, and the end of the heated (120 °C) sampling line (3.18 × 50 mm o.d.) of the mass spectrometer (Platform II, Micromass, Manchester, UK) was inserted into the headspace. Headspace was drawn into the sampling line of the mass spectrometer at 30 mL/min, and the signal for the volatiles reached a maximum within 20 s. The intensity of this signal was the value recorded for the headspace. Because of the large volume of the headspace in the flask (500 mL), and the short sampling times involved in the analysis, dilution of the headspace was insignificant, and the signal intensity recorded was taken as the equilibrium headspace concentration.

For breath analysis, 15-mL aliquots of the solutions were placed in-mouth and swallowed immediately. Breath was then exhaled, via either the nose or mouth, through a tube (8 mm i.d.) connected to the end of the mass spectrometer sampling line (which sampled breath at 30 mL/min). The maximum signal intensity obtained for the volatile in the exhaled breath was recorded, and it was compared with the signal obtained during headspace analysis.

**Mass Spectrometry.** The gas-phase volatile content was measured using a modified APCI−MS source (*12*). The dwell time of the mass spectrometer was set at 11ms (selected ion mode), and the compounds were ionized by a 4 kV corona discharge. The cone voltage was set to 18 V for all compounds, which were typically detected as the protonated molecular ion ($MH^+$).

**Breath Volume Analysis.** Breath volume was measured using a small flow meter (Interface Associates, 0.60 in. turbine coupled with transducer VMM-401, World Precision Instruments Ltd., Herts, UK) that was placed in-line with the nosepiece of the APCI−MS. When breath passed through the flow meter it spun an ultra-lightweight impeller blade. Infrared light-emitting diodes crossed the flow meter bore and the path of the impeller blade. As the impeller rotated it interrupted the light beams. These interruptions were detected by photo transitions that produced trains of pulses. By processing these signals the breath volume could be determined (one rotation of the impeller occurs for each 0.5 mL passing through the flow meter).

The signal from the transducer was passed to a PC via an RS232 interface. The data were logged every 10 ms by PC display software (Interface Associates, World Precision Instruments Ltd., Herts, UK). The data were then processed using a spreadsheet (Excel, Microsoft, Redmond, WA) and combined with the mass spectral data.

**Model Development.** Physicochemical parameters describing the volatile compounds were generated using the chemical modeling program Molecular Operating Environment (MOE, Chemical Computing Group Inc, Quebec). The parameters which explained the greatest amount of variation in the data set were selected using partial least squares (PLS) regression (MOE and Guideline + 7.2, Camo, Trondheim, Norway) before final model development in Design Expert 6.0.2 (Statease, Minneapolis, MN). Parameters which were statistically significant ($P < 0.05$) were used to generate the final model, which was validated with a test set.

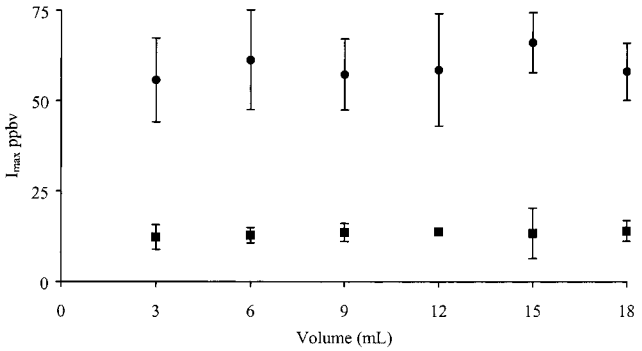


**Figure 1.** Effect of sample volume on the maximum breath volatile intensity (Imax) for 10 ppm solutions of linalool (●) and dimethyl pyrazine (■). Each value is the average of 6 replicate samples, consumed by 2 panelists. Error bars show the standard deviation.

## RESULTS AND DISCUSSION

**Dilution In-Mouth.** The study of flavor delivery from aqueous solutions in vivo eliminates those factors associated with bolus-to-saliva partitioning. This enables the effects of saliva dilution in-mouth prior to swallowing to be determined. Differences are likely to occur if the sample volume is changed, with smaller samples being more prone to dilution than larger ones. This hypothesis was tested by consuming a series of samples of different volumes. No significant differences were found in the maximum breath volatile concentration, even with a 6-fold variation in sample size (**Figure 1**). Further, changing the sample matrix by adding thickeners (hydroxy propyl methylcellulose) to water, did not significantly affect volatile delivery (*13*) despite the substantial increase in viscosity. Viscous solutions would not be expected to mix with saliva (and hence dilute) to the same extent as would wholly aqueous samples. It therefore appears unlikely that these samples were affected by dilution with saliva during consumption. Although this is the case for solutions swallowed immediately, the release of volatiles from other food matrixes may be more dependent on the amount of saliva in-mouth, for example if volatile release is dependent on hydration (dry foods), or if mass transfer from the bolus to saliva is limiting (*14*).

**Interactions with Salivary Components.** When the samples were consumed there was also the possibility that interactions between the volatiles and other salivary components, such as mucin, might have occurred. This may also have been dependent on the sample size, given a finite pool of saliva. Changing the sample volume did not affect the nosespace volatile content of linalool and dimethyl pyrazine (**Figure 1**) indicating that neither of these two compounds had interacted with salivary components. However, these two volatiles did not interact with either mucin or salivary salts in headspace partitioning studies (*15*), whereas the headspace concentration of other compounds such as decanal was significantly affected by the presence of mucin (ca. 85% decrease), and its breath concentration might therefore be influenced by sample volume. However, studies by Buettner and Schieberle (*16*), showed that when a solution of decanal was masticated, a substantial proportion could be recovered from spit-outs after 5 s, and it took 1min of mastication for losses to reach 50%. Therefore, it would appear that although substantial interactions with mucin can occur, the time course of the mucin−volatile interaction may be too slow to be a major factor in flavor release.

**Dilution in the Upper Airway.** The second major dilution event that may occur during flavor release involves the transfer of volatiles from the pharynx to the nose. The act of swallowing

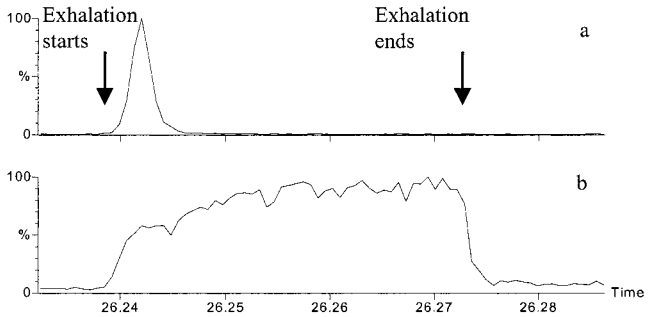


**Figure 2.** Breath volatile profiles (nosespace) for cymene (a) and acetone (b) in a single swallow breath expressed as a percentage relative to the maximum (100%). The exhalation started just before the 26.24 min time point of data acquisition.

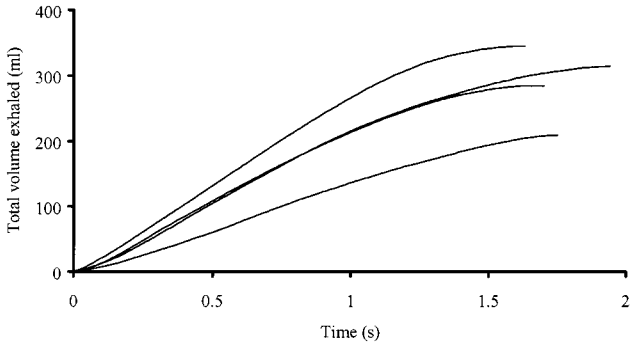


**Figure 3.** Breath volume recordings for 4 swallow breaths sampled from one nostril during the consumption of a solution of cymene in water (the other nostril remained open).

the sample will transfer gas-phase and liquid-phase samples from the mouth into the pharynx. The aqueous phase will coat the pharynx and may also act as a potential pool of volatile compounds. These processes will create a plug of volatile-laden gas in the pharynx just prior to exhalation. If this plug of gas passes through the nose without dilution by air from the lungs it should leave the nose as a discrete band. If, however, this plug of air has been thoroughly mixed with the air from the lungs then the volatile concentration in the breath will be similar at the start and end of the exhalation.

Using API, the concentration of volatiles in the breath can be followed over the time course of one single exhalation, such as the "swallow breath", i.e., the first exhalation to occur after swallowing. The profile for volatiles released from the sample can be compared with that observed for acetone. Acetone is generated in the liver during fatty acid metabolism and is exported in the breath, acting as a marker for exhalation. Cymene was selected as the test compound, because it would interact with the nasal mucosa to the least extent (*17*).

The cymene solution (10 ppm) was placed in the mouth and swallowed immediately; cymene was not detected throughout the entire breath but was exhaled as a discrete plug (**Figure 2**). The increase in the signal for cymene coincided with the start of the increase in the acetone signal (i.e., the start of the exhalation process) and finished 0.2 s later, long before the end of the exhalation. This would be consistent with minimal mixing or dilution of the exhaled aroma with breath from the lungs. Other compounds (consumed in aqueous solution) were also found to produce the most intense signal at the start of the exhalation, but some would still be present at significant concentrations (up to 60% of the maximum signal) throughout the rest of the exhalation due to their partitioning behavior (*17*).

Simultaneous with the API analysis of the breath cymene concentration, breath volume flow was measured (**Figure 3**) for the nostril used for API sampling. Experiments were performed with the second nostril either open or blocked, however this did not significantly affect the average volume of breath exhaled (517 ± 38 mL) or the duration of exhalation (1.77 ± 0.15s). The average duration of the cymene peak from start to finish was 0.2 s, with the majority of the cymene being detected over a 0.12 s period. During 0.12 s of the initial phase of exhalation, 20 ± 9 mL of breath was exhaled (**Figure 3**). This volume is very similar to the 5−15 mL of breath that Land (*18*) estimated to pass from the mouth into the pharynx during swallowing. This is also consistent with minimal mixing or dilution of volatiles in the gas phase as they pass from the pharynx through to the end of the nose.

The data presented by Vuilleumier and co-workers (*7*) showed that when the concentration of aroma compounds inhaled via an olfactometer was the same as that delivered retronasally, the perceived intensity was the same. Retronasal aroma delivery will have occurred as a narrow peak during the entire exhalation, whereas orthonasal inhalation from an olfactometer is likely to have delivered a similar volatile concentration to the nose throughout inhalation. It would appear, therefore, that it was the actual volatile concentration inhaled or exhaled that was the main factor affecting the intensity of perception, rather than the absolute amount of volatile passing through the nose.

If dilution in-mouth by saliva or in-nose by expired air are minimal factors affecting the delivery of volatiles from aqueous solutions, why are the breath volatile concentrations so much lower than those in comparable headspace analyses (*7*−*9*)? This was investigated using a wide range of volatile compounds in aqueous solution. It was considered that factors affecting the release of compounds into the breath in vivo may be affected by the physicochemical properties of the compounds themselves.

For each volatile, the equilibrium headspace concentration was measured (*8*) along with the mouthspace and nosespace volatile concentrations following consumption. By expressing the nosespace or mouthspace concentrations relative to the headspace concentration, differences expected on the basis of the air/water partitioning behavior of compounds were eliminated. For example, on the basis of the air/water partition coefficients, the headspace concentration of limonene would be greater than that of pyrazine (for similar concentrations of the volatiles in solution). Assuming dilution was the sole mechanism causing the decrease in mouthspace and nosespace concentrations, the nosespace concentration as a proportion of the headspace concentration should be the same for both volatiles.

The amounts of volatile in the breath relative to headspace, however, varied considerably between volatiles (**Table 1**). Butanol was present in the mouthspace at concentrations equal to 70% of those observed for headspace. By comparison, nonoxygenated terpenes had a mouthspace-to-headspace ratio of less than 1%. Clearly, there were major differences (over 100 fold) in the efficiency of volatile delivery, independent of any dilution factors (dilution would be expected to affect all compounds equally).

Given that neither volatile loss in vivo (*16*) nor dilution appear to be major factors affecting volatile delivery, what is reducing the breath volatile concentration? A significant correlation ($R^2 = 0.72$, **Figure 4**) was found between the mouthspace/headspace ratio (ms/hs) and the air/water partition coefficient, $K_{aw}$, despite the variation in the data observed between replicates (**Table**

**Table 1.** Values of Mouthspace and Nosespace Signal Intensities Relative to Those for Headspace (ms/hs and ns/hs respectively)

| compound[a] | ms/hs %[b] | ns/hs %[b] | compound[a] | ms/hs %[b] | ns/hs %[b] |
|---|---|---|---|---|---|
| 1-butanol | 70 | 6.4 | ethyl acetate | 22 | 5.3 |
| ethanol | 66 | 8.2 | octanol | 22 | 3.4 |
| propan-2-ol | 66 | 11 | octanone | 20 | 4.4 |
| propan-2-ol | 63 | 9.0 | acetaldehyde | 19 | 3.0 |
| pyrazine | 60 | 6.7 | menthone | 19 | 3.3 |
| pyrazine | 54 | 5.4 | 2-pentanone | 15 | 4.6 |
| dimethyl pyrazine | 51 | 7.6 | isoamyl acetate | 15 | 3.5 |
| 3-hexenol | 49 | 8.8 | 2-methylbutanal | 15 | 4.4 |
| furfuryl acetate | 48 | 8.5 | hexanal | 14 | 3.2 |
| diethyl methylpyrazine | 47 | 5.9 | ethyl butyrate | 13 | 2.4 |
| 2-methylbutanol | 45 | 5.0 | hexanal | 13 | 2.2 |
| butanone | 39 | 6.2 | butanal | 13 | 3.4 |
| carvone | 39 | 5.5 | ethyl hexanoate | 11 | 2.4 |
| hexanol | 38 | 6.6 | 2-methylbutanal | 9.9 | 3.3 |
| diacetyl | 37 | 4.8 | ethyl hexanoate | 9.4 | 2.5 |
| methyl acetate | 33 | 7.8 | ethyl hexanoate | 8.6 | 2.3 |
| benzaldehyde | 30 | 3.3 | methyl propanal | 7.8 | 3.8 |
| 2-hexenal | 29 | 3.5 | citronellal | 7.6 | 1.7 |
| guaiacol | 29 | 2.6 | decanone | 6.3 | 1.4 |
| carvone | 28 | 5.0 | octanal | 5.4 | 0.80 |
| linalool | 28 | 4.2 | decanol | 5.2 | 1.1 |
| terpineol | 28 | 4.1 | ethyl octanoate | 2.6 | 0.61 |
| 2-pentanone | 28 | 6.4 | decanal | 2.1 | 0.38 |
| 1,8-cineole | 26 | 5.8 | methyl furan | 1.1 | 0.39 |
| menthol | 24 | 5.5 | limonene | 0.68 | 0.44 |
| isobutyl methoxypyrazine | 23 | 5.6 | cymene | 0.58 | 0.15 |
| methyl salicylate | 23 | 2.7 | cymene | 0.52 | 0.16 |
| octanone | 22 | 4.4 | pinene | 0.17 | 0.09 |

[a] Where replicate solutions of a compound were prepared more than one value will be shown. [b] Each value is the median of 4 replicate samples, consumed by 2 panelists.

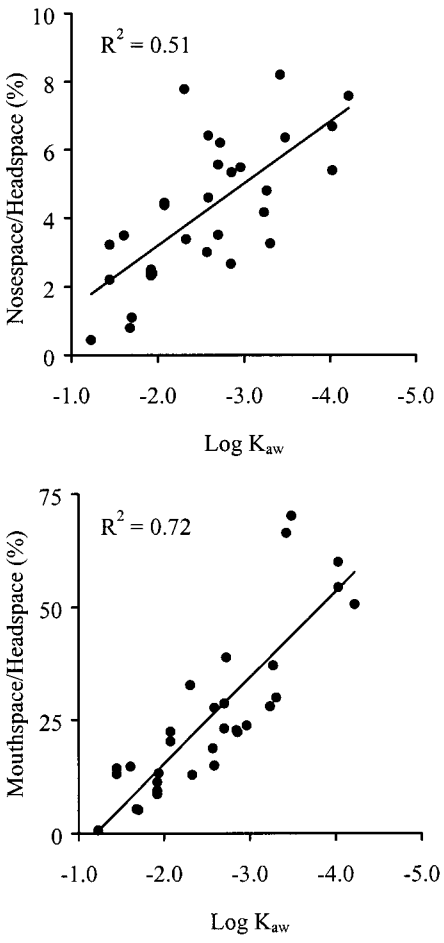


**Figure 4.** Relationship between the nosespace/headspace ratio and mouthspace/headspace ratio and the air/water partition coefficient.

**1**). A correlation of the data with $K_{aw}$ was not expected, given that the data were expressed relative to the headspace concentration and, in theory, independent of $K_{aw}$.

**Mass Transfer During Consumption in Vivo.** $K_{aw}$ has been identified as the major factor responsible for the differences between compounds in dynamic release studies, where volatiles partition between aqueous phases and the air above them (*8, 19, 20*). The relationship between $K_{aw}$ and the overall mass transfer coefficient ($k$) is shown in eq 1. $k_g$ and $k_l$ are the mass transfer terms for the gas and liquid phases respectively, which were similar for each compound (*19*).

$$\frac{1}{k} = \frac{1}{k_g} + \frac{K_{aw}}{k_l} \quad (1)$$

Compounds with low air/water partition coefficients (e.g., $1 \times 10^{-5}$) had much more stable headspace concentrations during gas-phase dilution (*19*), and reached equilibrium faster than those with higher $K_{aw}$ values (e.g., $1 \times 10^{-2}$). This may explain the observed correlation between $K_{aw}$ and the ms/hs ratio (**Figure 4**). The compounds with the lowest ms/hs values have the highest $K_{aw}$ values (and hence lower $k$) than those with the highest ms/hs ratios. It would therefore appear that mass transfer (as described by eq 1) could be a major factor affecting the amount of volatile that partitions into the breath during the consumption of aqueous solutions.

It has been reported (*4, 18*) that small volatile compounds could reach near equilibrium with aqueous systems within a few seconds, such that equilibration rates might not substantially affect volatile delivery in vivo. This was supported by Buettner and Schieberle (*21*), who found that the amount of ethyl butyrate in the breath after 5 s of in-mouth equilibration was just under half that of the maximum breath volatile content after longer periods of equilibration. For many volatiles, however, the mouthspace volatile concentrations observed in this study (**Table**

**Table 2.** Average Headspace and Nosespace Volatile Concentrations ($mg/m^3$) for Solutions Containing either Ethyl Hexanoate (5 ppm) or Octanol (10 ppm), and 25 g/kg Lipid Which Had Been either Crudely Blended or Homogenized at High Pressure

| | | ethyl hexanoate | | octanol | |
|---|---|---|---|---|---|
| sample[a] | | mean | SD | mean | SD |
| headspace | homogenized | 14.7 | 4.9 | 0.58 | 0.19 |
| headspace | crude mix | 11.7 | 2.7 | 0.60 | 0.18 |
| nosespace | homogenized | 0.72 | 0.30 | 0.17 | 0.07 |
| nosespace | crude mix | 0.28 | 0.09 | 0.10 | 0.02 |

[a] The nosespace values are based on 6 replicate samples consumed by 3 panelists; the headspace values are based on 3 replicates.

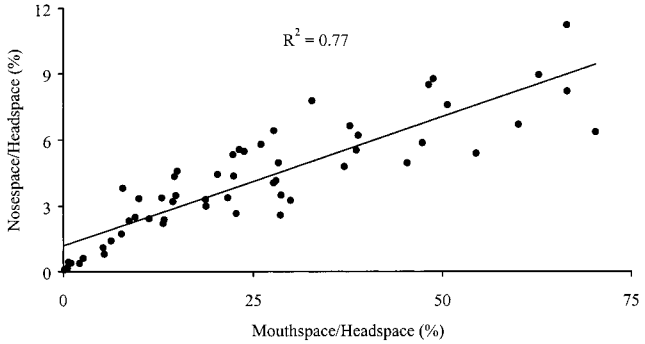


**Figure 5.** Relationship between the nosespace/headspace and mouthspace/headspace ratios. Data are from **Table 1**.

**1**) were far from those found during headspace analysis, suggesting a radical departure from the equilibrium.

If the dynamics of equilibration and mass transfer are important factors in the delivery of aroma compounds from mouth to nose, then food systems that alter either the partitioning behavior of compounds ($K_{aw}$), or their mass transfer in the aqueous phase ($k_l$) should affect flavor delivery. Adding a lipid emulsion to solutions of esters decreased their headspace concentration (*8*) and, hence, changed the partition coefficient (relative to the headspace above a totally aqueous sample). On consumption, both emulsion systems and water might be expected to show similar ns/hs ratios. However, in comparison with the aqueous solution, the nosespace content for lipid-containing systems was much greater than that expected on the basis of the headspace studies (*8*). This was attributed to dilution by saliva in-mouth affecting the air/emulsion partition coefficient (the air/emulsion partition coefficient is dependent on the oil fraction) as proposed by McNulty (*22*). It would have been necessary to dilute the emulsion 10-fold during consumption to increase the breath volatile concentration via changes in the air/emulsion partition coefficient alone. On the basis of current studies it seems more likely that it was changes in overall mass transfer ($k$), caused by changes in the partitioning behavior, that affected volatile delivery.

Emulsion systems with different droplet sizes might also be expected to show differences in release. Samples with different droplet sizes did not show significant differences in their in vitro partitioning behavior, and consequently had similar headspace concentrations (**Table 2**). They would also have been subjected to similar absorptive losses, and in-mouth or in-nose dilution. Despite their similar partitioning, dilution, and absorption, the nosespace concentration was higher for the samples with the smaller droplet size (**Table 2**). This would be consistent with changes in mass transfer within the sample, as the larger oil droplets would have restricted flavor diffusion and delivery.

The equation describing overall mass transfer (eq 1) was originally produced to describe the behavior of volatiles in the headspace above solutions while the gas phase was being diluted. It does, however, appear to have relevance for the in-mouth/throat situation where samples are under nonequilibrium conditions, principally driven by differences in $K_{aw}$, with $k_g$ as a constant and $k_l$ dependent on the sample matrix.

**Nosespace−Mouthspace Differences.** Comparison of the values obtained for the nosespace:headspace ratio (ns/hs) with those for the ms/hs ratio (**Table 1**; **Figure 5**) did show that there was a good correlation between the two (correlation coefficient, $R^2 = 0.77$). The values for the ns/hs ratio were, however, on average 8-fold lower than those for the ms/hs ratio. These differences may have been caused by absorptive losses as the volatile-laden air passed through the nose. Linforth and Taylor (*17*) showed that some compounds were very persistent in the breath, appearing in the exhalations following the swallow breath at concentrations up to 70% of those observed for the swallow breath. These compounds were also reported to show much wider swallow breath peaks, which was attributed to continuous absorption and desorption of the volatile between the air and nasal mucosa during exhalation (the "wash in−wash out" principle (*10*)). This is supported by the observation that dimethyl pyrazine was persistent on the breath, not only when consumed retronasally in solution, but orthonasally in the gas phase. The dimethyl pyrazine absorbed to the nasal epithelium, and then gradually desorbed, such that it was detected at 20% of its maximum concentration 1 min after initial orthonasal inhalation (unpublished Linforth).

The compounds showing the greatest absorption to the nasal epithelia, and hence persistence, were the more hydrophilic compounds, and those with the lowest vapor pressures (*17*). This is consistent with absorptive losses expected on the basis of models of upper airway function (*11*) and studies of volatile uptake by the respiratory tract (*10*). The model proposed by Keyhani and co-workers (*11*) relates directly to in-nose volatile absorption during inhalation, however, it seems reasonable to assume that similar processes will act during exhalation. Their model would predict in-nose losses of 15, 30, 60, and 75% for limonene, isoamyl acetate, butanol, and ethanol, respectively. The decrease in volatile concentration between the mouthspace and the nosespace was greater than that expected for all compounds (**Table 1**). The non-oxygenated terpenes would be expected to show the least absorptive losses, however the ratio between the ns/hs and ms/hs values was a factor of 2.6. If their breath concentration decreased by 15% due to absorptive losses this would leave a factor of 2.2 unaccounted for. This might be due to gas-phase dilution, given that the volume of the swallow breath that contained cymene (20 mL) was approximately twice the average volume Land reported (*18*) for the air displaced from the mouth to the nose during swallowing. Using a breath dilution factor of 2.2 and the absorptive losses of the Keyhani model, the nosespace volatile concentration for ethanol, butanol, and isoamyl acetate would be 8, 12, and 5% of the headspace concentrations, respectively. These are much closer to the ns/hs values actually observed (**Table 1**), than those based on the Keyhani model alone.

One consequence of the range of ns/hs values for different volatiles, is that compounds present at similar concentrations in equilibrium headspace samples could be present at substantially different concentrations in the breath following consumption. This may have significant implications for the analysis of beverages and high-water-content foodstuffs.

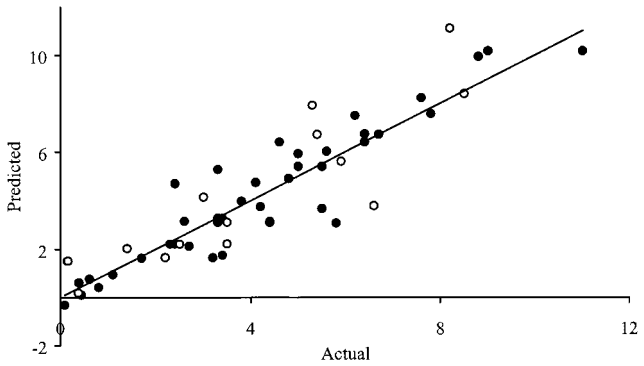


**Figure 6.** Actual and predicted nosespace/headspace values for the modeling data set (●) and the test set (○).

**Modeling the ns/hs Ratio.** Although the ms/hs data showed a good correlation with the $K_{aw}$ values available, the correlation between $K_{aw}$ and ns/hs data was much lower ($R^2 = 0.51$, **Figure 4**). This is likely to have been caused by the differential absorption of volatiles in the nose, which will have affected the compounds with the lowest $K_{aw}$ values the most. A series of physicochemical descriptors was calculated for the volatiles and compared with the ns/hs values. The octanol/water partition coefficient (Log *P*) showed the strongest correlation with the data, but the correlation coefficient ($R^2$) was only 0.49, which would have had limited predictive power.

A model was developed using a quantitative structure property relationship (QSPR) approach (*23*). Part of the data set (42 values) was used to develop the model, with the remaining 14 values serving as a test set for model validation. The parameters that were selected for the model (statistical significance $P < 0.05$) were Log *P*, parameters related to the hydrophobic van der Waals surface area (Q_VSA_HYD and vsa_hyd), and the fraction of the molecule's van der Waals surface area which was negatively charged and polar (PEOE_VSA_FPNEG). These will express the size and shape of the hydrophobic and hydrophilic regions of the molecule, which will affect both solubility and partition between aqueous and gaseous media. In addition, a count of the number of rotatable bonds divided by the total number of bonds (b_rotR) was found to improve the model, presumably reflecting differences in behavior between aromatic and aliphatic structures.

The final equation for the model (eq 2) contained linear, quadratic, and interactive terms. The model had a predictive correlation coefficient ($R_{cv}^2$) of 0.75 compared with an $R^2$ of 0.86 (**Figure 6**), indicating reasonable predictive power. However, with a model equation containing as many components as eq 2, it is necessary to validate its predictive power with a test set. The values predicted from the model for the test set were found to correlate with their actual values (**Figure 6**) with a correlation coefficient of 0.78. In addition, the intercept and the gradient of the regression line for the test set (0.12 and 1.04, respectively) were very close to those for the model itself (i.e., 0 and 1), further confirmation of the predictive power of the model.

$$\text{ns/hs \%} = 103\text{*PEOE\_VSA\_FPNEG} - 6.3\text{*Log } P + 63\text{*b\_rotR} + 0.043\text{*Q\_VSA\_HYD} + 0.14\text{*vsa\_hyd} - 219\text{*(PEOE\_VSA\_FPNEG)}^2 - 0.00093\text{*(vsa\_hyd)}^2 - 20\text{*Log } P\text{*b\_rotR} + 0.057\text{*Log } P\text{*vsa\_hyd} - 330\text{*PEOE\_VSA\_FPNEG*b\_rotR} - 13 \quad (2)$$

**Extrapolation to Longer Time Periods.** The samples consumed in these experiments were aqueous solutions and consequently were consumed within a few seconds, and may not reflect the situation over a longer eating time course. However, during the consumption of a range of solid foodstuffs the breath volatile concentration was still observed to be lower than the headspace volatile concentration (*9*). This might be attributed to effects such as differences in mass transfer, etc., however, the headspace system used in these studies was the retronasal aroma simulator or RAS (*24*), which was designed to mimic in-mouth aroma release.

One possible explanation for the ns/hs ratio is that during chewing the volume of air in the mouth is minimal, because the volume is largely occupied by the bolus which is confined by the tongue and cheeks as it is guided between the teeth. This air rapidly exchanges with the air in the pharynx during inhalation or exhalation as a result of mouth movements, such as swallowing or chewing actions (*21*). Chewing actions are the most frequent of these events, occurring at a frequency of around 2 per second. This may be sufficiently frequent to constantly remove and replenish the air phase in-mouth such that it fails to reach equilibrium with the volatile content of the bolus or saliva.

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